U.S. FOOD & DRUG ADMINISTRATION

# 2017 FDA Science Forum

## Mission Possible:
## FDA Research Enhancing Public Health

May 31 - June 1, 2017 • Great Room

www.fda.gov

# Contents

# A Message from FDA's Commissioner

**Scott Gottlieb, MD**
Commissioner, FDA

Addendum letter will be posted.

## Bio of Scott Gottlieb, MD

Dr. Scott Gottlieb was sworn in as the 23rd Commissioner of Food and Drugs on May 10, 2017. Dr. Gottlieb is a physician, medical policy expert, and public health advocate who previously served as the FDA's Deputy Commissioner for Medical and Scientific Affairs and before that, as a senior advisor to the FDA Commissioner.

He also worked on implementation of the Medicare drug benefit as a Senior Adviser to the Administrator of the Centers for Medicare and Medicaid Services, where he supported policy work on quality improvement and the agency's coverage process, particularly as it related to new medical technologies.

In 2013 Dr. Gottlieb was appointed by the Senate to serve on the Federal Health Information Technology Policy Committee, which advises the Department of Health and Human Services on healthcare information technology.

Dr. Gottlieb was previously a Resident Fellow at the American Enterprise Institute, and a Clinical Assistant Professor at the New York University School of Medicine in Manhattan, where he also practiced medicine as a hospitalist physician.

He completed a residency in internal medicine at the Mount Sinai Medical Center in New York, New York and is a graduate of the Mount Sinai School of Medicine and of Wesleyan University, in Middletown, Connecticut, where he studied Economics.

# A Message from FDA's Acting Chief Scientist

**Luciana Borio, MD**
Acting Chief Scientist, Office of the Chief Scientist, OC, FDA

We live in an age of speed, distinguished by instant access to a raft of products, the push button economy, and communication in 140 characters. Some may wonder why this characteristic of our times, which has reshaped other sectors of our economy, has not led to equally rapid medical product development. And so it bears noting that the app we use to hail a cab or order a book is, in fact, the outcome of many long hours of scientific research, collaboration, and investment.

The 2017 FDA Science Forum gives the American public the chance to understand and appreciate the unique scientific research and collaborative efforts of our 11,000 scientists, who investigate and apply new science and emerging technologies to inform FDA's regulatory decision-making and foster safe innovation.

Just as industry focuses on product development research and academia focuses on the scientific foundation, FDA research concentrates on creating test methods and developing a knowledge of processes to make sure that our products are safe and effective for consumers and patients--and that the harm from regulated tobacco products is reduced. The science we do at FDA is critical to product quality and safety, most especially because it's seldom undertaken by industry or academia.

For example, as the emerging technology of additive manufacturing is put into use and medical devices, produced by 3D printing, are increasingly cleared through FDA's Center for Devices, FDA bioengineers have positioned themselves at the forefront of knowledge and research about this process, conducting research into patient matching, imaging, and phantoms. With our proactive posture, FDA is paving the way for safe and effective innovation that will usher in life-saving advanced treatments for patients.

Or take the growing use of nanomaterials in FDA-regulated products. Silver nanoparticles are now used in wound dressing for their antimicrobial properties. And liposomal nanoparticles are used as drug carriers to reduce toxicity and increase circulation time in the blood. Characterizing these complex nanomaterials is challenging, and well developed methods are not available. But FDA research has produced analytical methods for characterizing nanomaterials in over-the-counter FDA-regulated products. This will help us with assessing risk, developing industry guidelines for characterizing nanomaterials, postmarket surveillance, and determining shelf life of nanomaterials in consumer products.

In the area of food safety, FDA has contributed to enhancing antimicrobial resistance monitoring in a collaborative effort with our sister agencies. Genomics studies conducted by FDA scientists have demonstrated that we can use the emerging technology whole genome sequencing as an effective tool for predicting antimicrobial resistance of certain foodborne pathogens.

The robust science that allows us to do all these things--from 3D printing to omics technologies—also promises to speed access to safe and effective medical products, and enable a swifter and more efficient monitoring of food safety. But not without the strong research that brings this vision to life. And the urgency of developing medical products rapidly and successfully has never been greater as infectious disease outbreaks like Zika become the new normal and put us all at risk.

Over the next two days we hope you gain a deeper understanding of the cutting-edge science we do at FDA to protect and promote the public health. And we look forward to sharing with you some of the exciting advances we're making with our partners in the scientific community.

## Bio of Luciana Borio, MD

Dr. Luciana Borio is FDA's acting chief scientist. In this capacity, she is responsible for leading and coordinating FDA's cross-cutting scientific and public health efforts.

The Office of the Chief Scientist works closely with FDA's product centers, providing strategic leadership and support for FDA's regulatory science and innovation initiatives, including the Advancing Regulatory Science Initiative, the Critical Path Initiative, scientific professional development, scientific integrity, and the Medical Countermeasures Initiative (MCMi).

Since 2011, Dr. Borio has served as the assistant commissioner for counterterrorism policy and director of the Office of Counterterrorism and Emerging Threats (OCET) in the Office of the Chief Scientist at FDA. In this capacity, Dr. Borio provided leadership, coordination, and oversight for FDA's national and global health security, counterterrorism, and emerging threat portfolios and led the MCMi.

Dr. Borio has been instrumental in coordinating FDA's response to the 2009 H1N1 influenza pandemic and continues to oversee FDA's preparedness and response activities for emerging threats, such as the avian influenza A (H7N9) virus and the West Africa Ebola epidemic. Before joining FDA as a medical reviewer in 2008, Dr. Borio served as a senior associate at the University of Pittsburgh Medical Center – Center for Biosecurity, assistant professor of medicine at the University of Pittsburgh, and advisor on biodefense programs for the U.S. Department of Health and Human Services. Dr. Borio received her MD from the George Washington University, and continues to practice medicine at the Johns Hopkins Hospital.

# **FDA Science Forum Agenda** (Day 1: May 31, 2017)

**8:30 – 8:40 AM** **Introduction**
Bernadette Johnson-Williams, MEd, Senior Advisor for STEM, Office of the Chief Scientist

**8:40 – 8:50 AM** **Welcome**
FDA Acting Chief Scientist, Luciana Borio, MD

**8:50 – 9:05 AM** **Remarks and Introduction of keynote speaker**
FDA Commissioner, Scott Gottlieb, MD

**9:05 – 9:45 AM** **Frontiers in Biomedical and Regulatory Science**
Keynote Speaker: Eric Lander, PhD,
President and Founding Director of the Broad Institute

**9:45 – 10:55 AM** **Poster Session 1 and Break**
Topics:
1. Identification and Evaluation of New Biomarkers
2. FDA Response to Urgent Public Health Needs

*10:55 – 12:40 PM* *Concurrent Sessions 1 & 2:*

## Concurrent Session 1: Identification and Evaluation of New Biomarkers

### Great Room Section B

Session Chair : Lisa Meier McShane, PhD, Chief, Biostatistics Branch, Biometric Research Program, National Institutes of Health /National Cancer Institute

**10:55 – 11:25 AM** **FDA/National Institutes of Health Interactions and BEST**
Lisa Meier McShane, PhD, Chief, Biostatistics Branch,
Biometric Research Program, National Institutes of Health/NCI

**11:25 – 11:35 AM** **Biomarker Qualification Program with Update, Case Studies and Challenges**
Christopher Leptak, MD, PhD,
Associate Director of Biomarker Development Regulatory Science Team, Center for Drug Evaluation and Research

**11:35 – 11:45 AM** **Biomarker Data in the Population Assessment of Tobacco and Health (PATH) Study**
Cindy M. Chang, PhD, MPH, Epidemiologist, Center for Tobacco Products

**11:45 – 11:55 AM** **Transcript, Proteo, and Metabol-omics as Tools for Translational Biomarker Discovery and Evaluation**
William B. Mattes, PhD, DABT, Director,
Division of Systems Biology, National Center for Toxicological Research

**11:55 – 12:05 PM** **CDRH Perspectives on Imaging Biomarkers Analytical Validation Expectations**
Daniel M. Krainak, PhD, Biomedical Engineer,
Center for Devices and Radiological Health

**12:05 – 12:15 PM** **Next Generation Sequencing (NGS): FDA Approval of the**

**1st NGS Companion Diagnostic**
Hisani Madison, PhD, MPH, Scientific Reviewer,
Center for Devices And Radiological Health

12:15 – 12:40 PM **Panel**
Lisa Meier McShane, PhD; Christopher Leptak, MD, PhD;
Cindy M. Chang, PhD, MPH; William B. Mattes, PhD, DABT;
Daniel M. Krainak, PhD; and Hisani Madison, PhD, MPH

## Concurrent Session 2: FDA Response to Urgent Public Health Needs

### Great Room Section C

Session Chair: RADM Palmer Orlandi, Jr, PhD, Chief Science Officer and Director of Research, Office of Foods and Veterinary Medicine

10:55 – 11:10 AM **FDA's Coordinated Response to Recent Foodborne Outbreaks,**
CDR Kari Irvin, MS, CORE Response Manager,
Center for Food Safety and Applied Nutrition

11:10 – 11:25 AM **Characterization and Analysis of Multidrug Resistant**
Foodborne Pathogens
Heather Tate, PhD, MS, Epidemiologist, Center for Veterinary Medicine

11:25 – 11:40 AM **Forensic Analysis of a Mass Poisoning in Mozambique Associated with a Homebrewed Beverage**
Travis Falconer, PhD, Chemist, Office of Regulatory Affairs

11:40 - 11:55 AM **Use of a FDA Real Time Mobile Communication Platform System during Medical Countermeasure Events: RAPID**
Henry "Skip" Francis, MD,
Director for Data Mining and Informatics Evaluation and Research,
Center for Drug Evaluation and Research

11:55 – 12:10 PM **Development of Total and Neutralizing Anti-Ebolavirus Antibody Assays for Deployment in West Africa to Evaluate Clinical Trials of MCM**
including Vaccines and Immunotherapies
Gerardo Kaplan, PhD, Principal Investigator,
Office of Blood Research and Review,
Center for Biologics Evaluation and Research

12:10 – 12:25 PM **Development of Mouse Models to Assess Efficacy and Potency of ZIKA Virus Therapeutics**
Daniela Verthelyi, MD, PhD, Lab Chief, Office of Biotechnology Products,
Center for Drug Evaluation and Research

12:25 – 12:40 PM **Q&A Session**

12:40 – 1:30 PM **Lunch**

## Concurrent Session 3: Microbiome and Human Health

### Great Room Section B

Session Chair: Ryan Ranallo, PhD, Program Officer, National Institutes of Health/National Institute of Allergy and Infectious Diseases

**1:30 – 1:45 PM** **Overview**
Ryan Ranallo, PhD, Program Officer, National Institutes of Health, National Institute of Allergy and Infectious Diseases

**1:45 – 2:00 PM** **MetaGenomeTrakr and Food Safety Microbiome Research at Center for Food Safety and Applied Nutrition**
Andrea Ottesen, Ph.D, Research Microbiologist, Center for Food Safety and Applied Nutrition

**2:00 – 2:15 PM** **MAIT Cells Alter the Murine Microbiome Reducing Colonization Resistance against *Clostridium difficile*.**
Paul Carlson, PhD, Senior Staff Fellow, Center for Biologics Evaluation and Research

**2:15 - 2:30 PM** **The Effect of Chlortetracycline on Swine Fecal Microbiome and Resistome**
Daniel A. Tadesse, PhD, Research Microbiologist, Center for Veterinary Medicine

**2:30 - 2:45 PM** **Interaction of Silver Nanoparticles Beyond Intestinal Bacterial Microbiota: Effects of Intestinal Virome and phages**
Sangeeta Khare, PhD, Research Microbiologist, National Center for Toxicological Research

**2:45 – 3:00 PM** **Impact of TNF Antagonist Treatment on the Gut Microbiome: An in Vivo Pilot Study**
Odile Engel, PhD, Researcher, Center for Drug Evaluation and Research

**3:00 – 3:30 PM** **Q&A Session**

## Concurrent Session 4: Advanced Manufacturing and 3D Printing

### Great Room Section C

Session Chair: Andy Christensen, President, Somaden LLC

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|---|---|
| 1:30 - 1:45 PM | **A Historical Perspective of 3D Printing in Clinical Medicine**<br>Andy Christensen, President, Somaden LLC |
| 1:45 – 2:00 PM | **Techniques for Performance and Process Evaluation of Advanced Manufacturing**<br>LCDR James Coburn, MS, Sr. Research Engineer,<br>Center for Devices and Radiological Health |
| 2:00 – 2:15 PM | **Continuous Manufacturing Technologies**<br>Celia Cruz, PhD, Division Director, Center for Drug Evaluation and Research |
| 2:15 – 2:30 PM | **Manufacturing the Seasonal Flu Vaccine**<br>Zhiping Ye, MD, PhD, Senior Investigator,<br>Center for Biologics Evaluation and Research |
| 2:30 – 2:45 PM | **Practical Microscale Technologies in the Assessment of Advanced Therapeutic Products in Center for Biologics Evaluation and Research**<br>Kyung Sung, PhD, Principal Investigator,<br>Center for Biologics Evaluation and Research |
| 2:45 – 3:00 PM | **Continuous Bio-manufacturing Technologies**<br>LCDR Cyrus Agarabi, PharmD, RPh, MBA, PhD, Regulatory Research Officer,<br>Center for Drug Evaluation And Research |
| 3:00 – 3:15 PM | **Advancing Characterization of 3D Printed Tissue Engineered Scaffolds**<br>Maureen Dreher, PhD, MS, Research Biomedical Engineer,<br>Center for Devices and Radiological Health |
| 3:15 - 3:30 PM | **Q & A Session** |
| 3:30 – 4:30 PM | **Poster Session 2**<br>Topics:<br>1. Microbiome and Human Health<br>2. Additive Manufacturing and 3D Printing<br>3. FDA Response to Urgent Public Health Needs |
| 4:30 PM | **End of Day 1** |

# **FDA Science Forum Agenda** (Day 2: June 1, 2017)

**9:15 – 10:15 AM** **Poster Session 3 and Break**

Topics:

1. Omics Technologies at the FDA
2. Patient and Consumer Engagement and Communication
3. FDA Response to Urgent Public Health Needs

*10:15 – 12:00 PM* *Concurrent Sessions 5 & 6*

## Concurrent Session 5: Omics Technologies at the FDA

### Great Room Section B

Session Chair: Minnie Sarwal, MD, FRCP, DCH, PhD, Professor of Surgery, Director Precision Transplant Medicine, University of California, San Francisco, FDA Science Board member

**10:15 – 10:30 AM** **Overview**
Minnie Sarwal, MD, FRCP, DCH, PhD, Professor of Surgery,
Director Precision Transplant Medicine,
University of California, San Francisco,
FDA Science Board member

**10:30 - 10:45 AM** **FDA led community-wide Sequencing Quality Control Consortium 2- (SEQC2)**
Weida Tong, PhD, Division Director,
Bioinformatics and Biostatistics, National Center for Toxicological Research

**10:45 – 11:00 AM** **FDA's GenomeTrakr Program: Advancing Food Safety Through Whole-Genome Sequencing of Foodborne Bacteria**
Errol Strain, PhD, Director, Biostatistics and Bioinformatics Staff,
Center for Food Safety and Applied Nutrition

**11:00 – 11:15 AM** **MicroRNA Biomarkers of Acute Pancreatic Injury Use**
Rodney Rouse, DVM, MBA, PhD, Acting Associate Director,
Division of Applied Regulatory Science, Office of Translational Science,
Center for Drug Evaluation and Research

**11:15 – 11:30 AM** **FDA-ARGOS Microbial Reference Genomes for Regulatory Use: Zika and Ebola**
Heike Sichtig, PhD, Subject Matter Expert, Principal Investigator,
Center for Devices and Radiological Health

**11:30 – 11:45 AM** **Glycomics Work-Flows for the Characterization of**
Vaccine Glycoprotein Antigens,
John Cipollo, PhD, Principal Investigator, Lab of Bacterial Polysaccharides,
Center for Biologics Evaluation and Research

**11:45 – 12:00 PM** **Q&A Session**

## Concurrent Session 6: Patient and Consumer Engagement and Communication

### Great Room Section C

Session Chair: Brian J. Zikmund-Fisher, PhD, Associate Professor of Health Behavior and Health Education, University of Michigan

**10:15 – 10:20 AM** **Overview**
Brian J. Zikmund-Fisher, PhD,
Associate Professor of Health Behavior and Health Education,
University of Michigan

**10:20 - 10:35AM** **Use of Flavored Tobacco Products: Findings from the Population Assessment of Tobacco and Health (PATH) Study**
Bridget Ambrose, PhD, MPH, Supervisory Epidemiologist,
Center for Tobacco Products

**10:35 – 10:50 AM** **Understanding Mothers' Attitudes and Motivations Regarding Menu Labeling: Testing Messaging Concepts and Treatments**
Kathleen Yu, MPH, Social Scientist,
Center For Food Safety and Applied Nutrition

**10:50 – 11:05 AM** **Development of Tools to Capture the Patient Perspective with Implantable Minimally Invasive Glaucoma Surgical (MIGS) Devices**
Michelle Tarver, MD, PhD, Medical Officer,
Center for Devices and Radiological Health

**11:05 - 11:20 AM** **Upper Limb Prostheses Patient Preference Study to Inform Clinical Trial Design and Regulatory Decisions**
Heather Benz, PhD, Medical Device Fellow,
Center for Devices and Radiological Health

**11:20 - 11:35 AM** **Advancing the Science of Patient Input in a Regulatory Setting through Internal Capacity Building and Research**
Million Tegenge, PhD, RPh, Visiting Scientist,
Center for Biologics Evaluation and Research

**11:35 - 11:50 AM** **Communicating Risk Information about Drugs: the Effect of Quantitative Information Type on Risk Perceptions and Understanding**
Paula Rausch, PhD, RN, Associate Director,
Research and Risk Communications,
Center for Drug Evaluation and Research

**11:50 - 12:00 PM** **Moderator's Comments and Closing Remarks**
Brian J. Zikmund-Fisher, PhD,
Associate Professor of Health Behavior and Health Education,
University of Michigan

**12:00 – 1:00 PM** **Lunch**

1:00 – 2:00 PM **Poster Session 4 and Break**
Topics:
1. Computational Modeling and Simulation at FDA
2. Current Progress in Nanotechnology Research at FDA

*2:00 – 3:40 PM* *Concurrent Sessions 7 & 8*

## Concurrent Session 7: Computational Modeling and Simulation at FDA

### Great Room Section B

Session Chair: Grace Peng, PhD, Director of Computational Modeling and Simulation, National Institutes Of Health/ National Institute of Biomedical Imaging and Bioengineering

2:00 – 2:10 PM **Overview**
Grace Peng, PhD, Director of Computational Modeling and Simulation, National Institutes of Health/National Institute of Biomedical Imaging and Bioengineering

2:10 – 2:20 PM **Advancing Regulatory Science at FDA with Modeling and Simulation**
Tina Morrison, PhD, Chair, Modeling and Simulation Working Group, Center for Devices and Radiological Health

2:20 – 2:30 PM **Computational Electromagnetic Modeling and Medical Devices**
Leonardo Angelone, PhD, Research Biomedical Engineer, Center for Devices and Radiological Health

2:30 – 2:40 PM **Using (Q)SAR Modeling to Inform Drug Safety Assessment**
Naomi Kruhlak, PhD, Chemist, Center for Drug Evaluation and Research

2:40 – 2:50 PM **Modeling the U.S. Blood Supply for Emergency Preparedness**
Mark Walderhaug, PhD, Microbiologist, Center for Biologics Evaluation and Research

2:50 - 3:00 PM **Potential Uses for Modeling and Simulation in Veterinary Medicine**
Marilyn Martinez, PhD, Senior Scientist, Center for Veterinary Medicine

3:00 - 3:10 PM **Contamination of Food by Radionuclides after a Nuclear Accident**
Danielle Larese, PhD, ORISE Fellow, Office of Regulatory Affairs

3:10 – 3:20 PM **Modeling and Simulation in Tobacco Regulatory Science**
Antonio Paredes, MA, MS, Lead Mathematical Statistician, Center for Tobacco Products

3:20 – 3:40 PM **Q&A Session**

## Concurrent Session 8: Current Progress in Nanotechnology Research at FDA

### Great Room Section C

Session Chair: Anil Patri, PhD, Director, Nanotechnology CORE,
National Center for Toxicological Research

**2:00 – 2:20 PM** **Current Progress in Nanotechnology Research at FDA (NTF, CORES,** Research Infrastructure Facilities)
Anil Patri, PhD, Director, Nanotechnology CORE,
National Center for Toxicological Research

**2:20 – 2:30 PM** **The Safety of Nanomaterials Using Silver Nanoparticles as an Example**
Mary Boudreau, PhD, Research Toxicologist,
National Center for Toxicological Research

**2:30 - 2:40 PM** **Drug Products Containing Nanomaterials**
Katherine Tyner, PhD, Acting Associate Director of Science,
Center for Drug Evaluation and Research

**2:40 - 2:50 PM** **Nanotechnology and Medical Devices**
Peter Goering, PhD, Research Toxicologist,
Center for Devices and Radiological Health

**2:50 - 3:00 PM** **Nanomaterial Based in Vitro Diagnostics for Pathogens**
Indira Hewlett, PhD, Laboratory Chief,
Center for Biologics Evaluation and Research

**3:00 - 3:10 PM** **Potential Exposure to Nanoparticles from Nanotechnology-Enabled** Food Contact Materials
Timothy Duncan, PhD, Research Chemist,
Center for Food Safety and Applied Nutrition

**3:10 - 3:40 PM** **Panel discussion**
Anil Patri, PhD; Mary Boudreau, PhD; Katherine Tyner, PhD;
Peter Goering, PhD; and Indira Hewlett, PhD

**3:40 – 4:00 PM** **Closing Remarks and Adjourn**
Carol Linden, PhD, Director, Office of Regulatory Science and Innovation

# Keynote Speaker

**Eric Lander, PhD**

*President and Founding Director of the Broad Institute Massachusetts Institute of Technology*

**Bio:** Eric Lander is president and founding director of the Broad Institute of MIT and Harvard. A geneticist, molecular biologist, and mathematician, Lander has played a pioneering role in all aspects of the reading, understanding, and biomedical application of the human genome. He was one of the principal leaders of the international Human Genome Project (HGP) from 1990 to 2003, with his center being the largest contributor to the mapping and sequencing of the human blueprint.

With his colleagues, Lander has developed and applied methods for discovering the molecular basis of rare genetic diseases, common diseases, and cancer. He has done pioneering work on genetic variation, population history, evolutionary forces, regulatory elements, long non-coding RNAs, three-dimensional folding of the human genome, and methods to systematically identify the genes essential for biological processes.

Lander is professor of biology at MIT and professor of systems biology at Harvard Medical School. He has served on governing and advisory boards for various government agencies, academic institutions, and scientific societies, and has co-founded several successful biotechnology firms.

In 2008, Lander was appointed by President Obama as co-chair of the President's Council of Advisors on Science and Technology. PCAST is a council of 20 of the nation's leading scientists and engineers, which advises the White House on matters including health, advanced manufacturing, energy policy, information technology, drug innovation, spectrum and communications policy, nanotechnology, and national security.

Lander's numerous honors and awards include the MacArthur Fellowship, the Woodrow Wilson Prize for Public Service from Princeton University, the City of Medicine Award, the Gairdner Foundation International Award of Canada, the AAAS Award for Public Understanding of Science and Technology, the Albany Prize in Medicine and Biological Research, the Dan David Prize of Israel, the Mendel Medal of the Genetics Society in the UK, the Breakthrough Prize in Life Sciences, and the James R. Killian Jr. Faculty Achievement Award.

He was elected a member of the U.S. National Academy of Sciences in 1997 and of the U.S. Institute of Medicine in 1999. In 2013, he was elected to the Royal Swedish Academy of Sciences. He has received honorary degrees from 10 colleges and universities.

Lander earned his BA in mathematics from Princeton University (1978) and his PhD in mathematics from Oxford University (1981) as a Rhodes Scholar.

# Speaker Bios and Abstracts

Session 1: Identification and Evaluation of New Biomarkers

**Lisa M McShane, PhD**
(Session Chair)
*Chief, Biostatistics Branch, Biometric Research Program, DCTD, NIH/NCI*

**Bio:** Dr. McShane is a Mathematical Statistician and Chief of the Biostatistics Branch in the Biometric Research Program in the Division of Cancer Treatment and Diagnosis (DCTD) at the U.S. National Cancer Institute (NCI). She advises programs in DCTD and NCI on matters relating to development and use of tumor markers for prognosis, therapy selection, and disease monitoring. She holds a PhD in Statistics from Cornell University and is a Fellow of the American Statistical Association. Her statistical research interests include biomarker-driven clinical trial design, analysis methods for high-dimensional omics data, multiple comparisons methods, surrogate endpoints, measurement error adjustment methods, and biomarker assay analytical performance assessment. She co-led efforts to develop Reporting guidelines for tumor marker prognostic studies (REMARK) and Criteria for the use of omics-based predictors in clinical trials. She is a coauthor of numerous statistical and biomedical papers and the book Statistical Design and Analysis of DNA Microarray Investigations. She is a frequent invited speaker at national and international oncology and statistics meetings.

Dr. McShane serves on the Scientific Advisory Board for Science Translational Medicine and is a member of the Editorial Board for BMC Medicine. She has served on several American Society of Clinical Oncology panels and committees, including those that developed guidelines for HER2 and hormone receptor testing in breast cancer, EGFR mutation testing in lung cancer, and use of tumor biomarkers in early stage breast cancer. She has served as a member of the Institute of Medicine Committee for Management of the Air Force Health Study Data and Specimens, the Consensus Committee on Management of the Air Force Health Study Data and Specimens-Report to Congress, and the Committee on the State of the Science in Ovarian Cancer Research.

**Abstract: FDA-NIH collaboration to harmonize terminology for biomarkers and endpoints, with the goal of strengthening quality and improving efficiency of translational science**

*Presenter: Lisa M McShane, PhD*
*Chief, Biostatistics Branch, Biometric Research Program, DCTD, NIH/NCI*

Biomarkers play important roles in biomedical research, medical product development, and clinical practice. Inconsistent use of terminology related to biomarkers and study endpoints has sometimes interfered with efficient translation of promising biomedical discoveries and led to misunderstandings about evidence required to support use of biomarkers for development of new therapeutics and other medical products. In the spring of 2015, the NIH-FDA Joint Leadership Council charged an interagency committee with developing a glossary of harmonized terminology for biomarkers and endpoints. The goals were to improve communication, sharpen scientific understanding, and align expectations for evidentiary requirements. That ongoing interagency collaboration has led to the development of the BEST (Biomarkers, Endpoints, and other Tools) Resource (https://www.ncbi.nlm.nih.gov/books/NBK326791/). The first phase of BEST comprises a glossary that clarifies important definitions and describes some of the hierarchical relationships, connections, and dependencies among the terms. Key concepts include the distinction between biomarkers and clinical endpoints, the multiple potential uses of biomarkers, the pivotal role of intended use in establishing evidentiary criteria, goals of biomarker qualification, and the multiple aspects of validation. Illustrative examples of biomarkers used as baseline indicators of risk, prognosis, and therapy benefit, and biomarkers indicative of therapy response or used as surrogate endpoints in clinical trials, are discussed to reinforce the key concepts and enhance understanding of linkage of terminology to evidentiary requirements. Ultimately it is hoped that the harmonized glossary will strengthen the quality of biomedical research involving biomarkers and make the clinical translation process more efficient.

**Christopher Leptak, MD, PhD**

*Associate Director of Biomarker Development*

**Bio:** Christopher Leptak, MD, PhD, completed his MD and PhD in microbiology/immunology at the University of California, San Francisco. After residency in Emergency Medicine at Harvard's combined Massachusetts General and Brigham program, he joined FDA in 2007 as a primary reviewer in OND's division of gastroenterology products, focusing on immunomodulators for inflammatory bowel diseases. In 2010, he joined OND's Guidance and Policy Team and became OND's first Biomarker and Companion Diagnostics Lead. His focus is on biomarker and diagnostic device usefulness in clinical trials and drug development, both for drug-specific programs as well as in his role as the OND Co-Director for the Biomarker Qualification Program. He is currently Director of OND's Regulatory Science Program, which aims to improve regulatory consistency and policy development in areas of emerging science and technology.

**Abstract: How can biomarkers be leveraged to improve drug development: A Regulatory Perspective**

**Summary**: The presentation will explore how biomarkers can aid in the drug development process and inform regulatory decisions. Although much focus is placed on biomarkers used as surrogate endpoints, biomarkers have multiple uses in a clinical trial context that can help to inform appropriate patient selection and predict future clinical events or patient responses to therapy. Biomarker information can come from several data sources and these sources can work together synergistically to inform scientific understanding about a given biomarker's usefulness. CDER's Biomarker Qualification Program will be discussed to highlight some of the above issues.

**Learning Objectives**: Explain how biomarkers can be used to improve probability of success or accelerate a drug development program; Describe the different classes of biomarkers; Describe a framework to support biomarker development; Describe the different pathways by which biomarker information can be used to inform regulatory decisions; Describe some of the challenges and solutions around development and use of an appropriate biomarker during a clinical program

**Cindy Chang, PhD, MPH,**

*Epidemiologist, Office of Science, FDA Center for Tobacco Products*

**Bio**: Cindy M. Chang, PhD, MPH joined the Center for Tobacco Products (CTP) at the US Food and Drug Administration (FDA) in 2012 as an epidemiologist. Previously, she was a postdoctoral fellow in the Division of Cancer Epidemiology and Genetics at the National Cancer Institute. In her current role as epidemiologist at CTP, she reviews tobacco product applications and conducts research in tobacco regulatory science. She leads the Office of Science Biomarker Work Group which recently organized two CTP biomarker workshops: Biomarkers of Tobacco Exposure and Biomarkers of Potential Harm. She also conducts research assessing the potential health risks of tobacco use, including the use of biomarkers.

*Selected publications:*

Center for Tobacco Products/ U.S. Food and Drug Administration. Biomarkers of Tobacco Exposure: A Public Workshop. 2015; Available from: http://www.fda.gov/TobaccoProducts/NewsEvents/ucm447723.htm

Center for Tobacco Products/ U.S. Food and Drug Administration. Biomarkers of Potential Harm: A Public Workshop. 2016; Available from: https://www.fda.gov/TobaccoProducts/NewsEvents/ucm481513.htm

Chang CM, Edwards SH, Arab A, Del Valle-Pinero AY, Yang L, Hatsukami DK. Biomarkers of Tobacco Exposure: Summary of an FDA-Sponsored Public Workshop. Cancer Epidemiol Biomarkers Prev. 2017 Mar;26(3):291-302. doi: 10.1158/1055-9965.EPI-16-0675. Review.

Rostron BL, Chang CM, van Bemmel DM, Xia Y, Blount BC. Nicotine and Toxicant Exposure among U.S. Smokeless Tobacco Users: Results from 1999 to 2012 National Health and Nutrition Examination Survey Data. Cancer Epidemiol Biomarkers Prev. 2015 Dec;24(12):1829-37. doi: 10.1158/1055-9965.EPI-15-0376.

Shiels MS, Katki HA, Freedman ND, Purdue MP, Wentzensen N, Trabert B, Kitahara CM, Furr M, Li Y, Kemp TJ, Goedert JJ, Chang CM, Engels EA, Caporaso NE, Pinto LA, Hildesheim A, Chaturvedi AK. Cigarette smoking and variations in systemic immune and inflammation markers. J Natl Cancer Inst. 2014 Oct 1;106(11). pii: dju294. doi: 10.1093/jnci/dju294.

**Abstract: Biomarker Data in the Population Assessment of Tobacco and Health (PATH) Study**

The Population Assessment of Tobacco and Health (PATH) Study is a nationally representative longitudinal study of the U.S. civilian, non-institutionalized population aged 12 years and older. The goal of PATH is to comprehensively assess tobacco use, its determinants, and its impacts to inform FDA's Center for Tobacco Products' regulatory research and activities. In this presentation, we describe the biospecimen collection and biomarker testing in PATH, which provides a valuable resource to characterize tobacco exposures and potential harm across different types of tobacco products, including novel products. Wave 1 data collection was fielded between September 2013 and December 2014 and included 32,320 adults (aged 18+ years) and 13,651 youth (12-17 years). Data collection of tobacco use, risk perceptions and attitudes, and health outcomes was conducted through Audio Computer-Assisted Self-Interviews for all participants. At Wave 1, consenting adults provided biospecimens such as urine, blood, and buccal cells. Biomarker testing was done for ~10,000 selected adult participants based on tobacco use status, including never, current, and former users of cigarettes, smokeless tobacco, traditional cigars, cigarillos, filtered cigars, pipe tobacco, hookah, and electronic cigarettes. Biomarkers measured include nicotine, heavy metals, tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and select markers of inflammation and oxidative stress. The study has also collected three annual waves of data and biospecimens and is currently in the fourth wave of data collection. In Wave 4, youth are asked to provide urine samples. Urine and blood are also collected from a replenishment sample of adults. Future analyses of biomarker data, in combination with tobacco use information, can address a broad range of research questions on tobacco exposures across different types of tobacco products used, including longitudinal changes in biomarkers as a result of changes in tobacco use, such as switching between products.

**William B Mattes, PhD, DABT, DABT**

*Director, Division of Systems Biology, NCTR*

**Bio**: Dr. Mattes is the Director of the Division of Systems Biology, part of FDA's National Center for Toxicological Research in Jefferson, Arkansas. The Division pursues a wide range of research that uses and develops innovative tools for assessing pharmaceutical safety and advancing public health. He has been an independent consultant as well as Director of Toxicology at the Critical Path Institute, where he developed and directed the Predictive Safety Testing Consortium (PSTC), a collaboration of 16 of the world's major pharmaceutical companies, with FDA and EMEA advisors, with the goal of qualifying new biomarkers for drug safety in a regulatory setting. This work resulted in the establishment of a formal process of biomarker qualification for FDA and EMEA, and FDA/EMA/PMDA qualification of new biomarkers of kidney injury. Dr. Mattes also developed the COPD Biomarkers Qualification Consortium, serving as its Senior Director and overseeing interactions with FDA. Dr. Mattes' other positions included senior scientific director of Toxicogenomics at Gene Logic, Associate Director of Toxicogenomics and Group Leader of Genetic Toxicology at Pharmacia Corp, Kalamazoo, MI, Group Leader of Experimental Toxicology and Metabolism at Ciba Pharmaceuticals, Summit, NJ, and Group Leader of Molecular and Cellular Toxicology, Ciba-Geigy Agricultural Chemical Division, Farmington, CT.

Dr. Mattes received his BA from the University of Pennsylvania and PhD in biological chemistry from the University of Michigan, Ann Arbor. He did his postdoctoral training in biochemistry at the Johns Hopkins University, and was a staff fellow at the National Cancer Institute,

the National Institutes of Health (NCI/NIH). In 1997 Dr. Mattes became a diplomate of the American Board of Toxicology. He is a full member of the Society of Toxicology and the American College of Toxicology (ACT) and has served on committees for both organizations. His research interests include bioinformatics and data science, cross-species comparisons of molecular responses, integration of safety assessment approaches, as well as group dynamics that lead to successful collaboration between scientists and changes in scientific policy. He also currently fills the guitar chair for the group Jazzicology at the ACT's annual meeting.

**Abstract: Transcript, proteo, and metabolomics as tools for translational biomarker discovery and evaluation**

The BEST (Biomarkers, Endpoints, and other Tools) Resource defines a biomarker as a "characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions." While the concept of 'omics in biomedicine is certainly pre-dated by its use in finance (i.e. econ-omics), the advent of tools that can measure a near-totality of genes, transcripts, proteins and metabolites has greatly facilitated the discovery of molecules that serve as biomarkers. Furthermore, these tools can be applied to studies to pursue 'translational' biomarkers, biomarkers that can bridge in vitro and animal models with clinical studies. Thus studies of global microRNA changes after toxicant treatment in rats identified one, miR-122, which appears to be specific for liver injury, and can be measured in serum. Studies in patients presenting with acetaminophen overdose show that similarly, serum miR-122 is elevated. Metabolomics provides another tool for discovering biomarkers that cross from non-clinical to clinical studies. Studies in mice, rats and acetaminophen-overdose patients all show increases in serum acylcarnitines that precede those of aminotransferases. Furthermore, it is possible that these serum metabolites might be indicative of mitochondrial, i.e. organelle, toxicity. These and other examples will be discussed.

### Daniel Krainak, PhD

*Biomedical Engineer, Division of Radiological Health, Office of In Vitro Diagnostics and Radiological Health, FDA's Center for Diagnostics and Radiological health*

**Bio**: Dr. Krainak obtained his PhD in biomedical engineering from Northwestern University. He began his career at FDA in 2011 through a postdoctoral fellowship with Sunder Rajan, PhD in the Office of Science and Engineering Laboratories at CDRH investigating diffusion tensor imaging. Dr. Krainak has been a reviewer in the Division of Radiological Health since 2012 with an emphasis on magnetic resonance imaging technologies. He also participates in the review of radiological devices, imaging biomarkers, and radiological imaging in therapeutic product clinical trials.

**Abstract: CDRH perspectives on imaging biomarkers-analytical validation expectations**

The Center for Devices and Radiological Health is responsible for radiological device pre-market reviews and participates in biomarker qualification review teams for imaging biomarkers through the Medical Device Development Tools Program (CDRH) and the Biomarker Qualification Program (CDER). CDRH's perspective on the evidentiary approach to quantitative imaging devices and imaging biomarkers will be presented. The interaction between claims and analytical validation expectations will be briefly explored through examples. Research within CDRH continues to refine and expand the available methods for assessing the performance of quantitative imaging biomarkers.


**Hisani Madison, PhD, MPH**
*Scientific Reviewer, OIR, CDRH, FDA*

**Bio**: Dr. Hisani Madison is a scientific reviewer in the Office of In Vitro Diagnostics and Radiological Health (OIR) in the Center for Devices and Radiological Health (CDRH). Dr. Madison leads the review of submissions in the Molecular Pathology and Cytology Branch in the Division of Molecular Genetics and Pathology (DMGP). The Molecular Pathology and Cytology Branch reviews a wide range of devices, including next-generation sequencing technologies and hybridization-based molecular techniques, to detect genetic alterations associated with cancer.

Dr. Madison specializes in review of devices intended to aid in selection of therapy for patients with solid tumors. Before serving in her current position, she was a postdoctoral fellow in the Hormonal and Reproductive Epidemiology Branch of the Division of Cancer Epidemiology and Genetics at the National Cancer Institute, where she conducted molecular epidemiologic research focusing on breast cancer etiology and heterogeneity. Dr. Madison obtained her PhD in Pathology from Duke University, where she did her doctoral research on identifying and characterizing genetic and epigenetic markers for the early detection, prognosis, and prediction of breast and ovarian cancer. She also has an MPH from Johns Hopkins Bloomberg School of Public Health, where she trained in epidemiology and biostatistics and received a Certificate in Health Disparities and Health Inequality.

**Abstract: Approval of the 1st NGS Companion Diagnostic**

FDA regulates drugs, devices, and biologics. Different types of products are subject to different regulatory requirements, and codevelopment requires understanding and coordinating the development processes to facilitate contemporaneous marketing authorizations. A companion diagnostic device is an in vitro diagnostic device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. On December 19, 2016 FDA granted accelerated approval to Rubraca (rucaparib) to treat women with a certain type of ovarian cancer and simultaneously approved the FoundationFocus CDxBRCA companion diagnostic for use with Rubraca. The FoundationFocus CDxBRCA test is the first NGS-based companion diagnostic approved by FDA. The NGS test detects the presence of deleterious BRCA gene mutations in the tumor tissue of ovarian cancer patients. If one or more of the mutations are detected, the patient may be eligible for treatment with Rubraca. This presentation will provide an overview of FDA's review of the FoundationFocus CDxBRCA test and highlight some of the regulatory considerations and emerging complexities that are unique to the precision medicine era.

**RADM Palmer Orlandi, Jr, PhD** (Session Chair)

*Chief Science Officer and Director of Research, Office of Foods and Veterinary Medicine*

**Bio**: Dr. Orlandi joined FDA in 1997 after his postdoctoral research training at the Walter Reed Army Institute of Research and the National Institutes of Health. After 11 years as a principal investigator at FDA's Center for Food Safety and Applied Nutrition he joined the Division of Field Science in the Office of Regulatory Affairs as a science coordinator, where he developed collaborative analytical methods programs for FDA field labs and the Food Emergency Response Network (FERN). From 2012 to 2015, he served as the senior science advisor to the Chief Science Officer in FDA's Office of Food and Veterinary Medicine. In this capacity, Dr. Orlandi served to integrate science and research efforts across all elements of FDA's foods program; and worked to ensure alignment of research and laboratory programs to the needs of regulatory field labs as they support FDA's evolving food safety mission. He is currently the acting Chief Science Officer and Research Director in the Office of Foods and Veterinary Medicine. He received a Commission as an officer in the US Army in 1981 and since 1991 has been an officer in the Commission Corps of the Public Health Service.

**CDR Kari Irvin, MS**

*CORE Response Manager, CFSAN*

**Bio**: CDR Irvin is currently the acting director for FDA's Coordinated Outbreak Response and Evaluation (CORE) Network. She has been with CORE since the organization was stood up in 2011, serving as a staff member, team leader, and response manager before assuming her current acting role. CDR Irvin joined FDA in 2008 as a microbiologist in the Center for Food Safety and Applied Nutrition (CFSAN), Office of Regulatory Science, where she performed research on identifying Salmonella sp. from various food matrices. She is a former CDC/APHL Emerging Infectious Diseases Training Fellow and former scientist with the state of Virginia's Division of Consolidated Laboratory Services (DCLS), a state public health lab. CDR Irvin received both her BS and MS in biology from Seton Hall University in South Orange, NJ.

**Abstract: FDA's Coordinated Response to Recent Foodborne Outbreaks**

The FDA Coordinated Outbreak Response and Evaluation (CORE) Network is a headquarter-based 34-member team that focuses on coordinating the flow of information during and after outbreaks involving human food, dietary supplements, and cosmetics. FDA CORE accomplishes this task by working across a paradigm of three outbreak phases: signals and surveillance, response, and post-response.

A different team in FDA CORE accomplishes each outbreak phase. The CORE Signals and Surveillance Team analyzes internal and external information for illness trends and potential clusters of illness. The CORE Response Teams coordinate information flow across organizations during an outbreak response, conduct traceback investigations, and evaluate environmental, epidemiologic, and laboratory data to inform assignments issued to FDA field offices during the outbreak investigation. The CORE Post-Response Team reviews outbreak responses and recommends prevention initiatives based on trends of outbreak data and lessons learned. In 2017, FDA CORE coordinated two high profile outbreak investigations: E. coli O157:H7 in soy nut butter and Listeria monocytogenes in soft cheese. Each of these outbreak responses highlights the importance of establishing an organized and systematic approach to identifying and responding to foodborne emergencies to protect the health and safety of consumers.

**Heather Tate, PhD, MS**

*Epidemiologist, NARMS, CVM*

**Bio**: Dr. Heather Tate is the lead epidemiologist in FDA's arm of the National Antimicrobial Resistance Monitoring System (NARMS), a national public health surveillance system that tracks changes in the antimicrobial susceptibility of enteric bacteria found in ill people, retail meats, and food animals in the United States. NARMS is housed in the Office of

Research in the Center for Veterinary Medicine (CVM). Dr. Tate is responsible for the design, collection, analysis and dissemination of data and findings from NARMS epidemiological studies, focusing on the potential public health impact of antimicrobial resistance in foodborne zoonotic bacteria and elaborating the link between animal drug use and resistance transfer to humans. Before coming to CVM in 2008 as a Commissioner's Fellow, Heather was Manager of Food Safety at the Association of Public Health Laboratories. She holds a doctorate in biomedical science from New York University and a Master's degree in epidemiology from the Harvard School of Public Health.

**Abstract: Characterization of a Multidrug Resistant Strain of Salmonella Isolated from Humans and Chickens in the United States**

Scientists from the National Antimicrobial Resistance Monitoring System (NARMS) sequenced the genomes of ten *Salmonella enterica* serovar Infantis containing *bla*$_{\text{CTX-M-65}}$ isolated from chicken, cattle, and human sources collected between 2012 and 2015 in the U.S. through routine NARMS surveillance and product sampling programs. FDA scientists also completely assembled the plasmids from four of the isolates. All isolates had a D87Y mutation in the *gyrA* gene and harbored between 7 and 10 resistance genes (*aph(4)-Ia, aac(3)-IVa, aph(3')-Ic , blaCTX-M-65, fosA, floR, dfrA14, sul1, tetA, aadA1*) located in two distinct sites of a megaplasmid (~316-323kb) similar to that described in a *bla*$_{\text{CTX-M-65}}$+ S. Infantis isolated from a patient in Italy. High-quality single nucleotide polymorphism (hqSNP) analysis revealed that all U.S. isolates were closely related, separated by only 1 to 38 pairwise high quality SNPs, indicating a high likelihood that strains from humans, chicken, and cattle recently evolved from a common ancestor. The U.S. isolates were genetically similar to the *bla*$_{\text{CTX-M-65}}$+ S. Infantis isolate from Italy, with a separation of 34 to 47 SNPs. This is the first report of the *bla*$_{\text{CTX-M-65}}$ gene and the pESI-like megaplasmid from S. Infantis in the United States, and illustrates the importance of applying a global One Health, human and animal perspective to combat antimicrobial resistance.

**Travis Falconer, PhD**

*Chemist, ORA*

**Bio**: Travis M. Falconer is a Chemist at the Forensic Chemistry Center in the Office of Regulatory Affairs at FDA. He earned a PhD in Chemistry from the University of North Carolina after obtaining a BS in Chemistry from the University of North Dakota. In his position with FDA, Travis specializes in non-targeted mass spectrometric analysis of foods, drugs, and dietary supplements for the presence of harmful and/or unlabeled substances. He also performs method development for difficult analyte/matrix combinations, conducts training for FDA and FERN (Food Emergency Response Network) scientists on the use of liquid chromatography and mass spectrometry instrumentation, and provides expert testimony regarding chemical analyses of foods, drugs, and dietary supplements. Before joining FDA, Travis spent two years as a Postdoctoral Scholar at the University of North Carolina designing and developing arrays of micro-scale cylindrical ion trap (CIT) mass spectrometers, capable of operation at higher pressure than conventional instruments, for use in a handheld device for field applications, such as the detection of chemical warfare agents.

**Abstract: Forensic Analysis of a Mass Poisoning in Mozambique Associated with a Homebrewed Beverage**

In January, 2015, 234 people became sick, 75 of whom died, in the Mozambique village of Chitima after attending a funeral. Symptoms included gastrointestinal distress, diarrhea, vomiting, muscle pain, and labored, rapid breathing. Investigation by the Mozambique Ministry of Health (MOH) and the U.S. Centers for Disease Control and Prevention (CDC) linked the incident to the consumption of a homemade, traditional African beer called pombe. Foul play was suspected and several media outlets reported that crocodile bile may have been used to poison the beer. The Forensic Chemistry Center (FCC) became involved at the request of CDC and Mozambique MOH. Samples of the suspect pombe received by FCC were subjected to a number of analyses and compared to a control sample. Ultimately, non-targeted

liquid chromatography-mass spectrometry screening revealed the presence of the potent toxin bongkrekic acid, and its structural isomer, isobongkrekic acid. Quantitative analysis measured potentially fatal levels of these toxins in the suspect pombe samples. Bongkrekic acid is known to be produced by the bacterium Burkholderia gladioli pv. cocovenenans. While this bacterium could not be isolated from the suspect pombe, bacteria identified as B. gladioli were isolated from corn flour, a starting ingredient in the production of pombe, obtained from the brewer's home. When the bacteria were co-plated with the fungus Rhizopus oryzae, which was also isolated from the corn flour, synergistic production of bongkrekic acid was observed. The results suggest a mechanism for bongkrekic acid intoxication, a phenomenon previously thought to be restricted to specific regions of Indonesia and China.

### Henry Skip Francis, MD

*Director for Data Mining and Informatics Evaluation and Research, Office of Translational Sciences, CDER*

**Bio**: Since March 11, 2013, Dr. Francis has been the Director of the Data Mining and Informatics Evaluation and Research Group in the Office of Translational Sciences, at FDA's Center for Drug Evaluation and Research (CDER). In this capacity he directs a trans-disciplinary group of senior scientists to test, create, and operate data analysis program facilitating the efficient use of scientific methods to evaluate complex data information to make regulatory decisions for drug approval and drug safety.

From October of 2007 until March 10, 2013, Dr. Francis was the Deputy Director of the Office of Surveillance and Epidemiology (OSE) at CDER. Dr. Francis worked with the OSE Director to lead five divisions of pharmacy and clinical scientists in the detection and study of adverse medical events (AE's) occurring after the release of new drugs into the American health market, also called the postmarket period. Dr. Francis' specific interest is in the development of data mining techniques to enhance pharmacovigilance capabilities in national medication use and health care databases.

Before joining FDA, Dr Francis was a basic and clinical researcher at the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (NIH). As an AIDS clinical investigator, he worked in several clinical and epidemiologic research projects conducting AIDS and tropical research projects in the Democratic Republic of the Congo (DRC, formerly known as Zaire) and other projects in the Caribbean and the South Pacific. In the DRC, Dr. Francis was the Director of the U.S. Public Health Service & Belgian Project SIDA (AIDS research) Research Laboratories in Kinshasa, DRC.

Dr. Francis served as the first Director of the National Institute on Drug Abuse's (NIDA) Center on AIDS and Other Medical Consequences of Drug Abuse (CAMCODA). CAMCODA's mission was to establish sustainable AIDS specific research projects in coordination with the other NIDA projects investigating drug abuse prevention and treatment.

As a clinician, Dr. Francis was an Assistant Professor of Medicine at the Johns Hopkins University School of Medicine's Division of Infectious Diseases where he served as the Principal Medical Officer of the Broadway Women's Drug Use Center and as the Ryan White Title III Investigator and Medical Director of the Baltimore City Department of Health's Sexually Transmitted Diseases clinics.

He completed his Internal Medicine residency training at the Long Beach Veterans Administration Hospital in Long Beach, California and his Infectious Diseases specialty training at the Johns Hopkins Hospital Division of Infectious Diseases, Baltimore, Maryland. He has published over 60 papers and book chapters on U.S. and international public health issues.

#### Abstract: Use of an FDA Real Time Mobile Communication Platform System during Medical Countermeasure Events: RAPID

FDA is responsible for protecting and advancing public health by helping to speed innovations that make medicines, devices, products, and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health.

In support of this mission, FDA has been developing the Real-Time Application for Portable Interactive Devices (RAPID) System to facilitate the real-time collection, analysis, and communication of medical countermeasures (MCM) product information during chemical, biological, radiological, nuclear (CBRN) and emerging infectious disease events. The RAPID system was created to provide a bi-directional information transmission pathway for drug safety information and provide real-time data analysis system to FDA scientists. The current RAPID capability can leverage innovative cloud, mobile and analytics technologies to address three use cases:

- Product Safety: Enable the transmittal, storage and processing of information contained in the FDA MedWatch 3500A or 3500B forms to facilitate the review of product safety issues.
- Risk Evaluation and Mitigation Strategies (REMS): Provide questions for providers, pharmacists and patients that are scored on a Likert Scale to support the assessment of the medication accessibility and prescription burden associated with REMS.
- Medication Errors: Provide an electronic data collection system for reporting medication errors with the option of including photo attachments.

The RAPID System will be able to support: Mobile data collection, including a speech-to-text functionality to capture patient and physician narratives with minimal effort. Integration of disparate data sources including electronic health record (EHR) data extracts from partner hospital system, within a cloud-based data lake. Enable analysis and visualization of data through the RAPID dashboard for post-market surveillance review and research; and bidirectional communication between FDA and reporters to promote transparency and information sharing.

**Gerardo Kaplan, PhD**

*Principal Investigator, Office of Blood Research and Review, CBER, FDA*

**Bio**: Dr. Kaplan received a Doctoral Degree in Biological Sciences in 1986 from the University of Buenos Aires, Argentina. After completing a Postdoctoral training at Columbia University, New York, he joined the Center of Biologics Evaluation and Research (CBER), FDA, in 1991. At CBER, he discovered the hepatitis A virus (HAV) cellular receptor 1 (HAVCR1), the first identified member of a family of molecules that have a significant role modulating asthmatic, autoimmune, graft-versus-host disease, anti-viral, and anti-cancer immune responses. He was tenured at CBER in 1998 due to his scientific contributions in HAV biology and regulatory work in hepatitis virus vaccines. From 2001 to 2008, he served as the Chief of the Laboratory of Hepatitis and Related Emerging Agents at the Office of Blood Research and Review (OBRR), CBER, where he continued working in hepatitis viruses. At OBRR, his work led to the identification of HAVCR family members as pattern recognition receptors (PRR) of apoptotic cells. In response to FDA's need to gain expertise in bioterrorism agents, Dr. Kaplan developed a filovirus program focused on vaccines based on the viral glycoprotein (GP) fused to the Fc fragment of human IgG1, BSL2 tests to evaluate anti-GP total and neutralizing antibodies based on replication-competent recombinant VSV containing GP, and the role of the HAVCR family in viral cell entry.

Dr. Kaplan is currently a Principal Investigator at the Lab of Emerging Pathogens, OBRR, CBER, where he continues his research in viral immunology and contributes to blood safety regulatory policy issues related to hepatitis viruses and filoviruses.

**Abstract: Development of total and neutralizing anti-Ebola virus antibody assays for deployment in West Africa to evaluate clinical trials of MCM including vaccines and immunotherapies**

The role of total and neutralizing antibodies during Ebola virus (EBOV) infection is not clear, and some survivors develop low levels or no neutralizing antibodies. The EBOV glycoprotein (GP) induces protective antibodies in animal models. Passive immunization with neutralizing monoclonal antibodies or immunoglobulin preparations from vaccinated nonhuman

primates (NHPs) that survived EBOV infection protected NHPs against lethal challenge with EBOV. Furthermore, NHPs that develop strong anti-GP total and neutralizing antibody responses with GP-vectored vaccines in general survive EBOV lethal challenge suggesting that development of high levels of antibodies could be used as a correlate of immunity for some vaccine candidates. Assessing total and neutralizing antibodies under BSL4 conditions is difficult and time-consuming. Therefore, there is a need for EBOV BSL2 assays to evaluate anti-GP total and neutralizing antibodies levels in clinical trials for vaccines and other medical countermeasures such as serum therapies.

We have developed BSL2 EBOV antibody assays to analyze total antibodies by a virus particle ELISA (VP-ELISA) and neutralizing antibodies by a fluorescence reduction neutralization test (FRNT), both assays based on replication-competent recombinant vesicular stomatitis virus (VSV) in which the VSV-G envelope was exchanged with the EBOV GP. We have evaluated these assays using serum samples from animal vaccine studies and human convalescent patients. The VP-ELISA showed a high degree of specificity and sensitivity. The FRNT in 96-well plates allowed the rapid determination of neutralization titers in 24-26 hours post-infection using a fluorescence plate reader. Our data showed that the EBOV BSL2 VP-ELISA and FRNT are robust assays that require minimal infrastructure. We are currently performing a field validation of these assays in Sierra Leone in collaboration with the Italian National Institute of Infectious Diseases "L. Spallanzani" to evaluate whether the VP-ELISA and FRNT could be used in the resource-limited settings.

**Daniela Verthelyi, MD, PhD**

*Lab Chief, Office of Biotechnology Products, CDER*

**Bio**: Dr. Verthelyi received her MD from the University of Buenos Aires and a PhD from the Virginia Tech in the U.S. She completed fellowship training in Immunology in the Retroviral Immunology Section of the Center for Biologics Evaluation and Research at FDA before joining the Laboratory of Immunology of Division of Therapeutic Proteins and eventually becoming its Chief. Dr. Verthelyi has authored over 80 peer reviewed articles and several patents, serves on the NIH Human Immunology advisory board, the NIH Immunology Interest Group and NIH-FDA Cytokine Interest Group, and has received FDA's Excellence in Laboratory Sciences Award, among other honors.

Research: Triggering of the innate immune system (IIS) leads to inflammation and initiation of cellular and humoral immune responses

Products that trigger the IIS receptors are called Innate Immune Response Modifiers (IIRMs) and are investigated in applications for cancer, vaccines, allergies and asthma. Conversely, impurities in therapeutic products that act as IIS activators can foster unwanted immune responses to self-proteins leading to autoimmune disease or enhance the immunogenicity of critical therapeutic proteins reducing their life-saving potential. The lab is focused on both effects developing tools to monitor and control innate immune and inflammatory responses. Working with murine and primate models it has been shown that modulating the innate immune and inflammatory response to pathogens can ameliorate lesion severity in rhesus macaques challenged with cutaneous leishmaniasis. In the studies assessing the safety and efficacy of using IIRM in the treatment of biothreats such as viral haemorrhagic fevers and Zika, several animal models have been developed including several models of infection in neonatal mice. These models can be used to assess and compare new therapeutics and vaccines, and to explore the immunopathogenesis of disease.

**Abstract: Development of mouse models to assess efficacy and potency of ZIKA virus therapeutics**

*Derek Ireland, Mohanraj Manangeeswaran, Leia Novak, Ian McWilliams, Jacob Sykes, Daniela Verthelyi**

The recent spread of Zika virus (ZIKV) and its association with increased rates of GuillainBarre and other neurological disorders as well as congenital defects that include microcephaly have created an urgent need to develop animal models to examine the pathogenesis of the disease and explore the

efficacy of potential therapeutics and vaccines. Recently developed infection models for ZIKV use mice defective in interferon responses. In this study we establish and characterize a new model of peripheral ZIKV infection using immunocompetent neonatal C57BL/6 mice and compare its clinical progression, virus distribution, immune response, and neuropathology with that of C57BL/6-IFNAR KO mice. We show that while ZIKV infected IFNAR KO mice develop bilateral hind limb paralysis and die 5-6 days post-infection (dpi), immunocompetent B6 WT mice develop signs of neurological disease including unsteady gait, kinetic tremors, severe ataxia and seizures by 13 dpi that subside gradually over 2 weeks. Immunohistochemistry show viral antigen predominantly in cerebellum at the peak of the disease in both models. However, whereas IFNAR KO mice showed infiltration by neutrophils and macrophages and higher expression of IL-1, IL-6 and Cox2, B6 WT mice show a cellular infiltration in the CNS composed predominantly of T cells, particularly CD8+ T cells, and increased mRNA expression levels of IFNg, GzmB and Prf1 at peak of disease. Lastly, the CNS of B6 WT mice shows evidence of neurodegeneration predominantly in the cerebellum that are less prominent in mice lacking the IFN response possibly due to the difference in cellular infiltrates and rapid progression of the disease in that model. The development of the B6 WT model of ZIKV infection will provide insight into the immunopathology of the virus and provides the Agency with a platform to facilitate the assessments of therapeutics and vaccines.

**Ryan Ranallo, PhD**
(Session Chair)

*Program Officer, National Institutes of Health/National Institute of Allergy and Infectious Diseases*

**Bio**: Dr. Ryan Ranallo is a Program Officer in the Division of Microbiology and Infectious Diseases (DMID) in the National Institute of Allergy and Infectious Diseases (NIAID), at the National Institutes of Health (NIH). Dr. Ranallo received a B.S. in Microbiology from Ohio University in Athens, Ohio and a PhD in Biochemistry and Molecular Biology from Colorado State University. He conducted his post-doctoral training at the National Cancer Institute, NIH in the Laboratory of Carl Wu, where he worked on chromatin structure and transcription initiation. In 2002, Dr. Ranallo changed the direction of his scientific career and took a position at the Walter Reed Army Institute of Research (WRAIR) in the Department of Enteric Infections. There he spent 10 years working on bacterial pathogenesis (Shigella and E. coli spp.) and vaccine development and successfully designed, constructed, and manufactured several live attenuated Shigella vaccines. In 2012, he transitioned to NIH/NIAID/DMID for a career in scientific administration, where he manages a portfolio of grants, contracts, and clinical trials involving Clostridium spp., Campylobacter spp., Botulinum neurotoxin and Staphylococcal Enterotoxin B. His areas of expertise include bacterial pathogenesis, vaccine development and non-traditional therapeutics involving the microbiome.

**Abstract: The Human Microbiota in Health and Disease**

The human microbiota, defined as the entire collection of microbial cells that live in and on humans, is seeded at birth and makes profound contributions to human development. Some essential functions of the microbiota include provision of key signals to the developing immune system, contributions to neurodevelopment, protection from microbial pathogens, metabolic functions, and essential metabolite production. Moreover, recent advances in culture independent technologies have dramatically enhanced our perspective on how the composition and function of the gut microbiota contribute to diseases, ranging from inflammatory disorders of the gut to cardiovascular illnesses. It is therefore no surprise to see an explosion of research aimed at exploiting this influence through microbiome manipulation. An introductory review of the human microbiome along with some recent advances in microbiome science will be presented.

**Andrea Ottesen, PhD**

*ORS, CFSAN*

**Bio**: Dr. Ottesen received her PhD from the College of Agriculture and Natural Resources (AGNR) at the University of Maryland, College Park in 2008. From 2003 to 2005 she worked for the Department of Plant Sciences (UMD) and the Division of Microbiology (DM) at the Center for Food Safety and Applied Nutrition (CFSAN) as a Master's student funded by a JIFSAN research assistantship to study agricultural microbial ecology and food safety. Ottesen's PhD work continued to apply metagenomic approaches to the study of bacterial and fungal microbiota associated with crops in response to organic and conventional agricultural management for food safety and sustainable agriculture questions.

At CFSAN, Dr. Ottesen has served as a Research Area Coordinator for Metagenomics and Food Microbiomes since 2009 in the Molecular Methods and Subtyping Branch (MMSB) of the Division of Microbiology in the Office of Regulatory Science (ORS). Ottesen's work for ORS focuses on supporting source tracking programs, such as GenomeTrakr, by providing molecular fingerprints to contribute to source tracking efforts as well as describing the microbial farm to fork continuum to identify critical control points for food safety. She is also working to implement a MetaGenomeTrakr program to expedite source tracking and continue to provide baseline microbial profiles for food and food ecologies.

**Abstract: MetaGenomeTrakr and Food Safety Microbiome Research at CFSAN**

The metagenomic description of microbiota

along the farm to fork continuum has been useful to identify environments and conditions that may play an important role in introducing pathogens to food commodities. Data from metagenomic research at CFSAN also support the evolution of Good Agricultural Practices (GAPs) and Food Safety Modernization Act (FSMA) regulations. Additionally, newly developing microbiome-based approaches to source tracking are improving response time by a degree that when leveraged correctly, could reduce the number of illnesses associated with any given foodborne outbreak by as many as 75% percent of total cases.

**Paul Carlson, PhD**

*Office of Vaccine Research and Review, CBER*

**Bio**: Paul Carlson, PhD is a principal investigator in FDA's Laboratory of Mucosal Pathogens and Cellular Immunology, Division of Bacterial, Parasitic, and Allergenic Products, Office of Vaccines Research and Review, CBER. He received his PhD from the University of Pittsburgh and did postdoctoral research at the University of Michigan in the laboratory of Phil Hanna. Since starting his laboratory at FDA in 2015, Dr. Carlson's research has focused on infections caused by the enteric pathogens Clostridium difficile and Vancomycinresistant Enterococcus (VRE) species. The laboratory has projects researching mechanisms of C. difficile pathogenesis, development of genetic tools to study C. difficile, host response to C. difficile, the role of the host microbiota in C. difficile colonization resistance, the influence of the host immune system on the microbiome, and bacteriophage therapy against VRE. Dr. Carlson is co-chair of the FDA microbiome working group and the Joint Agency Microbiome (JAM) working group, as well as a member of the Microbiome Interagency Working Group (MIWG). His regulatory responsibilities include product review for fecal microbiota transplantation (FMT), defined live biotherapeutic products, and bacteriophage therapies.

**Abstract: MAIT cells alter the murine microbiome reducing colonization resistance against *Clostridium difficile***

*Ashley D. Smith, Irma T. Zhang, Alyxandria M. Schubert, Nicole P. Giordano, Jessica E. Hastie, Siobhan C. Cowley, and Paul E. Carlson Jr.*

*Clostridium difficile* (Cd) is a gram-positive spore-forming bacterium and a leading cause of nosocomial and antibiotic associated infection. Cd infection (CDI) typically occurs following antibiotic usage, which perturbs the gut microbiota, leaving the host susceptible to Cd colonization. Mucosa-associated invariant T cells (MAIT) cells recognize intermediates of riboflavin biosynthesis presented on MR1, an MHC-I like molecule.

Since humans are not capable of riboflavin biosynthesis, MAIT cell development is dependent on the host microbiome. MAIT cells are found in high numbers at mucosal sites and are beneficial in combatting various pulmonary infections; however their role in gut infections is unknown. We hypothesized that MAIT cells would play a role in controlling CDI. To test this hypothesis, WT and MR1-/-(lacking MAIT cells) mice were treated with antibiotics and then infected with Cd spores. Stool was collected and plated to determine Cd colonization levels for several days post-infection. Contrary to our hypothesis, MR1-/- mice showed no signs of disease or detectable levels of Cd colonization. MR1-/- mice remained resistant to infection when a highly virulent strain was used. Fecal microbiota transplantation (FMT) was conducted to determine the role of the microbiota in this resistance phenotype. Susceptible WT mice given FMT from MR1-/- mice experienced dramatically lower colonization levels by day 7 and cleared detectable Cd by day 14, while WT mice given control FMT continued to exhibit high colonization levels. 16S rRNA sequencing of fecal samples from each strain revealed inherent phylum level differences in relative abundance of Bacteroidetes, Firmicutes, and Verrucomicrobia after antibiotics and FMT. Our data suggest the MR1-/- gut microbiome is resistant to Cd colonization and this resistance is transferrable via FMT.

**Daniel Tadesse, PhD**

*Research Microbiologist, Division of Animal and Food Microbiology, Office of Research, CVM, FDA*

**Bio**: Dr. Daniel A. Tadesse is a Research

Microbiologist at FDA's Center for Veterinary Medicine in the Office of Research, Division of Animal and Food Microbiology. Dr. Tadesse received his Doctor of Veterinary Medicine (DVM) degree from Addis Ababa University, Ethiopia and PhD in Molecular Epidemiology from The Ohio State University. He has conducted research on antimicrobial resistance and molecular subtyping of foodborne pathogens for more than 15 years. He has extensive experience in next-generation sequencing and microarray technologies. Dr. Tadesse established a microbiome research program that explores the effects of antimicrobials exposure on microbiome and resistome using metagenomics and metatranscriptomics approaches.

**Abstract: The Effect of Chlortetracycline on Swine Fecal Microbiome and Resistome**

The increasing prevalence of antibiotic resistance among bacteria is one of the pressing challenges in public health. The impact of antibiotic use in animal agriculture on human health is a complex issue that is not fully understood. A first step towards understanding these complex issues is to fully characterize the impact of antibiotic exposure on bacterial communities and resistance development within target animal species.

Twenty four piglets with no prior exposure to antibiotics were randomly assigned into 4 experimental groups and fed rations with different concentration of chlortetracycline: 50 gm/ton (growth promotion dose), 100 gm/ton (prophylaxis dose), 400 gm/ton (treatment dose), and none (control). All pigs consumed the same diet without CTC until formally beginning the feeding regimen assigned to their cohorts. Fecal samples were collected from all participants before treatment (day -21) and at days 0, 1, 2, 7, 14, 21, 28, and 35 post treatment.

In this study, we used targeted and shotgun metagenomics to investigate the effects of chlortetracycline (CTC) on swine intestinal microbiome and resistomes. The effect of CTC on the swine intestinal microbiota varied by dosage and duration; and that the changes in relative abundance were more at the genus and species level than at the phylum level. The microbial population structure of pigs fed 400 gm/ton concentrations diverged from that of control pigs starting from day 2, with a significant divergence between days 7 and 14. The majority of resistance-associated sequences found in control and treated pigs fecal samples belonged to tetracycline resistance. tet resistance gene alleles observed varied by dose, and their abundance increased as the duration of CTC exposure increased. Relative abundance of tetQ rose during the study period among pigs fed the treatment dose, while tetW increased among pigs receiving the prophylaxis dose. tetL and ermB alleles showed a similar relative abundance pattern, indicating the possibility of these genes being carried by the same group of bacteria or co-located on the same conjugative element. Pigs receiving 50 gm/ton CTC in feed showed an increase in tetA-P and tetQ alleles. Our study provides insights into the impact of CTC exposure on swine intestinal microflora.

### Sangeeta Khare, PhD

*OR, NCTR*

**Bio**: Dr. Sangeeta Khare is a Research Microbiologist in the Division of Microbiology, at FDA's National Center for Toxicological Research (NCTR). Dr. Sangeeta Khare leads an active team with a research emphasis on host-pathogen and host-microbiome interaction during perturbations with xenobiotic agents (nanoparticles, antibiotics and other drugs, natural products and additives). Dr. Khare's research group focuses mainly on 1) the gastrointestinal tract exposure using in vivo, in vitro and ex vivo models, and 2) the use of advanced technologies, such as next-generation sequencing, omics and systems biology approaches, in establishing innovative parameters of host toxicity.

Dr. Khare has extensive experience in working in BSL3 and A-BSL3 level laboratories. A recipient of numerous awards and honors for her research from various scientific organizations, she is a professional member of several scientific organizations and a member of the FDA Microbiome working group and high impact pathogen working group. She is an

expert reviewer for several journals and served as grant reviewer for FDA, U.S. Department of Agriculture, the National Institute of Environmental Health Sciences (NIEHS) and several other international grant organizations.

Dr. Khare has supervised several undergraduate, graduate and post-doctoral scientists and also served as a FDA Commissioner Fellow preceptor. Dr. Khare has organized several workshops and conferences and has been invited to share her research findings at other FDA Centers, several national and international conferences as well as medical institutes and universities with in the United States and abroad. These interactions have led to active collaborations with the scientists from several universities and with other federal agencies (NIEHS) and FDA centers [Center for Veterinary Medicine (CVM), and Center for Drug Evaluation and Research (CDER)]. The aim of these collaborative projects is to "Evaluate Innovative Emerging Technologies" and "Modernize Toxicology to Enhance Product Safety."

**Abstract: Interaction of Silver Nanoparticles Beyond Intestinal Bacterial Microbiota: Effects of Intestinal Virome and phages**

Incorporation of silver and silver nanoparticles (AgNP) into health-supplements, food packages, baby products and several household items has increased tremendously. The most likely targeted site is the gastrointestinal tract following intentional or accidental ingestion. Recent studies revealed the effects of chronic or acute exposure of AgNP on the gut-bacterial communities; however, the effect of AgNP on the intestinal virome community is mostly unknown.

This study had a two-fold objective: 1) to address the virus and phages inactivating properties of AgNP (in vitro), and 2) to assess the effect of AgNP on virome population and antiviral immunity in the gut using ex vivo and in vitro models, respectively. Representative enteric virus and bacteriophages were treated with variable sizes (10, 75, 110 nm) and doses (25, 50, and 100 µg ml-1) of AgNP to assess antiviral/antiphagic properties by evaluating appearance of cytopathic effects (CPE), detection of viral capsid protein, and the bacteriophages plaque forming units (PFU) ratio. Only 10 nm of AgNP were able to inactivate virus and phages in a dose-dependent manner. In the second objective, the interaction of 10nm of AgNP with intestinal virome (ex vivo) and T7-bacterophage and its effect on the gut immune response related gene expression (in vitro) was studied.

The whole genome sequencing data revealed AgNP inactivated intestinal phage population, as well as modified the T7-bacterophage-mediated expression of immune responses associated genes in the intestinal epithelial cells. Overall, it is evident that the small size AgNP could lead to perturbations of the gut microbial ecosystem, leading to inactivation of resident gut viruses and phages that play an important role in gut-associated immune responses and in gastrointestinal health. Taken together, the results of this study support the notion to integrate data from intestinal toxicity as an additional endpoint in the risk assessment of the nanoparticles to enhance product safety.

### Odile Engel, PhD

*CDER*

**Bio**: Odile Gabay Engel pursued her education at Pierre and Marie Curie University in Paris France and received a PhD in Physiology and Pathophysiology with her MD medical training. She also received a Virology certificate from Pasteur Institute, Paris. She joined National Institutes of Health in the National Institute of Arthritis and Musculoskeletal and Skin Diseases in 2008 as a post-doctoral fellow in the Cartilage Biology and Orthopedic Branch, and then served in the immuno-regulation section, auto-immunity branch of the Clinical Center as a research fellow from 2013 to 2014, before joining FDA. Dr. Engel has gained substantial experience working with animals and managing transgenic, immuno-deficient, knockout and Germ Free mouse colonies since 2008. Her major areas of expertise are inflammation, arthritis and auto-immune diseases, epigenetics, immunology, TNF family/anti-TNF therapies and Microbiome.

**Abstract: The impact of TNF antagonist treatment on the gut microbiome in vivo.**

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*Jonathan, FDA/CDER; Wunderlin, Grant, FDA/CDER; Tiffany, Linda, FDA/CDER; Wells Wu, FDA/CBER; Simonyan Vahan, FDA/CBER and Clouse, Kathleen A. FDA/CDER.*

*Plain Language Synopsis:*

**Auto-immune diseases are in constant progression in the U.S. Biologic therapeutics have been used successfully to treat these diseases but have presented some unique regulatory challenges. Although they can be very efficacious, the response to these therapeutics can initially be quite variable among patients, and patients who are initially responsive can develop resistance to them over time. We propose that the gut microbiome could play a role in the initial variability and have an impact on the response to treatments.**

*Abstract:*

Germ Free (GF) mice colonies have been successfully developed in a sterile environment in the CBER/CDER Animal Facility. We used these GF mice to test human monoclonal antibodies and fusion proteins, following a treatment regimen consistent with patient regimens, to evaluate the role of the microbiome when GF mice are compared to conventional mice controls treated in parallel. Our pilot study is analyzed from two different approaches: assessment of taxonomy changes and an immunologic variation in the mouse gut.

Our results show a break in the symbiosis of the commensal bacteria communities after TNF antagonist treatment. These mice present a shift in the ratio Firmicutes/Bacteroidetes with a statistically significant increase in this latter family over uncultured bacteria. Differences are reported between males and females and between young (3-month-old) and old (9-month-old) mice. When the mucosal immune system is explored, comparing conventional and GF mice, it appears that the Innate Lymphoid Cells (ILCs) colonizing the lamina propria of the gut have two very different profiles and therefore, are likely to respond differently to cross-talk with commensal bacteria in the gut. A preliminary mechanistic link to the plasticity between ILC1 and LC3 is suggested in older mice.

Our results show that the Microbiota indeed plays a regulating role in TNF antagonist treatment, involving a dysbiosis and a regulation through ILCs. This observational study should be followed by in vivo functionality studies.

# Speaker Bios and Abstracts

Session 4: Advanced Manufacturing and 3D Printing

**Andy Christensen**
(Session Chair)
*President, Somaden LLC*

**Bio**: Mr. Christensen has been active in the 3D printing (aka additive manufacturing) industry since the mid 1990's, the entire time with a focus on medical device applications. He is a graduate of the University of Colorado at Denver, with a BS in Business. From 2000 to 2014 he was the Founder and President of Medical Modeling, Inc., a leading global medical device 3D Printing service bureau based in Golden, Colorado. Medical Modeling created entirely new toolsets in the areas of patient-specific anatomical modeling, virtual surgical planning, personalized surgical guides/implants and 3D printed metallic implants. In 2014 Medical Modeling was acquired by 3D Systems and saw Andy lead the creation of a new business vertical for 3D Systems in the Healthcare sector. Andy left 3D Systems in 2015 to pursue other interests.

*Additional Information:*

Mr. Christensen has lectured nationally and internationally on the ecosystem surrounding 3D printing in medicine to include clinical applications, materials, regulations and technology. For the past 20 years he has been a frequent contributor to surgical and engineering meetings. He has lectured at conferences put on by Google, FDA, the Mayo Clinic, Materialise, Euromold, Inside 3D Printing, National Institutes of Health (NIH), Advanced Digital Technology in Head and Neck Reconstruction (ADT), the American Association of Oral and Maxillofacial Surgeons (AAOMS), Time Compression Technologies (TCT), SME's RAPID, Singularity University's Exponential Medicine and more. He has several issued patents in his name, has authored three book chapters and has authored or co-authored a number of articles on the use of 3D Printing technology for medical device applications.

He has been involved with the Society of Manufacturing Engineers RTAM (Rapid Technologies and Additive Manufacturing) technical community for many years, including serving as Chair of the SME/RTAM Steering Committee in 2011. He is a recipient of the 2009 SME/RTAM Industry Achievement Award, an award given for groundbreaking work in the additive manufacturing industry. He is Associate Editor of a new journal titled 3D Printing in Medicine, published by Springer. He also runs a small consulting business, Somaden LLC, which focuses on providing assistance to the medical device industry.

Abstract:
Although 3D printing was developed in the 1980s, its use in medicine has increased dramatically in volume over the past ten years. Typically, these applications use the power of 3D printing to produce complex, patient-matched solutions which are used for pre-operative planning, intra-operative reference, and simulation. 3D printing in metal for the production of implants has recently gained popularity due to its ability to produce complex, porous geometries often used as bone in-growth surfaces. In this talk the history of 3D printing will be reviewed with a focus on the progression of use within the medical field.

**LCDR James Coburn, MS**

*Sr. Research Engineer, FDA/CDRH*

**Bio**: LCDR Coburn began his career in clinically based experimental research. He then spent time building a tissue engineering background through a research fellowship at the National University of Ireland. For the past five years he has been using these techniques to study the way medical devices interact with and affect patient and user function. This patient-centric focus enables him to work in many cross-cutting areas from orthopedic implants to degradable tissue engineering scaffolds. LCDR Coburn is co-chair of the FDA Additive Manufacturing Working Group and leader of the Additive Manufacturing of Medical Products Core Facility atFDA's White Oak Campus.

**Abstract: Techniques for Evaluating Advanced Manufacturing Performance and Processes**

Additive manufacturing (AM), also known as 3D printing, has created avenues of potential in the medical products industries through newly enabled design possibilities and personalized

medicine capabilities. Forecasts project significant growth of AM in the medical device space by 2025 (Smithers, 2015). Regulatory science research at CDRH and the other medical product Centers is helping FDA develop an understanding of the unique technical aspects and measurement challenges that are present with 3D printed patient matched or complex lattice geometries. Two research projects provide examples of these efforts.

Patient-matched devices and some non-patient matched devices often use 3D-printed patient-matched cutting guides. These guides are purported to reduce the workload for surgeons and improve outcomes for patients. However, there are not clear tests for these metrics applied to guides. We developed a custom set of guides and asked surgeons of varying skill levels to use them along with a traditional instrument set. The guide creation workflow was evaluated for repeatability and robustness to anatomical variation. The mock surgeries were evaluated objectively for accuracy and participants were also asked to rate the experience using both sets of guides.

A second research project evaluated the effectiveness of cleaning protocols for 3D-printed lattice structures. To be suitable for patient contact or implantation support materials, manufacturing residues, and debris must be sufficiently removed from devices. The challenges of each geometry are specific to the device and the AM technique used. While test devices have been produced to characterize the resolution and build capabilities of 3D printers, no test device has yet been designed to challenge cleaning protocols with complex geometries relevant to AM medical devices. Ongoing research has designed a cleaning challenge device that mimics several relevant geometric features for medical devices. Preliminary tests have shown that it can be used to assess cleaning effectiveness and differentiate between cleaning cycles.

**Celia Cruz, PhD**

*Division Director, OPQ, CDER*

**Bio**: Dr. Cruz is an Acting Branch Chief in the Office of Process and Facilities (OPF) within CDER's Office of Pharmaceutical Quality. She has a PhD in Chemical Engineering from Carnegie Mellon University. Before joining FDA in 2010, Dr. Cruz worked at Merck for 11 years, where she led a team responsible for the development of solid oral drug products, materials characterization, and new manufacturing technologies. This included hot melt extrusion for low solubility products and robust roller compaction platforms for "drop in" approach to formulation development. Her industrial experience includes the design and management of manufacturing process development plans from Phase 2 through commercialization, and global technical operations. As a black belt in Six Sigma, she was an early adopter of Quality by Design and the use of quality risk assessments for guiding drug product development.

Within FDA, Dr. Cruz has served as a reviewer for new and generic drugs, a QbD liasion for team reviews, and as Lead of the CDER Nanotechnology Working Group. She has presented on the use of risk assessments for product development and review and is currently involved in the review of continuous manufacturing processes in FDA applications.

In her new position as Acting Branch Chief in OPF, Dr. Cruz is responsible for secondary reviews and supervision of process assessment for solid oral dosage forms and transdermal products in ANDAs and NDAs.

**Abstract: Continuous Manufacturing Technologies**

With the promise of ensuring quality, reducing cycle times, and footprint, increasing manufacturing flexibility, and improving the robustness of drug supply, continuous manufacturing (CM) has been identified as an emerging technology by FDA. The pharmaceutical industry and academia have been focusing on technology development for in-line methods, equipment redesign, and material understanding, to advance the adoption of CM. Similarly, FDA has committed to CM innovation via programs such as the Emerging Technology Team, regulatory research, and the engagement of FDA experts in professional, regulatory, and academic groups focused on

CM. The recent approvals of drug products using continuous manufacturing, Orkambi and Prezista, have demonstrated that with earlier and more regular engagement with the sponsors, manufacturing innovation that translates to drug product quality can become a reality. But what's next? This talk will discuss the salient features of previous and emerging proposals for CM, the technical and regulatory challenges, and the opportunities for advancing pharmaceutical manufacturing.

**Zhiping YE, MD, PhD**

*Senior Investigator, DVP/OVRR/CBER*

**Bio**: Dr. Zhiping Ye is a Senior Investigator in CBER. He received a PhD degree in Virology from the Department of Microbiology, University of Virginia and an MD degree in Medicine from the Shanghai First Medical University, China. During the 1980s, he worked with Dr. Chi-Ming Chu at the Institute of Virology, Beijing, China, where he was involved with studying both the epidemiology of influenza and the development of influenza vaccines. While studying under Dr. Robert Wagner at the University of Virginia, he worked extensively with the influenza virus and the vesicular stomatitis virus.

Since 1998, Dr. Ye has worked on the influenza research program with respect to the improvement of production and safety of the influenza virus vaccine at FDA. He and his research team refined his earlier investigations of influenza virus matrix protein structure-function relations and have since performed novel studies in the field of influenza viruses, in particular those used in influenza virus vaccines. Their research effort has been facilitated by the introduction of the method termed "reverse genetics." By using this technique, his team has explored the role of influenza genes in viral replication and pathogenesis. His research program includes the development of molecular techniques for influenza virus vaccine analysis and improvement, thus providing knowledge to enhance the safety and efficacy of influenza virus vaccines.

Dr. Ye has been an Advisor, representing FDA, for the World Health Organization Annual Consultation on the Composition of Influenza Vaccine Update since 2001.

**Abstract: Manufacturing the Seasonal Influenza Vaccines**

The commercially available influenza vaccines in the United States are made using different production technologies. Three types of seasonal influenza vaccines, inactivated, live attenuated and recombinant, are approved in the U.S. Each vaccine contains three or four antigens representing the hemagglutinin (HA) of the strains included in the vaccine.

Live attenuated and most inactivated vaccines are made using egg-based vaccine manufacturing process, the most common technology. Each vaccine virus is propagated in embryonated chicken eggs. The allantoic fluid containing each virus is harvested, concentrated, and purified. For inactivated vaccines, the virus is chemically inactivated and disrupted using a non-ionic detergent producing a "split virus". The split virus is further purified and then re-suspended in the buffer. For cell-based inactivated vaccine, the vaccine viruses are propagated in cell culture, chemically inactivated, concentrated, purified, chemically disrupted and further purified. Antigens from the three or four viruses included in the vaccine are produced separately and then combined to make the trivalent or quadrivalent formulation. The recombinant vaccine approved by the U.S. is produced in a continuous insect cell line grown in serum-free medium. Each of the HA antigens is expressed in this cell line using a baculovirus vector, then extracted from the cells with a non-ionic detergent, purified and formulated. Standard doses are filled and finished by the manufacturers into final containers such as vials, syringes, and sprayers. Safety, purity and potency of each lot of the vaccine are tested.

In parallel with vaccine manufacturing, CBER develops and calibrates potency reagents, which are provided to the vaccine manufacturers. Manufacturers and CBER use these reagents to test the vaccines for potency and identity before formulation of the influenza vaccines for U.S. distribution. Manufacturers submit their vaccine testing results, along with samples from each lot, to CBER for "lot release."

**Kyung Sung, PhD**

*Principal Investigator, OTAT, CBER*

**Bio**: Dr. Kyung Sung is a biomedical engineer with expertise in developing functional and practical microscale in vitro tools for medical and biological applications. Dr. Sung's main research interests lie in studying cell-materials interactions and exploring cell behavior in various tissue micro-environmental conditions.

Dr. Sung received her Ph.D. in Chemical Engineering in 2007 at the University of Michigan, and worked as a post-doctoral researcher in the Department of Biomedical Engineering at the University of Wisconsin-Madison, where she also worked as a Principal Investigator before she joined FDA in 2015. She also worked as a patent examiner in Biotechnology at the US Patent and Trademark Office. During her previous research, she used principles from tissue and microsystems engineering to develop tissue-like structures such as blood vessels and mammary ducts in microfluidic channels to develop new practical tools to conduct cancer research in vitro. The microscale in vitro systems provide unique capabilities when studying complex interactions occurring in tissue microenvironment, by providing more precise controls of biochemical and biomechanical factors than traditional platforms. In addition, the small scale of the microfluidic screening platforms allow high-throughput screening experiments using a small number of cells from patient samples and expensive reagents. She has been able to create innovative opportunities and strategies for researchers to explore biology in different ways – particularly in understanding the role of the tissue microenvironment in regulating cellular functions.

**Abstract: Practical microscale technologies in the assessment of advanced therapeutic products in the Center for Biologics Evaluation and Research (CBER)**

As described in the 21st Century Cures Act, products eligible for Regenerative Medicine Advanced Therapy (RMAT) designation comprise cell therapy, therapeutic tissue engineered products, human cell and tissue products, or any combination products that use such therapies or products. Multipotent stromal cells (MSCs) and induced Pluripotent Cells (iPSCs) have been popular sources for manufacturing RMAT products due to their ability to undergo lineage-specific differentiation in distinct manufacturing conditions.

Despite great promise, successful clinical translation of such cell-based products is often hindered by the manufacturing hurdles associated with them and the lack of reliable markers that can predict the products' in vivo performance. For instance, mounting evidence suggests that MSCs are very heterogeneous and responsive to their surrounding environment, resulting in distinct subpopulations of cells with potentially different potencies based on their tissue sources and in vitro cell culture conditions for isolation and expansion.

Given the fact that there are numerous biochemical and biomechanical factors regulating the functions of MSCs, it is critical to develop reliable high-throughput assays that enable the efficient exploration of large and complex parameter spaces for functional cellular endpoints. Microscale in vitro systems possess practical potential to meet this unmet need. Several simple microfluidic channel arrays have been successfully implicated in screening the influence of paracrine mediators and various tissue microenvironment components in the regulation of cellular functions. In addition, using simple physics and channel geometry, three-dimensional tissue-like structures, such as blood vessels, have been incorporated into the high-throughput cell-based screening platforms in efforts to provide more in vivo-like conditions than traditional two-dimensional platforms. This presentation will give an overview of practical microscale technologies that are simple to operate while enhancing throughput, relevance, and reliability and will discuss how such technologies could be employed in the assessment of regenerative advanced therapeutic products in CBER.


**LCDR Cyrus Agarabi, PharmD, RPh, MBA, PhD**

*Regulatory Research Officer, OPQ, CDER*

**Bio**: Before joining FDA in 2009, Dr. Agarabi pursued his PharmD, two Masters Degrees, and PhD at the University of Rhode Island, and performed his research in branded and generic pharmaceutical, biotechnology, and contract manufacturing organizations. His research has included small and large molecules over a wide array of dosage forms and delivery systems, including: multi particulate sustained release oral dosage forms, topical OTC products, and lyophilization (freeze-drying). His lyophilization work began during his MS research at an innovator Biotech company, where he focused on lyophilization cycle optimization, formulation, and container closure for a CBER-regulated recombinant therapeutic protein.

Since joining FDA, he has helped lead the Division of Product Quality Research's efforts to expand and develop state-of-the art infrastructure for lyophilization and bioprocessing, to study PAT and QbD and impacts on product quality. Since 2010, Dr. Agarabi has acted as both the lead and a collaborator on lyophilization focused RSR projects funded for FY 2011 and FY 2012, respectively, and the CDER liaison for two lyophilization-focused projects with the National Institute for Pharmaceutical Technology and Education (NIPTE). Dr. Agarabi has successfully co-authored two journal articles and two poster presentations on QbD approaches to lyophilized formulation and PAT approaches to processing of a model monoclonal antibody. He is a member of the DPQR biotechnology research team, which is actively studying advances in lyophilization technology such as controlled ice nucleation, Manometric Temperature Measurements, and NIR/Raman Spectroscopy to support the PAT and QbD guidance documents.

**Abstract: Continuous Bio-manufacturing Technologies**

Consistent high quality monoclonal antibody yield is a key goal for commercial cell culture bioprocessing. This endpoint is typically achieved in commercial settings through product and process engineering of bioreactor parameters during development. Traditionally, biotechnology products have been manufactured in batch mode, and may have utilized selective continuous manufacturing technologies at a unit operation level (such as, perfusion cell culture) where necessary to overcome product quality challenges associated with long hold times or protein stability concerns. Continuous "end to end" bioprocessing platform systems have been proposed to quickly and efficiently provide large quantities of protein products, while potentially reducing operating costs. These new approaches have a unique set of scientific and regulatory challenges that require further scientific evaluation. This presentation will provide an overview of OBP's lab capabilities in the bioprocessing area and regulatory research focusing on monoclonal antibodies production. These lab-based capabilities are being leveraged to study continuous bioreactor cell culture production, equipment and Process Analytical Technology (PAT) tools. Case studies will be presented of the ongoing collaborative lab regulatory research being conducted in these areas to support regulatory decision making.

**Maureen Dreher, PhD, MS**

*Research Biomedical Engineer, OSEL, CDRH*

**Bio**: Dr. Dreher is a researcher in CDRH's Office of Science and Engineering Laboratories, with expertise in mechanical durability and degradation of materials. She has conducted research to advance characterization of 3D-printed tissue engineered scaffolds and is leading the development of an ASTM standard guide on the use of microCT imaging for scaffold characterization.

**Abstract: Advancing Characterization of 3D Printed Tissue Engineered Scaffolds**

The microstructure (e.g., pore structure, size, organization) of tissue engineered scaffolds plays an important role in structural support and facilitating matrix deposition. In addition, changes to scaffold microstructure during degradation may continue to influence the eventual repair. Additive manufacturing is an efficient production method to generate scaffolds with a wide variety of microstructural

designs and variation in porosity. One of the primary advantages of using additive manufacturing is the ability to control the scaffold pore space and its organization at a high resolution.

A wide variety of printer types and process parameters exist to accomplish this objective. However, this results in the need for significant optimization to achieve the desired quality. In addition, the complex geometries at the microscale level in tissue engineered scaffolds results in difficulties for measuring quality. Device quality is multi-faceted and requires controls for the raw material and processing as well as multiple metrics for assessment on the finished part.

This presentation will discuss the use of non-destructive x-ray based imaging (microCT) as a tool to characterize the microstructure of scaffolds and optimization procedures to increase part fidelity for tissue engineered scaffolds manufactured from custom and proprietary materials. In these studies, we designed multiple scaffolds with varying pore size (400 microns – 1mm) and organizations based on a modular approach for fabrication using continuous digital light processing. We used both commercially available materials and custom materials, namely poly(propylene fumarate).

MicroCT was able to identify statistically significant differences in key microstructural features for parts built horizontally and vertically. However, part quality was highly dependent on the material chosen and the microstructure design. The results from these studies highlight microCT as a quantitative tool for assessing build quality, key aspects for future standardization of the method, and the interplay between scaffold design and material on build quality.

# Speaker Bios and Abstracts

Session 5: Omics Technologies at the FDA

**Minnie Sarwal, MD, FRCP, DCH, PhD**
(Session Chair)
*Professor of Surgery, Director, Precision Transplant Medicine, University of California, San Francisco, FDA Science Board member*

**Bio**: Dr. Minnie Sarwal is Professor of Surgery and Director of the Precision Transplant Medicine Initiative at the University of California San Francisco (UCSF). She is on Faculty for the Masters in Translational Medicine Program at the Haas School of Business, Berkeley and UCSF and the FDA Science Board. She is an elected TTS Councilor, and Professor of the Faculty of Health Sciences in Odense University, Sweden. Previously, she was the Medical Director of the Pediatric Kidney Transplant Program and Professor of Pediatrics/ Immunology/Surgery at Stanford University, Senior Lecturer at Guys Hospital, London and Lecturer at Addenbrookes Hospital, Cambridge, UK.

She received her MD from Calcutta Medical College, India and Guy's Hospital, London, UK, before completing a doctorate in molecular genetics at Cambridge University, Cambridge, UK with Nobel Laureate Sydney Brenner. She obtained her diploma in child health (London University, UK) and membership and fellowship to the Royal College of Physicians (MRCP, London).

Dr. Sarwal serves on NIH study sections and serves for transplant organizations (TTS, AST, ASTS, IPTA) in leadership capacities. She is the chief editor for Frontiers in Nephrology (Nature) and assistant editor for Clinical Transplantation and AJT. Dr. Sarwal has over 250 peer reviewed publications and is the recipient of multiple awards, such as the Order of Excellence in Scientific Research (Cambridge, UK, 2002), Dean's Graduate Teaching Award (Stanford University, 2005), Junior Faculty Award (Stanford University, 2003-6), Key Opinion Leader in Organ Transplantation (TTS; 2007-2009), Senator at Large (Stanford Faculty Senate), TTS-Roche Award for Outstanding Achievement Transplantation Science (Clinical, 2010), and the Cuneo Richardson Award for Scientific Excellence (National Kidney Foundation, 2012).

Dr. Sarwal has directed the Sarwal lab since 1997 and manages high-caliber clinical and scientific staff. She founded Organ-I, a company for personalization of transplant medicine, spun out of Stanford and recently acquired by Immucor. Minnie has leadership skills in the development, delivery, and evaluation of services in large medical centers in the USA and England, securing pharma and NIH funding, and expanding clinical services in a competitive environment. She is a key opinion leader in the field of renal and transplant medicine, genomics, proteomics and immunology. As principal investigator for industry and NIH multicenter clinical trials, she leads trial design, execution, and human subject safety policies.

Abstract:
Precision medicine encompasses customization of individual health care, accounting for biological, behavioral, and environmental measurements. Precision medicine is driven by data science, generated by new omic hypothesis generating approaches, which need to be subsequently validated by biological testing, and for eventual application back to the patient to improve patient care, need endorsement from payers and should meet critical public health needs. These omic studies are facilitated by interrogation of large patient and sample cohorts, which are increasingly being organized by health systems, as they gather their patient populations and partner with others to make larger cohorts. These health systems engage their populations, getting them more involved in research. This talk will review the recent technologies and general approaches that facilitate precision medicine.

**Weida Tong, PhD**

*Division Director, Bioinformatics and Biostatistics – NCTR*

**Bio**: Dr. Tong is Director of the Division of Bioinformatics and Biostatistics at FDA's National Center for Toxicological Research (NCTR/FDA). He has served as a science advisory board member for several large

projects involving multiple institutes in Europe and the U.S. He also holds several adjunct positions at universities in the U.S. and China. His division at FDA develops bioinformatic methodologies and standards to support FDA research and regulation and to advance regulatory science and personalized medicine. The most visible projects from his group are 1) leading the Microarray Quality Control (MAQC) consortium to develop standard analysis protocols and quality control metrics for emerging technologies to support regulatory science and precision medicine; 2) developing liver toxicity knowledge base (LTKB) for drug safety; 3) in silico drug repositioning for the enhanced treatment of rare diseases; and 4) developing FDA's bioinformatics system, ArrayTrackTM suite, to support FDA review and research on pharmacogenomics. In addition, his group also specializes in molecular modeling and QSARs with specific interest in estrogen, androgen, and endocrine disruptors. Dr. Tong has published more than 230 papers and book chapters.

**Abstract: FDA led community-wide Sequencing Quality Control Consortium 2- (SEQC2)**

The application of constantly evolving high-throughput genomics technologies to assess safety and efficacy of FDA regulated products raises concerns about their reliability and robustness to support regulatory decision-making in FDA. The Microarray/Sequencing Quality Control (MAQC/SEQC) consortium is led by FDA to address concerns such as reproducibility, precision, specificity/sensitivity and interpretation. The consortium completed three phases, (MAQC I-III): MAQC-I focused on the technical aspects of microarray-based gene expression measurements, MAQC-II focused on validation of microarray-based predictive models and the Sequencing Quality Control (SEQC) and MAQC-III focused on assessing the performance of RNA sequencing (RNA-seq). The results of MAQC I-III provided the basic parameters for fit-for-purpose application of these new genomic data streams in regulatory environments, and the solutions are available from over 30 publications in the peer-reviewed literature. This presentation will discuss the 4th phase of MAQC, named the Sequencing Quality Control Phase 2 (SEQC2/MAQC-IV) project, which will be focused on developing quality control metrics, assessing reproducibility and continuity, and benchmarking bioinformatics approaches for clinical use of whole genome sequencing and target gene sequencing data generated through next-generation sequencing (NGS) approaches. The project's ultimate goal is the development of standards for using NGS data that will provide FDA with objective criteria and metrics for data integrity assessment that can be applied in regulatory settings and to inform for precision medicine.

### Errol Strain, PhD

*Director, Biostatistics and Bioinformatics Staff, OAO, CFSAN*

**Bio**: Errol Strain is the Director of the Biostatistics and Bioinformatics Staff at the Center for Food Safety and Applied Nutrition (CFSAN). He joined CFSAN in 2008 as a Bioinformaticist on the Statistical Applications Team.

**Abstract: FDA's GenomeTrakr Program: Advancing Food Safety through Whole-Genome Sequencing of Foodborne Bacteria**

In 2013 CFSAN set up a pilot project at the national level using whole genome sequence data (WGS) to track foodborne outbreaks. This pilot, now a mature network, is called GenomeTrakr. In this network, public health agencies collect and publicly share WGS data in real time. This high-resolution, rapidly growing database is actively being used in outbreak investigations at the state, national, and international level.

### Rodney Rouse, DVM, MBA, PhD

*Acting Associate Director, Office of Translational Science, Office of Clinical Pharmacology, Division of Applied Regulatory Science, CDER*

**Bio**: After graduating from LSU School of Veterinary Medicine, Dr. Rouse spent 20 years developing multiple mixed and small animal hospitals while acquiring an MBA. Dr. Rouse completed a PhD with research on the impact of pre-natal exposure to inhaled environmental pollutants on adult immune function. During his eight years at CDER, Dr. Rouse has served as a

research veterinary medical officer completing eight multi-year projects, authoring or co-authoring over 25 peer reviewed publications, and supporting animal research at FDA. As part of his duties, Dr. Rouse consults on pre- and post-market safety issues with human drugs and oversees animal research development in support of FDA's regulatory mission. His laboratory research projects have included development of biomarkers of tissue injury, histopathology methods in biomarker qualification, the use of digital pathology, animal modeling of drug-induced pancreatitis, and strategies to suppress emerging antibiotic resistance. He was recently appointed acting Associate Director of the Division of Applied Regulatory Science.

*Selected publications:*

Goodwin D, Rosenzweig B, Thompson K , Xu L, Stewart S, Zhang J, Rouse R. 2014. Evaluation of miR-216a and miR-217 as potential biomarkers of acute pancreatic injury in rats and mice. Biomarkers 25: 1-13.

Rouse R, Min M, Francke-Carroll S, Mog S, Zhang J, Shea K, Stewart S, Colatsky T. 2015. Impact of Pathologists, Blind Evaluations, and Sampling Methods on Performance Assessment of the Kidney Injury Biomarker, Kim-1. Toxicol Pathol 43(5): 662-674.

Rouse, R. 2015. Regulatory Forum Opinion Piece: Blinding and Binning in Histopathology Methods in the Biomarker Qualification Process. Toxicol Pathol 43(6): 757-759.

Noel A, Xiao R, Perveen Z, Hasanuzzaman M, Rouse RL, Paulsen D, Penn AL. 2016. Incomplete lung recovery following sub-acute inhalation of combustion-derived ultrafine particles in mice. Part Fibre Toxicol (2016) 13:10. DOI 10.1186/s12989-016-0122-z.

Rouse R, Rosenzweig B, Shea K, Knapton A, Stewart S, Xu L, Chockalingam A, Zadrozny L, Thompson K. 2017. MicroRNA Biomarkers of Pancreatic Injury in a Canine Model. Exp Toxicol Pathol 69(1): 33-43.

**Abstract: MicroRNA Biomarkers of Acute Pancreatic Injury**

MicroRNAs (miRNAs) are highly conserved short noncoding RNA molecules that modulate gene expression by binding to complementary sequence in messenger RNA (mRNA) and blocking translation of that mRNA to protein or marking the mRNA for degradation. This presentation provides an overview of the investigation by the Division of Applied Regulatory Science (DARS) of miRNAs enriched in pancreatic tissue as non-invasive tissue-specific biomarkers of acute pancreatic injury. These miRNAs have tremendous potential as biomarkers because they rapidly appear in a highly stable form in serum and urine following pancreatic injury and have highly conserved sequence between species. Multiple acute pancreatic injury models were used in rats and mice and the caerulein model of acute injury was used in dogs to characterize the response and translatability of the miRNAs as biomarkers across species. Characterization data for the miRNA in all models and species are very similar to the traditional acute pancreatic injury biomarkers, serum amylase and lipase. However, the miRNAs are more sensitive and specific than the traditional biomarkers. Next Generation Sequencing (NGS) on tissue samples linked specific miRNA changes to specific mRNA changes during acute injury and recovery and identify a role for the miRNAs in autophagy and apoptosis, the processes histologically identified in experimental samples. This study demonstrates the usefulness of NGS, a large data format, in defining a mechanistic anchor that further enhances the profile of these miRNAs as biomarkers of acute pancreatic injury. During this project, NGS was also used to address an annotation problem that highlights the necessity of miRNA sequence validation in each species when considering translational value.

**Heike Sichtig, PhD**

*Subject Matter Expert, Principal Investigator, OIR, CDRH*

**Bio**: Dr. Heike Sichtig is a principal investigator (PI) and subject matter expert (SME) in FDA's Office of In-Vitro Diagnostics and Radiological Health in the Division of Microbiology Devices. She directs, as sole PI, the highly collaborative effort on developing FDA-ARGOS: FDA database

for Regulatory Grade micrObial Sequences. For her exceptional leadership on this project, Dr. Sichtig was awarded the Commissioners' Special Citation award in 2016. Dr. Sichtig joined the Division of Microbiology Devices in 2012 and is primarily focused on enabling next-generation sequencing (NGS) based technologies for clinical diagnostics. Dr. Sichtig leads a multidisciplinary team developing and implementing concepts for validation and evaluation of NGS-based infectious disease diagnostic devices. She obtained a BS / MS in Computer Science/ Statistics from Kean University in 2002 and 2003, respectively, and a PhD in Biomedical Engineering from Binghamton University in 2009. Subsequently, Dr. Sichtig completed postdoctoral training at the University of Florida/Genetics Institute in Gainesville FL in pathogen signatures, transcriptional regulation, and epigenetics.

**Abstract: FDA-ARGOS Microbial Reference Genomes for Regulatory Use: Zika and Ebola**

FDA and collaborators established a publicly available database for Reference Grade microbial Sequences called FDA-ARGOS. With funding support from FDA's Office of Counterterrorism and Emerging Threats (OCET) and DoD, the FDA-ARGOS team is collecting and sequencing 2000 microbes that include biothreat micro-organisms, common clinical pathogens, and closely related species. Manufacturers who develop sequence-based tests to identify infectious agents and/or to detect resistance or virulence markers can use FDA-ARGOS to further their development programs and support the regulatory science review of such tests. This presentation will focus on the FDA-ARGOS sequencing pipeline, including Zika and Ebola reference genomes. For more info, visit the FDA-ARGOS reference genome database project website: https://www.fda.gov/MedicalDevices/ScienceandResearch/

### John Cipollo, PhD

*Principal Investigator, Lab of Bacterial Polysaccharides, OVRR, CBER*

**Bio**: Dr. Cipollo earned his PhD at the State University of New York at Albany. He did post doctoral work at Boston University School of Dental Medicine and served as a Research Assistant Professor at Boston University School of Medicine Department of Biochemistry. During this period Dr. Cipollo developed methods in high field NMR and mass spectrometry to study structure–function relationships of glycoconjugates. He was the first to report the Caenorhabditis elegans N-glycome and went on to report the O-glycome and its importance in the nematode's interactions with pathogens.

After joining CBER DBPAP in 2007, he focused on development of mass spectrometry-based glycoanalytics, supporting software for glycoproteomics and glycan array analysis. Areas of interest include polysaccharide and polysaccharide conjugate vaccine structure and other glycoconjugate antigens. A major area of interest is influenza virus and its carbohydrate dependent interactions with its hosts, specifically how the virus's glycoproteins are masked by carbohydrate, how its glycosylation patterns influence interaction with host immune system lectins and what carbohydrates hemagglutinin recognizes as the virus propagates through zoonotic and human hosts. Methods used in these studies are adaptable to other glycoproteins and conjugates.

**Abstract: Mass Spectrometry Based Characterization of Influenza Hemagglutinin Glycoprotein Antigens**

Influenza hemagglutinin glycoprotein is the major antigen in seasonal and pandemic influenza vaccines. As the virus propagates through the human population it tends to gain glycosylation sites as part of its adaptive process. This is especially the case for H1N1 and H3N2 influenza strains, two of the three virus subclasses present in the seasonal vaccine. Depending on the virus the surface can have as few as four and as many as 14 glycosylation sites and these tend to be in regions of defined antigenicity implying an antigenic "masking" component of this adaptive process. Some regions may differentially interact with the host's innate immune system depending on the subclass of glycans present. In the context of vaccines, a number of cell substrates are now used to propagate virus for vaccine production.

These include hen egg, MDCK, Vero and Sf9 cell systems with others currently in development. All of these systems will produce cell specific glycosylation patterns, which may affect vaccine performance by influencing antigen uptake, antibody production, innate immune interactions and interaction with components of the host cellular immune system. In this lecture glycomics analytics used in influenza antigen characterization will be discussed. Three cases will be presented revealing aspects of hemagglutinin glycosylation that may impact the influenza vaccines and the virus's interactions with the human host.

**Brian J. Zikmund-Fisher, PhD** (Session Chair)

*Associate Professor of Health Behavior and Health Education, University of Michigan*

**Bio**: Brian J. Zikmund-Fisher, PhD, is an Associate Professor of Health Behavior and Health Education at the University of Michigan (UM) School of Public Health and a Research Associate Professor in the UM Department of Internal Medicine. In addition, he is an Associate Director of the UM Center for Bioethics and Social Sciences in Medicine, a member of the UM Health Informatics Program, and an Associate Editor of the Journals Medical Decision Making and Medical Decision Making: Policy and Practice. Dr. Zikmund-Fisher received his PhD in Behavioral Decision Theory (a combination of decision psychology and behavioral economics) from Carnegie Mellon University. He uses this interdisciplinary background to study factors that affect individuals' ability to use data to inform their health and medical decision making.

An author of over 100 articles and book chapters, Dr. Zikmund-Fisher researches the design of communications to make health risk and test data more intuitively meaningful. In particular, he considers the effects of numeracy (people's ability to use numbers to inform their health decisions) on health communication. Past projects have included the National Survey of Medical Decisions (often called the DECISIONS Study), an NIEHS-funded grant studying perceptions of risk from dioxin exposure within affected communities, an American Cancer Society award regarding the development and testing of visual displays of risk, and several projects examining how patient testimonials influence risk perceptions and decision making. He is currently the principal investigator of a multi-year grant from the Agency for Healthcare Research and Quality (AHRQ) to design more intuitively meaningful displays of laboratory test results for use in patient online portals. At Michigan, Dr. Zikmund-Fisher teaches graduate courses in risk communication and designing health messages.

**Bridget Ambrose, PhD, MPH**

*Epidemiologist, FDA's Center for Tobacco Products*

**Bio**: Bridget Ambrose, PhD, MPH has been an Epidemiologist in the Center for Tobacco Products' (CTP) Office of Science since September 2011. As an Epidemiologist within the Division of Population Health Sciences, her duties at CTP include collaborative efforts with other federal agencies in the design, planning, implementation and analysis of the Population Assessment of Tobacco and Health (PATH) Study, as well as national tobacco surveillance efforts. Dr. Ambrose has co-authored articles published in JAMA and the New England Journal of Medicine related to patterns of tobacco use among U.S. youth and adults, including work focused on use of flavored tobacco by youth in the United States. In addition, she reviews tobacco product applications to assess the potential impact on population health, and was most notably involved in evaluating the first modified risk tobacco product application submitted to CTP. Dr. Ambrose received her PhD in Epidemiology from the Johns Hopkins Bloomberg School of Public Health, her MPH from the George Washington University, and her BA from the College of the Holy Cross.

**Abstract: Use of Flavored Tobacco Products: Findings from the Population Assessment of Tobacco and Health (PATH) Study**

Most tobacco products include flavoring additives that improve the palatability and lessen the harshness of inhaled tobacco smoke. Reviews of internal tobacco industry documents indicate that some manufacturers historically added flavors to tobacco to attract young consumers. In the interest of preventing youth smoking initiation, the 2009 Family Smoking Prevention and Tobacco Control Act (FSPTCA) banned the inclusion of constituents or additives that impart characterizing flavors (e.g., candy, fruit) other than tobacco and menthol in cigarettes, but not other tobacco products. Cigars, electronic cigarettes and hookah/waterpipe tobacco are commonly sold in flavor varieties that include candy and fruit flavors, which can increase the appeal of these tobacco products to young people.

National cross sectional surveys have shown

that a high proportion of youth tobacco users report use of flavored products. This presentation will describe recent findings on flavored tobacco use from the Population Assessment of Tobacco and Health (PATH) Study. A joint collaboration between FDA and the NIH, the PATH Study is the largest prospective study of tobacco use and health in the Unites States, enrolling more than 46,000 civilian, non-institutionalized youth and adults via household-based interviews.

Study findings to be discussed include patterns of flavored tobacco use among youth, young adults and older adults, including a description of types of flavors used across tobacco products, as well as results from a prospective assessment of the association between first trying a flavored product and current tobacco use at study follow-up. Findings from this research provide insight into the role characterizing flavorings may play in promoting the use of non-cigarette tobacco products, particularly among youth and young adults.

**Kathleen Yu, MPH**

*Social Scientist, OAO, CFSAN*

**Bio**: Kathleen Yu is a Social Scientist in the Consumer Studies Branch at the Center for Food Safety and Applied Nutrition (CFSAN), where she leads qualitative research studies to assist the Center with education and outreach efforts and exploratory research. She previously worked at the Center for Cancer Prevention and Control Research, a joint program of the UCLA Fielding School of Public Health and the Jonsson Comprehensive Cancer Center, where her work focused on cancer disparities research, particularly with socially and medically underserved populations. Her research background and interests include nutrition and chronic diseases, particularly as they intersect with health disparities and community-based participatory research. She earned her BA in Public Health from the University of California, Berkeley, and her MPH from Columbia University Mailman School of Public Health.

**Abstract: Understanding mothers' attitudes and motivations regarding menu labeling: Testing messaging concepts and treatments**

Studies show that providing nutrition information at restaurants can help people make healthier choices. The availability of nutrition information through menu labeling would provide Americans with additional information to make informed choices about their diets. On December 1, 2014, FDA published a Final Rule requiring restaurants and similar retail food establishments that are part of a chain with 20 or more locations to provide calorie and other nutrition information for standard menu items, including food on display and self-service food. To support the implementation of the rule, FDA will develop educational materials to help consumers interpret and use the information. As a first step in this effort, qualitative data were collected from s16 consumer focus groups to explore and understand how to reach and communicate with consumers on menu labeling. These focus groups targeted US middle-income mothers of children ages 3-10 years who ate food away from home at least once a week. The groups were segmented by race/ethnicity, and conducted in two phases from June through November 2016: the first phase tested concepts, and the second tested mock advertisements. Attitudinal, motivational, and behavioral questions surrounding food eaten away from home were asked of participants in both phases. In phase 1, the concepts that emphasized actionable tips (e.g. simple swaps) resonated best with middle-income mothers. In phase 2 testing of mock advertisements, the easily employable tips continued to resonate most with participants. In both phases, there were a minority of groups whose participants identified with a healthy eating lifestyle (e.g., happy healthy children) versus the tips. There were no outstanding differences in reactions to concepts or mock advertisements by ethnicity. Overall, the focus groups highlighted the importance of targeted messaging and provided valuable findings in better understanding middle-income mothers' motivations when eating out.

**Michelle Tarver, MD, PhD**

*Medical Officer, ODE, CDRH*

**Bio**: Michelle Tarver is a medical officer in FDA's Center for Devices and Radiological Health. She attended Spelman College in Atlanta, GA, where she received a BS in biochemistry. She completed the MD/PhD program at The Johns Hopkins University Bloomberg School of Public Health in 2002, earning her doctorate in clinical epidemiology. She completed her MD at the Johns Hopkins School of Medicine in 2003. Following her internal medicine internship, Dr. Tarver completed a residency in ophthalmology with fellowship training in ocular inflammation at the Wilmer Eye Institute (Johns Hopkins). She is board certified in ophthalmology and was previously an Assistant Professor in the Department of Ophthalmology at the Johns Hopkins University School of Medicine in the Division of Ocular Immunology.

In 2009, Dr. Tarver joined FDA, where she works on ensuring that ophthalmic devices are safe and effective before entering the U.S. marketplace. She is actively involved in research on ophthalmic devices and efforts aimed at incorporating the patient's voice in the evaluation of medical devices. She led the development of a patient-reported outcome measure for LASIK surgery and continues to be involved with developing these measures for other ophthalmic conditions. Dr. Tarver has also led the development of a patient preference study for glaucoma. She has received numerous awards for her research efforts from FDA and the American Academy of Ophthalmology. She continues to see uveitis patients through her privileges at The Johns Hopkins Wilmer Eye Clinic and Solomon Eye Associates.

**Abstract: Development of Tools to Capture the Patient Perspective with Implantable Minimally Invasive Glaucoma Surgical (MIGS) Devices**

Glaucoma is a chronic and potentially blinding eye disease that damages the optic nerve. Increased eye pressure, called intraocular pressure (IOP), is a known modifiable risk factor for glaucoma progression. Most therapies are aimed at lowering IOP. Many treatment options are available to treat glaucoma, including prescribed eye drops, laser therapy, and surgery. Recent innovation in glaucoma procedures has led to the development of minimally invasive glaucoma surgical (MIGS) devices which are increasingly being used to lower pressure in the eye. These devices claim to be safer than other glaucoma surgical procedures and to reduce the need to use eye drops. Prior evaluation of these devices has not incorporated patient preference information. Patient preference information provides insight on the relative desirability and acceptability of the benefits and risks of therapies in treating medical conditions. Similarly, patient-reported outcome (PROs) measures are often incorporated in clinical trials for MIGS devices to capture ocular symptoms and visual function but may not have undergone a development process sensitive to mild to moderate glaucoma patients undergoing surgery. To develop tools sensitive to capture preferences and PROs in patients with mild to moderate glaucoma, FDA has partnered with the Johns Hopkins University and the University of California San Francisco/Stanford Centers for Excellence in Regulatory Science and Innovation (CERSIs). For the patient preference study, focus groups with mild to moderate glaucoma patients have been conducted leading to the development of a survey which is being used to quantitatively assess preferences with this disease. For the PRO measure development, focus groups with mild to moderate glaucoma patients across the US have been completed. The PRO measure derived from these discussions is being constructed on a web-based platform. These efforts will facilitate the incorporation of patients' perspectives into the MIGS device development and evaluation process.

**Heather Benz, PhD**

*Medical Device Fellow, OCD, CDRH*

**Bio**: Dr. Heather Benz is a Medical Device Fellow in FDA's Center for Devices and Radiological Health (CDRH). She conducts research on patient preferences in the Office of the Center Director in support of the Center strategic priority, "Partnering with Patients" with a focus on the application of patient preference information to neurological device review. She also collaborates with researchers in the

Center's Office of Science and Engineering Laboratories on outcome measures for advanced upper limb prostheses. Dr. Benz received a BS in Biomedical Engineering from Case Western Reserve University, Cleveland, Ohio and a PhD in Biomedical Engineering from Johns Hopkins University School of Medicine, Baltimore, Maryland.

**Abstract: Upper Limb Prostheses Patient Preference Study to Inform Clinical Trial Design and Regulatory Decision**

The needs of individuals with upper limb amputation and congenital limb difference are not being fully met by current prostheses, evidenced by prosthesis rejection, non-wear, and reports of pain and challenging activities. Emerging technologies such as electrical stimulation for the restoration of sensation, implantable neural interfaces for improved prosthetic control, bone-anchored devices, and dexterous sensorized robotic limbs have the potential to provide novel benefits, but pose additional risks. These implantable technologies will require careful assessments of benefit-risk tradeoffs. Prosthesis users' perspectives on such benefits and risks can inform device development, clinical trial design, and regulatory benefit-risk assessments. Patient preference surveys may be used to assess upper limb prosthesis user perspectives on benefits and risks. Upper limb prostheses are inherently preference-sensitive, as patients work closely with clinicians to choose a prosthesis that fits their needs and on a daily basis decide whether to use the device. Qualitative interviews, focus groups, and surveys with individuals with upper limb amputation or congenital limb difference were conducted to identify attributes for use in developing patient preference surveys. These patient preference surveys provide a more complete understanding of how novel technologies could address patient concerns and inform implementation of new technologies and regulatory decision-making.

**Million Tegenge, PhD, RPh**

*Visiting Scientist, FDA's Office of Biostatistics and Epidemiology, CBER*

**Bio**: Dr. Million A. Tegenge is a visiting scientist (pharmacologist) at Center for Biologics Evaluation and Research U.S FDA. His research and regulatory review focuses on quantitative pharmacology/toxicology modeling, personalized medicine, pharmaceutical outcomes, patient preferences and benefits-risks assessments. Previously, he was a neurology postdoctoral fellow at Johns Hopkins School of Medicine and received his doctoral degree from Germany in Systems Neuroscience. Dr. Tegenge is a registered pharmacist.

**Abstract: Advancing the science of patient input in a regulatory setting through internal capacity building and research**

Qualitative patient input can be collected through patient representatives on advisory boards, interactions with advocacy groups, and Patient-Focused Drug Development meetings. In recent years, there has been an increased scientific demand for more systematic and quantitative approaches to incorporate patient input throughout the medical product lifecycle, including informing regulatory benefit-risk assessments. The use of patient preference information (PPI), elicited using established scientific methods, is a promising strategy for accomplishing this. In this presentation, an overview of FDA's Center for Biologics Evaluation and Research (CBER) experience with and current initiatives on advancing the science of patient input (SPI) in a regulatory setting will be provided. Current CBER activities include capacity building through the launch of the CBER SPI Initiative and inter-center collaboration on the regulatory application of patient reported outcomes and patient preference studies. To further advance the application of SPI in a regulatory framework, a list of example resources that support the design and conduct of PPI studies will be provided. The presentation also highlights some examples of potential opportunities and prevalent challenges in the use of PPI in regulatory decision-making. Finally, the results of specific ongoing regulatory projects that aim to incorporate quantitative patient preference data for informing preference-sensitive decisions will be presented.

**Paula Rausch, PhD, RN**

*Associate Director, Research and Risk Communications, OCOMM, CDER*

**Bio**: Paula Rausch, PhD, RN, is the associate director of Research and Risk Communications in FDA's Center for Drug Evaluation and Research (CDER) Office of Communications (OCOMM). In this position, she oversees development of FDA's Drug Safety Communications, the Agency's primary non-regulatory tool for communicating new and emerging post-market information to the public, including health care professionals and patients, about safety risks and adverse events associated with therapeutic uses of prescription and over-the-counter drugs. In addition, she directs OCOMM's social science risk communication research program, conducting formative and evaluative qualitative and quantitative studies aimed at gathering evidence used to improve CDER's communications. Before FDA, she worked as a strategic communications program manager for the National Cancer Institute; as an instructor and researcher and the University of Florida (UF) College of Journalism and Communications; in public affairs at UF, including for the College of Medicine and at the University level; as a journalist for newspapers and broadcast media; and as a registered nurse in two urban teaching hospitals.

**Abstract: Communicating Risk Information about Drugs: The Effect of Quantitative Information Type on Risk Perceptions and Understanding**

The goal of this study was to assess experimentally how different types of quantitative information that might be included in a communication about a new drug safety issue affects perceptions of severity and susceptibility to that issue and understanding/recall. This experiment was undertaken to obtain generalizable data that could be used to enhance FDA's Drug Safety Communications.

Respondents with diabetes (n=1477) or constipation (n=1453) read a paragraph that reported information about a safety issue with a medication used to treat their respective conditions. Participants in each group were randomly assigned to one of six conditions.

All five experimental conditions included the number of adverse events reported to FDA. Three included a source (FDA's Adverse Event Reporting System). Two included frequency information: quantitative (fewer than one in a million) and qualitative (very rarely). The control included source but no quantitative or frequency information.

Across both medical conditions, inclusion of quantitative frequency information resulted in lower perceptions of risk severity, and risk susceptibility for both the self and the typical person compared to the control condition. Qualitative frequency information also resulted in decreased risk severity perceptions compared to when frequency information was lacking. In the constipation group only, respondents who viewed a communication with quantitative adverse event information only were more likely to have accurate understanding/recall compared to all other groups. In both medical conditions, respondents who saw quantitative information and additional qualitative frequency information had the poorest recall.

These findings suggest adding either qualitative or quantitative information about the frequency of drug adverse events can lower risk perceptions about a safety issue, which can help reduce potential unintended effects of these types of communications. However, the inclusion of qualitative frequency information has the additional effect of reducing information understanding. In some, but not all, cases, quantitative information alone can increase understanding/recall.

# Speaker Bios and Abstracts

Session 7: Computational Modeling and Simulation at FDA

**Grace Peng, PhD**
(Session Chair)
*Director of Computational Modeling and Simulation, NIBIB, NIH*

**Bio**: Grace C.Y. Peng, PhD is the Director of Computational Modeling, Simulation and Analysis at the National Institute of Biomedical Imaging and Bioengineering (NIBIB) within the National Institutes of Health (NIH) in the US Department of Health and Human Services (DHHS). In this capacity she has programmatic oversight of extramural activities in these areas.

Dr. Peng received the BS degree in electrical engineering from the University of Illinois at Urbana, the MS and PhD degrees in biomedical engineering from Northwestern University. She performed postdoctoral and faculty research in the department of Neurology at the Johns Hopkins University. In 2000, she became the Clare Boothe Luce professor of biomedical engineering at the Catholic University of America. Her research focused on developing computational models of the vestibular system in control of the head and neck, and analytical tools for studying the oculomotor system in patients with vestibular dysfunction. Since 2002, Dr. Peng has been a Program Director in the NIBIB, overseeing various programs promoting the development of mathematical and statistical modeling and analysis methods;: medical simulation tools,; and next generation engineering systems for rehabilitation, robotics, neuroengineering, and surgical systems. In 2003, Dr. Peng led the creation of the Interagency Modeling and Analysis Group (IMAG), which now consists of program officers from multiple federal agencies of the U.S. government. Since 2004, IMAG has supported funding initiatives targeted to multiscale modeling of biomedical, biological and behavioral systems. Since 2006, IMAG has facilitated the activities of the Multiscale Modeling Consortium of investigators. Dr. Peng is committed to promoting the development and use of intelligent tools and reusable models to accelerate biomedical research and translate scientific knowledge to the clinic and community.

**Abstract: Multiscale Modeling in Biomedical, Biological and Behavioral Systems**

Over the last decade the biomedical community is beginning to recognize not only the usefulness of models, but the essential role models play to integrate disparate fields of knowledge, identify gaps and present testable hypothesis to drive experiments. Multiscale modeling, in particular, is at the forefront of making a significant impact in biomedical discoveries, applied science and medicine.

In the U.S., a confluence of events resulted in the 2003 formation of the Interagency Modeling and Analysis Group (IMAG) and subsequent release of the first interagency solicitation for multiscale modeling of biomedical, biological and behavioral systems. That solicitation funded 24 projects creating the Multiscale Modeling Consortium (MSM) in 2006. The IMAG MSM Consortium now has over 100 multiscale modeling related projects. During this time, many other multiscale modeling initiatives have emerged from the multiple government agencies of IMAG with more than 80 program directors managing programs for modeling and analysis and biomedical, biological and behavioral systems.

One of the main activities of IMAG is to coordinate the MSM Consortium. The MSM mission is to grow the field of multiscale modeling in biomedical, biological and behavioral systems by: 1) promoting multidisciplinary scientific collaboration among multiscale modelers; 2) encouraging future generations of multiscale modelers; 3) developing accurate methods and algorithms to cross the interface between multiple spatiotemporal scales; 4) promoting model sharing and the development of reusable multiscale models; and 5) disseminating the models and insights arrived from the models to the larger biomedical, biological, and behavioral research community.

The MSM Consortium is actively addressing many pressing issues facing the multiscale modeling community. Of particular focus are the challenges of model sharing and model translation – in particular model accessibility, reproducibility, reusability and credibility.

**Tina Morrison, PhD**

*Chair, Modeling and Simulation Working Group, Office of Science and Engineering Laboratories, CDRH*

**Bio**: Tina Morrison is chair of the new FDA-wide working group on Modeling and Simulation, sponsored by the Office of the Chief Scientist, which launched in 2017. She has been serving as the Regulatory Advisor of Computational Modeling for the Center for Devices and Radiological Health (CDRH) since 2012. In that capacity, she leads the Regulatory Review of Computational Modeling working group, which has developed guidance documents on the use of modeling and simulation in the regulatory evaluation of medical devices. She dedicates much of her energy towards advancing regulatory science through modeling and simulation because she believes the future of medical device design and evaluation, and thus enhanced patient care, lies with computation and enhanced visualization. She is chair of the ASME Verification and Validation Committee, and of the ASME V&V40 Subcommittee on Computational Modeling of Medical Devices, where she is leading the development of a strategy to assess the credibility of computational models. She is also working with a team at CDRH to implement this strategy into the review of premarket submissions that leverage computational modeling. For seven years, she was a scientific reviewer on a variety of medical device premarket submissions in Cardiovascular Devices. She is a mechanical engineer who received her PhD in Theoretical and Applied Mechanics from Cornell University in 2006.

Abstract:
In its 2011 Advancing Regulatory Science Report[1], FDA identified an important role for modeling and simulation to further regulatory science and FDA's strategic priorities. In that document, FDA identified eight regulatory science priority areas, four of which had identified a specific method or approach for modeling and simulation.

Four Science Priority Areas

- Modernize Toxicology to Enhance Product Safety
- Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes
- Ensure FDA Readiness to Evaluate Innovative Emerging Technologies
- Harness Diverse Data through Information Sciences to Improve Health Outcomes

Proposed Methods and Approaches

- Computer models of cells, organs, and systems to better predict product safety and efficacy
- Virtual physiologic patients for testing medical products
- Clinical trial simulations that reveal interactions between therapeutic effects, patient characteristics, and disease variables
- Knowledge-building tools for data mining, visualization and high throughput synthesis
- Mechanism for sharing data/models/algorithms

This presentation will give an overview of the different types of modeling disciplines used for the different products that FDA regulates, at different phase of a product's lifecycle. Moreover, this presentation will showcase key projects on advancing regulatory science with modeling and simulation. Finally, this presentation will introduce the new FDA-wide working group on modeling and simulation, and what our goals and objectives are for advancing the role of modeling and simulation in regulatory decision-making.

**Leonardo Angelone, PhD**

*CDRH*

**Bio**: Leonardo M. Angelone is a Research Biomedical Engineer at FDA's Office of Science and Engineering Laboratories, Center of Devices and Radiological Health. Dr. Angelone leads a Research program that focuses on assessment of energy deposition and heating induced in the human body by medical devices using electromagnetic energy. The investigation is

1 http://www.fda.gov/downloads/ScienceResearch/SpecialTopics/RegulatoryScience/UCM268225.pdf

based on a combination of anatomically precise computational models and experimental measurements applied to several areas of clinical significance, including RF safety of human subjects during interventional MRI and analysis of safety and effectiveness of MR Conditional deep brain stimulators. The projects, supported by both FDA and external funding, have been carried out in collaboration with groups at leading academic research institutes and industry organizations, delivering over 100 peer-reviewed journal articles and conference proceedings, as well as publicly available software. Dr. Angelone completed a laurea in Electronic Engineering (University "La Sapienza", Rome, Italy), a PhD in Biomedical Engineering (Tufts University, Medford, MA), and a Research Fellowship at the Department of Radiology of the Massachusetts General Hospital, Harvard Medical School. Before joining FDA, Dr. Angelone was a consultant with the Research and Development Department in the Surgical Products Division of Hologic, Inc.

**Abstract: Computational Electromagnetic Modeling and Medical Devices**

The EM modeling group in the Office of Science and Engineering Laboratories at CDRH supports the FDA regulatory and guidance role by advancing our knowledge on the complex interactions of electromagnetic (EM) fields and the human body. The research combines anatomically precise computational models and experimental measurements applied to several areas of clinical significance, including the: 1) analysis of radiofrequency (RF)-induced heating in patients with passive implanted medical devices who undergo magnetic resonance imaging (MRI); 2) analysis of the safety and effectiveness of MR Conditional active implants (e.g., deep brain stimulators and pacemakers) during MRI; 3) RF safety of human subjects during interventional MRI; and 4) analysis of patient safety with respect to gradient-induced heating and unintended nerve stimulation undergoing MRI. These projects are conducted with active collaborations between several researchers, within FDA and worldwide, at leading academic research institutes and industry organizations. There is a direct impact to the regulatory mission of the Agency, as the tools developed by our research are extensively used by industry in pre-market evaluation for the safety and effectiveness of medical devices.

**Naomi Kruhlak, PhD**

*CDER*

**Bio**: Dr. Naomi Kruhlak has worked for the FDA Center for Drug Evaluation and Research (CDER) as a computational toxicologist for 15 years, developing and applying (quantitative) structure-activity relationship ((Q)SAR) models to support regulatory review decisions. She is currently the Lead for the Division of Applied Regulatory Science Chemical Informatics Program and is the Principal Investigator on three FDA/CDER Research Collaboration Agreements with commercial (Q)SAR software vendors. Dr. Kruhlak has published 30 peer-reviewed articles describing data standardization, transformation, and classification for modeling purposes, as well as the creation and refinement of (Q)SAR models with chemical interpretability. She has extensive experience in the technical aspects of (Q)SAR modeling as well as their application in a regulatory context, most recently contributing to the International Conference on Harmonization (ICH) M7 guideline describing the use of computational models in place of conventional testing for the safety assessment of drug impurities. Dr. Kruhlak holds BSc and PhD degrees in chemistry from the University of Salford, England, and the University of Calgary, Canada, respectively.

**Abstract: Using (Q)SAR Modeling to Inform Drug Safety Assessment**

The FDA Center for Drug Evaluation and Research (CDER) maintains a robust regulatory research program supporting the development and application of (quantitative) structure-activity relationship ((Q)SAR) models for regulatory decision-making. (Q)SAR models can make predictions of toxicity based solely on a chemical structure and are used by CDER's Chemical Informatics Program to provide predictions for chemical substances under review in the absence of adequate experimental data. For example, (Q)SAR models are routinely used to predict the mutagenicity of drug impurities as a replacement for traditional

testing in accordance with international regulatory guidance. Additionally, (Q)SAR models are used to provide supplemental toxicity predictions—such as for carcinogenicity or liver damage—during regulatory safety review when traditional testing data are limited or conflicting. More recently, (Q)SAR models that predict whether a synthetic street drug binds to opioid receptors have been developed to support the legal classification of newly identified substances. This presentation will provide an overview of (Q)SAR modeling efforts at CDER, as well as supporting activities in database development and structure-based searching conducted by the Chemical Informatics Program.

**Mark Walderhaug, PhD**

*Office of Biostatistics and Epidemiology, CBER*

**Bio**: Mark Walderhaug is an interdisciplinary scientist in FDA's Center for Biologics Evaluation and Research (CBER). He works in the Office of Biostatistics and Epidemiology where he is the Associate Office Director for Risk Assessment. He is currently working on incorporating the computational resource, High-Performance Integrated Virtual Environment (HIVE), into the regulatory structures of CBER and supporting the HIVE in the development of high-performance computing solutions that protect and promote health. In the past, he developed quantitative risk assessments on babesiosis, avian influenza/pandemic flu, malaria, and the impact of emerging infectious diseases on biologics. The quantitative risk assessments have incorporated health data from CMS Standard Analytical administrative files as well as other health data sources. He assists in managing text mining and health surveillance modeling for CBER. He is a member of CBER's Computational Science Review Committee, and represents CBER on FDA's Scientific Computing Board. Before joining CBER, he worked at FDA's Center for Food Safety and Applied Nutrition where he was a member of the Food Safety Initiative's Microbiological Risk Assessment Team where he worked on FDA's Vibrio parahaemolyticus and Listeria monocytogenes risk assessments and USDA's E. coli O157:H7 risk assessment for ground beef. He later served as a temporary advisor for the Joint FAO/WHO Expert consultation on Microbial Risk Assessment of Vibrio spp. in seafood. He earned his Ph.D. at Vanderbilt University and held a postdoctoral appointment at the University of Chicago in the department of Molecular Genetics and Cell Biology before coming to FDA.

**Abstract: U.S. Blood Supply**

*Arianna Simonetti, Hussein Ezzeldin, Richard Forshee, Mark Walderhaug*

*Office of Biostatistics and Epidemiology (OBE), Center for Biologics Evaluation and Research*

To ensure a resilient blood supply in the nation, a model of the U.S. blood supply was developed to explore the variable effects of changes in the supply of blood resulting from pandemic or inter-regional disruptions that had an impact on blood donations.

The model provides a simulation, from blood donation to testing to storage, in blood collector inventories to hospital inventory and finally to a patient. Stochastically derived daily amounts of donations and blood demand to simulate the supply and demand of a stock and flow model. The age of blood units in the simulation is monitored to allow for the withdrawal of units that have expired. Initially, the model only had one collection stock and one distribution stock, but the model has been extended to modeling four interacting regions of collection stocks where one region may request blood units from another. The model has been used to simulate the effect of a pandemic and the impact of changing the expiration date of stored blood on the blood supply.

**Marilyn Martinez, PhD**

*CVM*

**Bio**: Dr. Martinez is a Senior Biomedical Research Scientist for the Food and Drug Administration, Center for Veterinary Medicine (CVM). In addition to her responsibilities at the CVM, her activities include her role as a voting member of the Veterinary Antimicrobial Susceptibility Testing Subcommittee of the Clinical Laboratory Standards Institute, Chair of the Veterinary International Conference on Harmonization bioequivalence expert

working group, Adjunct Professor in the College of Veterinary Medicine, North Carolina State University, Federal Liaison to OrBITO, member of the Editorial Board of the Journal of Veterinary Pharmacology and Therapeutics, and Associate Editor of the AAPS Journal. She is the recipient of the 2015 Lloyd Davis Lifetime Achievement Award and was elected as Fellow of the American Association of Pharmaceutical Scientists and the Controlled Release Society. She received her PhD from the Department of Physiology and Biophysics, Georgetown University School of Medicine.

**Abstract: Potential Uses for Modeling and Simulation in Veterinary Medicine**

Unlike that encountered in human medicine (21 CFR 320), there is no statutory requirement for PK data when submitting a new animal drug application. Accordingly, to date, there have been only a handful of examples where modeling and simulation (M&S) strategies have been included in new animal drug applications. Nevertheless, the FDA Center for Veterinary Medicine (CVM) recognizes the power of M&S as a tool for addressing the challenges and concerns we encounter. Therefore, CVM has been actively exploring the use and development of a range of M&S strategies for purposes such as:

- Extrapolating (PK), data characterized in normal healthy animals to:
  - Diseased animal patients?
  - Across breeds (e.g., dogs)?
- Predicting the likelihood of food effects in dogs.
- For farm animals, some medicines are administered in food. By developing models that describe the within farm and between farm factors influencing the food intake, M&S can be used to anticipate the variability in drug exposure resulting from the administration of medicines
  - Across animals within a farm
  - Across farms
- Sometimes, the critical clinical pharmacology questions regarding canine formulations or clinical study designs are based upon data generated in humans. M&S can support interspecies extrapolations. It can also be used to support an understanding of mechanisms responsible for altered oral drug absorption across a variety of formulation strategies.

Given CVM's successes when applying M&S strategies for the purposes described, the goal is to continue refining these tools to optimize the efficiency of our product evaluation process and in so doing, encourage the submission of the therapeutic products needed to insure the health of companion and food-producing animal species.

### Danielle Larese, PhD

*ORA/WEAC*

**Bio**: Danielle Larese is an ORISE Fellow at the FDA Winchester Engineering and Analytical Center. She obtained a PhD in theoretical molecular physics from Yale University. She graduated from Michigan State University in 2008 with degrees in chemical physics and mathematics. As an ORISE Fellow she works on developing mathematical models to organize, interpret, and extrapolate experimental data from the team's laboratory.

**Abstract: Contamination of Food By Radionuclides After a Nuclear Accident**

*Danielle Larese, Brian Sweeney, Anthony Wetherby Jr, and Cong Wei*
*Winchester Engineering and Analytical Center, U.S. Food and Drug Administration*

*Olivier Vitrac*
*INRA and AgroParisTech UMR 1145 Food Processing Engineering, F-91300 Massy, France*

This project presents an ongoing study on the use of computational modeling to increase the efficiency and rate of laboratory sample analysis following a nuclear accident. Although critical to the safety of the food supply, the testing and analysis of food samples for radionuclide contamination can be slow, difficult to scale, and expensive. The team is proposing an approach which combines map-based estimates of contamination, physics-based descriptions of permeation through packaging/food systems, and efficient modeling techniques for studying radionuclide transport phenomena to construct

a sample triage mechanism.

The 2011 Fukushima nuclear accident was a modern demonstration of how large amounts of processed and packaged food can be contaminated by radionuclides at all points of the supply chain. Previous research into radionuclide contamination of food has focused on crops in fields, but little is currently known about the permeability of post-harvest products or packaged foods to radionuclides. The research involves grouping foods and food packaging materials into categories that reflect their resistance and response to routes of radionuclide exposure. Conservative simulations have been devised to transform real-time data into maps that can depict the likely contamination of food and food packaging by category.

Results will be available to emergency responders within hours and can be updated in real time as conditions evolve. This may allow for a more efficient triage process through an enhanced sample targeting strategy. In addition, different laboratory analyses that require substantially less time may be applied to sample categories with limited radionuclide permeability, enabling rapid information transfer to emergency response decision makers.

**Antonio Paredes, MA, MS**

*Lead Mathematical Statistician, CTP*

**Bio**: Antonio Paredes is a Lead Mathematical Statistician in the Statistics Branch, Division of Population Health Science, Office of Science, in FDA's Center for Tobacco Products, where he has been since August, 2012. Antonio's responsibilities include managing the work of Mathematical Statisticians working in regulatory activities and tobacco research at CTP, including: regulatory activities associated with modified risk tobacco product applications and research activities associated with modeling and simulation investigating the impact of tobacco products on the US population as a whole. From 2008 to 2012 Antonio worked as a Mathematical Statistician in the Drug Safety Program of the Center for Drug Evaluation and Research Office of Biostatistics. Before coming to FDA, from 2003 to 2008, Antonio worked as a Mathematical Statistician in the regulation of virus and bacterial vaccines in the Statistic Unit of the USDA Center for Veterinary Biologics. Over the years Antonio has developed extensive maturity in the research and application of statistical science in a regulatory environment. Antonio is passionate about his responsibilities as a public servant, in particular of his contributions to the mission and vision of CTP in implementing the Family Smoking Prevention and Tobacco Control Act.

**Abstract: Modeling and Simulation in Tobacco Regulatory Science**

Tobacco use is the single largest preventable cause of disease and death in the United States. The Family Smoking Prevention and Tobacco Control Act (FSPTCA, Public law 111-31) signed on June 22, 2009 grants authority to FDA to regulate tobacco products. This law also gave FDA regulatory authority to regulate Modified Risk Tobacco Products (MRTP). An MRTP is any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products. FDA can issue an order authorizing the marketing of a product as a MRTP only if the evidence submitted in an application (MRTPA) meets the requirements of Section 911 of the FSPTCA, including, showing that the product will or is expected to benefit the health of the population as a whole, taking into consideration users and non-users of tobacco products. This presentation presents several modeling and simulation strategies currently under development at CTP with desirable properties amenable to investigating the impact of a new tobacco product on the population as a whole, including: agent-based models, system dynamic models, and social network analysis. Although these procedures/methods are well-established in many areas of applications of the social and physical sciences, their applications to tobacco research and regulation are relatively new.

# Speaker Bios and Abstracts

Session 8: Current Progress in Nanotechnology Research at FDA

**Anil Patri, PhD**
(Session Chair)
*Director, NCTR-ORA Nanotechnology Core Facility, National Center for Toxicological Research (NCTR), FDA*

**Bio**: Dr. Anil Patri conducts Nanotechnology research at NCTR and coordinates research, training, funding, and nanotechnology related standards development activities for the agency as the Chair of NTF. He serves on the National Nanotechnology Initiative (NNI) as a member from FDA in the Nanoscale Science, Engineering and Technology (NSET) Subcommittee and the Nanotechnology Environmental and Health Implications (NEHI) working group. He serves as the Co-chair of the US-EU Communities of Research on Characterization to coordinate international engagement in the field through NNI. He is a member of ISO TC229, serves on the Executive Committee of ASTM E56, a member from FDA for OECD WPMN and engages industry and government agency stakeholders on standards development activities relevant to the agency. Dr. Patri works with global regulatory agency stakeholders through the International Pharmaceutical Regulators Forum (IPRF) Nano working group. His laboratory conducts nanotechnology research and collaborates with several divisions at NCTR and other Centers at FDA.

Before joining FDA in August 2014, Dr. Patri served as the Deputy Director of the Nanotechnology Characterization laboratory (NCL) at the Frederick National Laboratory for Cancer Research. In a decade long tenure at NCL, he assisted collaborators from industry and academia towards clinical translation of nanomedicines intended for cancer. He led a collaborative multi-disciplinary team of scientists at NCL and oversaw 85 projects through preclinical assessment that included proposal review and guidance, material characterization, in vitro and in vivo studies on different nanomaterial platforms intended for drug delivery, gene delivery, and imaging. From 2006-2014, he served as a guest scientist at NIST and helped co-develop the first Nanosized gold reference material standards. Dr. Patri served at the University of Michigan Medical School, Center for Biologic Nanotechnology, and developed targeted drug delivery and imaging agents until 2005. He is a co-author of over 65 publications, serves on editorial boards of Molecular Pharmaceutics, Nanomedicine & Nanobiotechnology, and has organized many meetings and conferences.

Dr. Patri earned a PhD degree in Chemistry from the University of South Florida followed by a post-doctoral training at the University of Michigan. He worked at Astra Zeneca and as a lecturer in Chemistry before graduate school.

**Abstract: Overview Introduction to Nanotechnology Research at FDA**

The global advance in nanotechnology research is resulting in a gradual increase in novel nanomaterial- containing medical, food, and consumer products. To keep pace with these rapid developments, the US FDA established the Nanotechnology Task Force (NTF) to identify knowledge gaps, conduct research, train reviewers, develop guidance documents, and enable collaborative standards development with industry to facilitate responsible development of these much needed technologies. FDA engages other government agencies through the National Nanotechnology Initiative for nanotechnology-related activities, and with industry through Standards Development Organizations. This presentation will provide an overview of nanotechnology research and the advanced infrastructure facilities available to conduct research relevant to products regulated by FDA.

**Mary Boudreau, PhD**

*Research Toxicologist, NCTR*

**Bio**: Mary D. Boudreau has BS and MS degrees in Human Nutrition and a PhD in Veterinary Medical Sciences, Toxicology. She serves as a senior research toxicologist in the Division of Biochemical Toxicology within FDA's National Center for Toxicological Research, where she performs fundamental and applied research activities. In this capacity, Dr. Boudreau has served as principal investigator for six National Toxicology Program funded projects.

She plans, implements, and manages all phases of research for projects that are focused on evaluating the biological effects of exposure to a wide variety of disparate, yet potentially toxic agents; defining the complex mechanisms that govern their toxicity; understanding critical biological events in the expression of their toxicity; and developing methods to improve assessment of human exposure, susceptibility, and risk. The test agents may include, but are not limited to, natural and synthetic dietary supplements, natural and synthetic chemicals, nanoscale materials, and topical and ingested drugs. Dr. Boudreau integrates her expertise in toxicology, physiology, nutritional biochemistry, and photobiology and provides professional leadership and direction for a broad, coordinated program that focuses on critical mission-related activities and issues.

Dr. Boudreau prepares scientific literature reviews, research proposals, technical reports, and collaborates with other FDA scientists, other Federal agencies, and contractors as appropriate. She is a recognized expert in toxicology and photobiology, and is sought by peers and others to discuss research matters and serve in various advisory and leadership roles. These interactions include discussions with representatives from government agencies, scientific and academic societies, industry investigators, and the international scientific community.

**Abstract: The Safety of Nanomaterials Using Silver Nanoparticles as an Example**

In the 16th century, Paraclesus stated that "All things are poison and nothing is without poison", and this statement is as true today as it was then. Nanoscale materials hold great promise for products that perform better and improve the quality of life, but safety is not an inherent property of nanoscale materials.

New consumer products containing nanoscale material enter the market at a rate of 3 – 4 per week in the form of cosmetics, food packaging, detergents, and others. The safety or toxicity of nanoscale materials to humans and the environment depends on the potential exposure concentrations at which the materials will be used, as well as the specific chemical composition, coating material, size, and morphology of the material.

Metal nanoparticles are among the most widely used nanoscale material, and silver nanoparticles (AgNP) have a greater market share than other metallic nanoparticles. The increasing number of food, cosmetic, and medical applications of AgNP and the general lack of toxicological and pharmacological data raised public health safety concerns within FDA and prompted the nomination of AgNP for studies under the National Toxicology Program.

A focus of this presentation will be the NTP studies that were conducted at NCTR and the approaches that were used to address the safety of silver nanoparticles (AgNP). The presentation will address the selection of exposure assessments (route of exposure, medium, and monitoring), methods used for AgNP characterization (assessment of available analytical methods, limitations, physico-chemical properties), exposure conditions (dose administration and measurements), and the results from the NTP studies and other research collaborations on AgNP at FDA's NCTR.

### Katherine Tyner, PhD

*Acting Associate Director of Science, CDER*

**Bio**: Dr. Katherine Tyner is the Associate Director of Science (acting) in the Immediate Office of the Office of Pharmaceutical Quality (OPQ), Center for Drug Evaluation and Research at the Food and Drug Administration (FDA). As Associate Director, Dr. Tyner leads the OPQ Science Staff in coordinating the intersection between science, review and policy in OPQ as well as facilitating interactions between other CDER offices and FDA Centers. She received her PhD in Chemistry from Cornell University and joined FDA in 2007 as a chemist specializing in nanotechnology. While at FDA, Dr. Tyner has investigated the quality, safety, and efficacy of drug products containing nanomaterials, and she currently leads the CDER nanotechnology working group and is active in other CDER and FDA nanotechnology initiatives. Dr. Tyner is the author of multiple book chapters and journal articles concerning the appropriate

characterization and biological impact of nanoparticle therapeutics.

**Abstract: Drug Products Containing Nanomaterials**

In recent years there has been an increased focus on developing drug products containing nanomaterials. With this increased focus, there has been a corresponding increase in applications for drug products containing nanomaterials to FDA submitted for Agency review. Although subject to the same rigorous regulatory standards as any other drug product, unique properties that arise from the small size and large surface area of nanomaterials may lead to additional scientific considerations when following current FDA guidelines and practices. Such considerations may extend to determining the correct analytical techniques to characterize and control the drug product. This presentation will discuss the trends of drug products containing nanomaterials seen to date by FDA, scientific considerations, and current regulatory perspectives.

**Peter Goering, PhD**

*Research Toxicologist, CDRH*

**Bio**: Dr. Goering is a Research Toxicologist and serves as Deputy Director of the Division of Biology, Chemistry and Materials Science, Office of Science and Engineering Laboratories within the Center for Devices and Radiological Health. His research and regulatory science contributions have focused on nanotechnology/ nanotoxicology, evaluating liver and kidney toxic injury, elucidating new biomarkers of toxicity, and understanding mechanisms of metal toxicity.

The division's nanotechnology research is aimed to address key challenges associated with the evaluation of nanomaterial characterization and toxicity for biocompatibility testing, such as test sample preparation, interference with biochemical assays, dosimetry, size, cell uptake and intracellular localization of particles, clinically relevant in vitro and in vivo models, and developing health risk assessment approaches for exposure to nanomaterials.

A second goal is to understand how nano-engineered surfaces and coatings on medical devices influence biological responses of cells, tissue, and proteins. The information guides development of non-clinical models that facilitate and improve understanding the safety and efficacy of FDA regulated products incorporating nanotechnology. Dr. Goering currently serves as CDRH liaison to the ASTM E56 standards committee on Nanotechnology: Physical, Chemical, and Toxicological Properties. He has co-authored over 100 peer-reviewed publications and book chapters, is a Diplomate of the American Board of Toxicology, and a Fellow of Academy of Toxicological Sciences. He served as President of the Society of Toxicology in 2015-16.

**Abstract: Nanotechnology and medical devices: A regulatory science approach involving research, standards, and risk assessment**

Because of their unique properties, engineered nanoscale materials and structures can impart desirable characteristics to medical devices, and are currently used in bone void fillers, dental resins, antimicrobial coatings on wound dressings and catheters, medical imaging contrast agents, and in vitro diagnostic devices. Nanotechnology encompasses two distinct forms in medical devices: 1) discrete nano-objects, e.g., nanoparticles, and 2) immobilized surface nanostructures.

A growing trend in the medical device industry is to engineer device surfaces with nanoscale features to promote desirable responses from surrounding tissues, such as superior integration (orthopedic and dental implants), and faster regeneration (tissue engineering medical products) and repair (skin, airway, bladder and hernia repairs). CDRH has developed an integrated program of laboratory research, guidance and standards development, and health risk assessment to enhance regulatory decision-making for devices incorporating nanotechnology. We developed a comprehensive research paradigm for evaluation of medical devices incorporating nanoparticles and nanostructures, including fabrication of prototypic nanostructured surfaces and characterization of their physical-chemical properties and cell responses. We

address safety concerns by characterizing the release of nano-objects from devices, such as silver nanoparticles from anti-biofouling coatings; by evaluating biological testing issues, such as test sample preparation, assay interference, dosimetry, and cell uptake; and by developing clinically relevant in vitro and in vivo models.

Findings from these studies translate to international consensus standards and guidance documents for nanotechnology. In spite of results from empirical research, uncertainties exist related to potential health risks of nano-objects released from medical devices. Therefore, using a risk assessment approach, we established a provisional tolerable intake exposure level, i.e., an amount released from a device that does not pose appreciable harm to human health, for products incorporating silver nanoparticles. In summary, the CDRH nanotechnology program of research, standards, and risk assessment has positioned CDRH to enhance review of nanotechnology product submissions.

**Indira Hewlett, PhD**

*Laboratory Chief, CBER*

**Bio**: Indira Hewlett, PhD, is chief of the Laboratory of Molecular Virology in CBER at FDA. Dr Hewlett received her PhD from the State University of New York at Albany and completed postdoctoral fellowships at the Imperial Cancer Research Fund in London, UK and the NCI, NIH in Bethesda, Maryland. She joined CBER in 1985 as Senior Staff Fellow in the Division of Virology to initiate a program on HIV/AIDS.

In 1987, she became Chief of the Genetics Section in the Lab of Retrovirology of the Division of Blood and Blood Products and Chief of the Laboratory of Molecular Virology in the Division of Emerging and Transfusion Transmitted Diseases of the Office of Blood in 1992, a position she has held until the present time.

Her major research focus has been HIV/AIDS and recently, the field of nanotechnology diagnostics as it relates to pathogen detection. Dr. Hewlett's group is investigating the molecular evolution of HIV and the diagnostic and pathogenic significance of HIV genetic diversity by characterizing novel and diverse HIV strains from blood banks in Cameroon, a hot spot for new variants.

In the field of nanotechnology diagnostics, her group is investigating nanoparticles, biosensors and other nanotechnology and microarray approaches to further enhance sensitivity and simplify assay formats for rapid point-of-care testing for a variety of blood- borne and biodefense pathogens including HIV and pandemic influenza. She has received several outside and internal grants to support her research program.

At FDA, Dr. Hewlett's group participates in the review and licensure of HIV tests for screening blood donors and for diagnostics. Dr. Hewlett has received numerous awards in recognition of her contribution to public health research and blood safety.

*Abstract:*
*Indira K. Hewlett, Laboratory Chief, CBER, FDA, Silver Spring, MD; Jikun Liu, CBER/FDA, Silver Spring, MD; Mohan Haleyur Giri Setty, CBER/FDA, Silver Spring, MD; Jikun Liu, CTP/FDA, Alex Sposito, CBER/FDA, Jiwen Zheng, CDRH/FDA; Jiangqin Zhao, CBER/FDA, Silver Spring*

In vitro tests used for blood donor screening and all HIV and human retroviral diagnostics are regulated by FDA's Center for Biologics Evaluation and Research (CBER) During the past few decades nanomaterials have found a myriad of applications in healthcare through their use in devices for collection, processing and storage of blood, and in vitro diagnostics for screening of blood donations and patient diagnosis (e.g. fluorescent, metal nanoparticles, etc.). Materials at the nano-scale have different physico-chemical properties than conventional materials and therefore merit further investigation for clinical application.

Nanoparticles as novel nanomaterials are particularly attractive as probes or reaction substrates for rapid, ultrasensitive and multiplex detection of bio-analytes due to their unique physiochemical properties. Our laboratory in CBER has shown that nanoparticle

probes provide detection sensitivity limits in the sub-pg/mL range for the detection of HIV, influenza and anthrax lethal toxin antigens and low copy number detection of genomes of HIV and influenza. Further, the use of nanomaterials also facilitated the adaptation of assays to point-of-care formats such as microfluidic devices while maintaining good sensitivity and specificity.

However, accuracy and reproducibility of results may be significantly affected by batch-to-batch consistency of nanomaterials and the bio-conjugation process. This presentation will focus on research being performed at CBER to provide insights on the usefulness of various nanoparticles to improve sensitivity and adaptability to point-of-care and the impact of variations in nanomaterial preparation and bio-conjugation on assay performance. These studies will help identify possible issues in nanomaterial preparation and establish quality control processes to improve quality of nanoparticle based in vitro devices for pathogen detection.

*Disclaimer:*

This presentation reflects the views of the authors and should not be considered as representing FDA's views or policies.

**Timothy Duncan, PhD**

*Research Chemist, CFSAN*

**Bio**: Timothy Duncan received his undergraduate degree in chemistry in 2000 from Haverford College, PA. He earned his PhD in physical/inorganic chemistry in 2006 from the University of Pennsylvania, where he synthesized and studied the photophysical properties of conjugated porphyrin arrays designed for medical diagnostic and optoelectronic applications under Professor Michael J. Therien. After graduation, he did a post-doc at the University of Pennsylvania in the lab of Professor So-Jung Park, where he built a single-molecule fluorescence imaging system to study the light emission properties of novel quantum-dot based bio-imaging agents and devised a new method to synthesize color-tunable conducting polymers. Since 2009, he has been a research scientist at the FDA's Division of Food Processing Science and Technology, where he studies the potential exposure to nanomaterials from food contact materials and the development of nanotechnology-enabled sensors for food safety.

**Abstract: Potential exposure to nanoparticles from nanotechnology-enabled food contact materials**

Polymer nanocomposites (PNCs) are materials in which nanoscale fillers are dispersed within a polymer host matrix. Despite excitement surrounding potential applications, there is a desire for additional information on whether the embedded nanofillers may become released into the nearby environment during product lifecycles. Our group adopts a bottom-up experimental strategy to study potential exposure pathways that relies on well-characterized model systems, which are designed to investigate specific relationships between nanofiller characteristics and release kinetics. Here we present several model systems developed in our laboratory based on semiconducting quantum dots or other nanofillers. We incorporated well-characterized nanofillers into appropriately chosen polymers by melt compounding and immersed the resulting model PNCs in liquid media intended to simulate the properties of certain foods or environmental media. Using chemical and materials analysis techniques, our model systems provide a different view of the effect of the external medium chemistry, environmental conditions, and particle size/composition on release phenomena, as well as draw fundamental conclusions about primary release mechanisms. This work shows that model systems offer a powerful experimental compliment to the use of commercial PNCs when it comes to lifecycle analysis of nanotechnology-enabled materials.

## Closing Remarks

**Carol Linden, PhD**
Director, Office of Regulatory Science and Innovation, Office of the Chief Scientist

# Poster Session 1 (Day 1, AM)

Scientific Topic: Identification and Evaluation of New Biomarkers

1. **Antibody microarray analysis of protein level changes in an in vitro blood-brain barrier model following exposures to silver-nanoparticles: focusing on apoptosis signaling proteins**

*Authors: Gu, Qiang, FDA/NCTR; Lantz, Susan, FDA/NCTR; Cuevas, Elvis, FDA/NCTR; Ali, F. Syed, FDA/NCTR; Kanungo, Jyotshna, FDA/NCTR; Paule, G. Merle, FDA/NCTR; Zhang, Yongbin, FDA/NCTR (now FDA/CDER); Krauthamer, Victor, FDA/CDRH*

Abstract:

Microarray experiments are a centerpiece of post genomics life sciences and the current efforts to develop systems diagnostics for personalized medicine. In the present study, antibody microarrays were utilized to detect proteomic changes in rat micro-vessel endothelial cells (rMVECs), an in vitro model of the blood-brain barrier, following exposure to nanoparticles. When cells became confluent, typically two weeks post-seeding, they were exposed to various concentrations (0.01 – 50 ug/mL) of 20 nm diameter citrate-coated silver nanoparticles (AgNPs). The physiochemical properties of the AgNPs were characterized using transmission electron microscopy and dynamic light scattering. The dose-dependent cytotoxic effects of these AgNPs were determined using lactate dehydrogenase, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, and FluoroJade-C assays. Based on the cytotoxicity profile, a toxic dose of AgNPs (10 ug/ml) was applied to rMVEC cultures for subsequent proteomics analyses. After 24-hours of treatment, proteins were extracted from AgNP-treated and control cultures and relative protein levels were quantified using antibody microarrays that targeted 1,358 proteins from a variety of biological signaling pathways. Our initial focus was on apoptosis signaling pathways because of the known cytotoxic effects of AgNPs. Among the two-dozen apoptosis pathway-associated proteins examined, fourteen were significantly down-regulated while three showed significant up-regulation, indicating that these proteins may play an important role in AgNP-induced toxicity. To further confirm these antibody microarray results, seven rat protein-specific antibodies were selected and used for capillary electrophoresis based immuno-blot analyses. The results confirmed that AgNP-exposure induced changes in the levels of expression of these proteins which include BAD, BAX, caspase 2, caspase 9, cytochrome C, I-kappa-B-alpha, and MCL-1. The changes in expression of apoptosis-associated proteins may represent molecular signature biomarkers of AgNP-induced cytotoxicity. Identifying such proteins should further elucidate the molecular mechanisms associated with nanoparticle-induced cytotoxicity and aid in the effective characterization and regulatory review of potential toxicities following human exposure to nanomaterials.

Supported by NCTR Protocol E0746001.

2. **Detection of hypercoagulant condition caused by elevated prothrombin with a thrombin generation assay**

*Authors: Chang, William, FDA/CBER; Machlus, Kellie, University of North Carolina at Chapel Hill/Department of Pathology and Laboratory Medicine; Wolberg, Alisa, University of North Carolina at Chapel Hill/Department of Pathology and Laboratory Medicine; Ovanesov, Mikhahi, FDA/CBER*

Plain Language Synopsis: High prothrombin levels are associated with blood clot risk. The thrombin generation assay may be usable to detect high prothrombin. We reanalyzed data from a published experiment to find a new way to separate high prothrombin samples from other high thrombin activity samples. This holds potential for future clinical use.

Abstract:
Hypercoagulability resulting from elevated prothrombin is associated with increased thrombosis risk. Patients with elevated prothrombin include those with the G20210A mutation that represents the 2nd most common risk factor for venous thrombosis in European Caucasians. We hypothesized that the commercial thrombin generation assay (TGA), which measures the rate of synthetic thrombin substrate consumption, may detect procoagulant condition caused by elevated prothrombin.

Normal pooled plasma was treated with prothrombin or other coagulation factors to increase their levels to 200% or 400% of normal. TGA was performed using the Calibrated Automated Thrombogram (CAT) platform (Stago, USA) and analyzed with and without correction for substrate consumption. As expected, addition of elevated coagulation factors resulted in elevated TGA as measured by thrombin peak height (TPH) and endogenous thrombin potential (ETP). ETP allowed detection of up to 83% of spiked samples with 200% prothrombin but TPH has failed to distinguish these samples from the procoagulant samples obtained with other coagulation factors.

For samples with 400% prothrombin, CAT failed to return values for TPH for 40% of samples and ETP for 100% of samples but the remaining 60% of TPH values were detected as the procoagulant samples. By analyzing the raw fluorescence data directly, we found that elevated prothrombin, but not other coagulation factors, resulted in thrombin substrate depletion which allowed us to correctly distinguish all samples within our dataset regarding whether or not they had added prothrombin.

We conclude that the commercially available TGA has limited utility in distinguishing samples with elevated prothrombin but additional analysis of data can address this problem. With this additional analysis, TGA may be developable into new point-of-care test to diagnose patients with hypercoagulant conditions undetectable by currently used clotting assays.

### 3. Novel Gametocyte Biomarkers for Detection of the Plasmodium falciparum Infectious Reservoirs

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Plain Language Synopsis: We have identified a novel biomarker on Plasmodium falciparum gametocyte stage parasite that may allow superior detection of reservoirs of malaria who do not have any clinical symptoms, but are still capable of transmitting malaria in mosquito vectors.

Abstract:
Eradication efforts as well as the optimal use of transmission-reducing malaria interventions requires further knowledge of the submicroscopic infectious reservoirs among asymptomatic individuals. Even sub-microscopic levels of Plasmodium falciparum gametocytes can be infectious to mosquitos and promote onward transmission. Most efforts to identify gametocyte carriers rely upon PCR amplification of the gametocyte-specific transcript Pfs25, a sexual stage antigen. We have utilized gene expression profiling microarrays of blood stage and gametocyte stage P. falciparum parasites to identify potentially novel gametocyte-enriched transcripts that could be sensitive biomarkers for gametocyte detection in asymptomatic individuals. This has led to identification of over 200 molecules that are uniquely expressed in sexual parasite stages. One candidate in particular, Pfg17, exhibited superior analytical sensitivity against a reference panel of gametocyte-spiked whole blood detecting as few as 10 gametocytes/mL of blood; in comparison Pfs25 detected only 25.3 gametocytes/mL of blood. Pfg17 also exhibited

superior clinical sensitivity; identifying 19.1% more samples among blood-film negative Ghanaian children and 40% more samples from asymptomatic adults as gametocyte positive. Cumulatively, our results suggest that Pfg17 could serve as a novel biomarker to detect asymptomatic infectious reservoirs who would be otherwise missed by the most sensitive molecular method available. Our study has also improved the repertoire of transmission stage antigens available for evaluation as candidate vaccines.

**4. MicroRNAs as potential biomarkers in live attenuated Leishmania vaccine induced protective immunity**

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Plain Language Synopsis: No licensed vaccines or donor screening tests are available against blood borne protozoan parasitic agents. Live attenuated parasite vaccines have the potential to reduce disease burden and enhance the blood transfusion safety. We identified novel biomarkers of safety and immunogenicity that enable more robust product evaluation.

Abstract:
No vaccine exists against visceral leishmaniasis. In an attempt to develop effective vaccines, we have reported extensively on the immunogenicity of live attenuated LdCen-/- mutant in animal models. Currently several vaccines and diagnostic tests for Leishmania are under development that will be evaluated by the FDA. The biomarkers that are in common use cannot predict the effectiveness of anti-leishmanial vaccines in preclinical studies. To identify biomarkers of safety and immunogenicity associated with live attenuated parasitic vaccines, we have undertaken studies using LdCen-/- parasites. We infected macrophages derived from healthy human blood donors with LdCen-/- or LdWT parasites ex vivo and compared the early gene expression profiles. In addition to altered expression of immune related genes, we identified several microRNAs that regulate important cytokine genes, significantly induced upon LdWT infection compared to LdCen-/- infection. Importantly, we found a strong induction of microRNA-21 (miR-21), which has been shown to control IL12 mRNA expression. IL12 produced by DCs is critical for priming a host protective Th1 cell response during Leishmania infection. To validate the role of miR-21 in regulating IL12 during Leishmania infection and its role in LdCen-/- mediated protection, we altered the miR-21 expression in murine DCs infected with LdWT or LdCen-/-. Silencing of miR-21 using specific inhibitors resulted in an augmented induction of IL12 in LdWT infected BMDCs, illustrating the role of miR-21 in LdWT mediated suppression of IL12. In contrast, LdCen-/- infected BMDCs, showed a strong induction of IL12, and miR-21 silencing resulted in a further increase in IL12 levels. Our studies demonstrate that LdCen-/- infection suppresses miR-21 expression, enables IL12 mediated induction of adaptive immunity including proliferation of antigen experienced CD4+ T cells and development of a Th1 immunity. Similar miR-21 mediated IL12 regulation was also observed in normal human macrophage infections with Leishmania donovani indicating that miR-21 plays a role in early IL12 mediated immunity and could be an important biomarker for LdCen-/- vaccine immunity in human clinical trials.

**5. The role of nickel in promoting Toll-like receptor 4-mediated angiogenesis using a novel technique of in vivo implantation of angioreactors**

*Authors: Snapper, Dustin, FDA/CDRH; Srivastava, Anup, FDA/CDER; Wood, Steven, FDA/CDRH*

Plain Language Synopsis: Nickel titanium alloy (nitinol) is used in a number of biomedical applications. However, nitinol can induce inappropriate blood vessel formation during wound healing responses. We have developed a method in mice that recreates aberrant blood vessel formation that will offer important insights into the biocompatibility testing of metals.

Abstract:

Nickel titanium alloy (nitinol) is used in a number of biomedical applications, including cardiac, peripheral vascular, and fallopian tube stents. There are significant biocompatibility issues of metallic implants that may be missed because humans respond to nickel ions and nano/micro sized alloy particles much differently than rodents. We have recently shown that micron size wear particles from metal on metal hips utilize the innate immune signaling Toll-like receptor 4 (TLR4). In particular, nitinol can generate nanoparticles in vivo (i.e. "wear" particles) that have not previously been studied for their potential to induce inflammation and angiogenesis. Previous studies demonstrated that nickel can induce contact hypersensitivity, dependent on the human, but not mouse TLR4, but a potential role for nickel in nitinol stent revascularization, and thus stent failure is unknown. We demonstrated that 60nm nitinol nanoparticles or nickel chloride, both shown to be free of endotoxin, induce cytokine and chemokine expression in human endothelial and monocyte cell lines in vitro that are both pro-inflammatory as well as proangiogenic, a property that might contribute to stent failure. To determine a role for nickel in inducing angiogenesis in vivo, we implanted 1 cm silicon angioreactors subcutaneously in athymic (T cell-deficient) nude mice. The implantation of angioreactors represents a novel method that allows for quantitation of angiogenesis in vivo. Angioreactors were filled with Matrigel a gelatinous protein mixture that resembles extracellular matrix, in addition to either PBS (negative control), fibroblast growth factor-2 (FGF-2) (positive control), or nitinol nanoparticles. After 15 days the angioreactors were removed and the contents were extracted and enzymatically digested for cell isolation. Cells were stained for flow cytometry using fluorochrome-labeled anti-CD31 and anti-MAdCAM-1 to quantitate endothelial cell migration into the angioreactor. In contrast to negative control, angioreactors containing FGF-2 exhibited a large infiltration of endothelial cells. Analysis of angioreactors containing nitinol nanoparticles demonstrated, as predicted, a lack of induced angiogenesis, since athymic nude mice only express the murine, not human TLR4. To further address this, future studies will utilize mice that are transgenic for human TLR4, to determine whether angioreactors containing nitinol nanoparticles induce endothelial and inflammatory cell influx. These studies will provide important information for evaluating the biocompatibility of nitinol used in various medical applications.

**6. Application of FDA High-Performance Integrated Virtual Environment (HIVE) Supercomputer to Analysis of Cardiac Safety Clinical Trials**

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Plain Language Synopsis: We present parallelizable software that runs in the HIVE supercomputer which enables automated analysis of millions of ECGs from clinical trials submitted to FDA. A dataset with 5,232 ECGs from an FDA-sponsored clinical trial was used to validate the HIVE implementation and to assess throughput differences between PC and HIVE.

Abstract:
Through an analysis of preclinical ion channel and clinical digital electrocardiograms (ECG) from 34 thorough QT clinical trials submitted to FDA and 2 FDA-sponsored clinical studies, we recently identified novel ECG biomarkers to better identify drug effects on ion channels of cardiac cells. We developed ECG analysis software to assess these ECG biomarkers automatically in clinical studies. We present a highly parallelizable version of our ECG software that runs in the High Performance Integrated Virtual Environment (HIVE) supercomputer and that will enable automated analysis of millions of ECGs from clinical trials submitted to FDA. All ECG analysis code was written in C++, and the analysis workflow was implemented using the HIVE Application Program Interface to facilitate and manage

parallelization. A dataset with 5,232 ECG signals from an FDA-sponsored clinical trial was used to validate the HIVE implementation and to assess differences in throughput (number of ECGs analyzed per second) between PC and HIVE. We assessed HIVE's throughput distributing analysis using multi-cores in subsets (average number of ECGs per core, number of cores) of (10, 200), (50, 105), (100, 53), (500, 11), (1000, 6) and (5,232, 1). Statistical analysis was done in R 3.2.3(R Foundation for Statistical Computing, Vienna, Austria). There were no differences in the results produced by the PC and HIVE versions. Parallelization of ECG analysis in the HIVE reduced total execution time required to analyze all the ECGs when compared to PC version from 30 minutes to 40 seconds. Maximum throughput of 140 ECGs per second was achieved when dividing the data in groups of 10 ECGs (i.e. 524 simultaneous processes executed in 200 cores). We plan to use the HIVE to analyze 9.5+ million ECGs from 400+ thorough QT studies submitted to FDA since 2005 to assess appropriate thresholds and performance for application in early phase 1 clinical trials. The process of parallelization and design of this analysis establishes a work stream so that this and subsequently developed algorithms can be quickly applied and compared across QT studies. Results of this analysis will allow implementing novel ECG biomarkers into regulatory review of new drugs.

**7. RNA-Seq profiling of colorectal cancers reveals increased expression of genes pertaining to inflammation and innate immunity but decreased expression of genes pertaining to cells mediating adaptive immune responses**

*Authors: Xu, Lai, FDA/CDER; Wang, Rong, FDA/CDER; Rosenberg, Amy, FDA/CDER; Wu, Wells, FDA/CBER; Phue, Jenie, FDA/CBER; Shen, Rong-Fong, FDA/CBER; Wu, Leihong, FDA/NCTR; Fang, Hong FDA/NCTR*

Plain Language Synopsis: CRC remains second leading cause of cancer death in US. It is known that the immune system has a multi-faceted role in CRC. By using next generation sequencing (NGS), we found that immunologically relevant genes are differentially expressed in CRCs and have the potential to be used as CRC biomarkers.

Abstract:
CRC remains a major public health challenge worldwide. Depicted as a multi-step evolving disease, CRC develops slowly over several years and progresses through histologically distinct states, from single crypt lesions through adenoma, to malignant carcinoma with the potential for invasion and metastasis. It has been recognized that inflammation and immunity actively participate in the pathogenesis, and progression of CRC. Advances in immunology and molecular biology have shown that CRC can be immunogenic and that the host immune response influences survival. Since next generation sequencing (NGS) has a number of advantages over hybridization-based techniques, such as annotation-independent detection of transcription, improved sensitivity and increased dynamic range, we used NGS to explore the differential expression of immune related genes between 79 colorectal cancers and 79 healthy patient matched colonic tissues. We found that CXC chemokines, IL1B, IL6, and tumor necrosis factor superfamily members (TNFSFs) which promote inflammation were upregulated, and CCL chemokines and receptors, IL6R, IL16, IL18, and tumor necrosis factor receptor superfamily members (TNFRSFs) were downregulated in the majority of CRCs. We further found that cell surface markers of neutrophils, myeloid-derived suppressor cells (MDSCs), and macrophages were upregulated, while cell surface markers of T and B cells were downregulated in the majority of CRCs. Further analysis of genes pertaining to natural killer cells (NK), dendritic cells (DC), and immune checkpoint (ICP) cell surface markers, revealed that CRCs can be classified into 3 molecular subtypes regardless of their histological stages and grades: subtype I (60% of cohort) had upregulation of NK, DC and ICP surface markers compared to normal samples; subtype II (25% of cohort) had downregulation of NK, DC and ICP surface markers compared

to normal samples; and subtype III (15% of cohort) exhibited an expression profile of NK, DC and ICP surface markers similar to that of normal samples. These immune subtypes could potentially provide clear immunological interpretability and the basis for targeted interventions. Our study provides a detailed portrait of the immunologic content of the tumor microenvironment and will facilitate the understanding of the role played by innate and adaptive immune system in the local progression and metastasis of CRC.

**8. Tumor-cell resistance to death receptor 5-induced apoptosis through persistent expression of the intermediate filament protein keratin 8**

*Authors: Bozza, William, FDA/CDER; Zhang, Yaqin, FDA/CDER; Luo, Shen, FDA/CDER; Zhang, Baolin, FDA/CDER*

Plain language synopsis: This project aims to identify biomarkers to predict tumor response to death receptor targeted therapies. The anticipated results could facilitate the development of safe and effective cancer therapies.

Abstract:
Therapeutic targeting of death receptors (DRs) 4 and 5 can selectively induce apoptosis in cancer cells without harming most normal cells, making them attractive targets for cancer treatment. The DR4/DR5 apoptosis pathways can be activated through the use recombinant TNF-related apoptosis inducing ligand (TRAIL) or receptor agonistic antibodies. In early phases of clinical trials these agents were shown to be well tolerated but their therapeutic effects were limited likely due to the emergence of tumor resistance. This project aims to identify biomarkers that can predict tumor response to DR4/DR5 targeted therapies. Using a combination of molecular and proteomic approaches, we found that the intermediate filament protein keratin 8 is a key determinant of breast cancer cell resistance to DR5 mediated apoptosis. The protein levels of keratin 8 are consistently higher in TRAIL resistant cells compared to TRAIL sensitive cells in a panel of breast cancer cell lines. Keratin 8 appears to negatively regulate the surface expression of DR5 as targeted knockdown of keratin 8 restored surface expression of DR5, and as a result, sensitized TRAIL-resistant cells to TRAIL-induced apoptosis. Together these results suggest that upregulation of keratin 8 could be a predictor of tumor resistance to DR5-targeted therapies.

**9. Quantitative biomarkers for brain injury based upon sensory-evoked electroencephalography and diffusion correlation spectroscopy**

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Plain Language Synopsis: Quantitative diagnostic "gold standards" for detecting and assessing TBI are generally lacking. We explored candidate biomarkers that could be measured noninvasively through portable device technology. Using infrared optical and electroencephalographic monitoring techniques, we observed significant alterations in sensory-evoked cerebral blood flow and electrical neural signals following injury.

Abstract:
Traumatic brain injury (TBI) is a leading cause of death and disability in persons under the age of 45 years. Quantitative biomarkers or diagnostic "gold standards" for detecting TBI, however, are generally lacking. Clinical imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) can provide quantitative and anatomically specific data, however mild TBI (mTBI) is more difficult to detect. Also, clinical imaging modalities are costly and not available in active field settings. Recently, we found that cortical somatosensory evoked electroencephalographic potentials (SSEPs) can be used to detect and track acute brain injury in a mouse model [1]. Evoked hemodynamic and metabolic responses to the same somatosensory stimuli, which have been recorded using non-invasive near

infrared (NIR) optical techniques, are also potential biomarkers. Although EEG and NIR optical techniques have been performed in isolation of each other, the two techniques measure signals—electrical activity and the ensuing hemodynamic and metabolic response—that jointly report on the integrity of the neurovascular unit, the dynamic coupling between individual neurons and nearby microvasculature. We sought to explore whether a correlate of these injury-induced alterations in sensory activation could be detected at the level of local cerebral blood flow (CBF). Diffuse correlation spectroscopy (DCS) is an emerging portable diffuse NIR modality that has been used for measurements of CBF [2]. Among other optical techniques, DCS has the unique advantage of sensing primarily CBF in microvasculature. We explored potential sensory-activated CBF biomarkers in vivo by performing continuous DCS measurements during somatosensory stimulation of the median nerve in mice following controlled cortical impact (CCI). CCI acutely reduced the peak amplitude of stimulus-evoked CBF responses as well as SSEPs. These signals gradually recovered to baseline amplitude values within 30 min, although the amplitude of the CBF responses appeared to recover to pre-injury values more rapidly compared with SSEPs. These preliminary results suggest that combined measurements of somatosensory-evoked CBF and SSEPs can jointly offer new quantitative metrics for brain injury.

[1] J. Fisher et al., IEEE Trans. Neural Sys. Rehab. 24, 1003 - 1012 (2016).

[2] T. Durduran et al., NeuroImage 85, 51 – 63 (2014)

## 10. Identification of Predictive Biomarkers of Doxorubicin-induced Clinical Cardiotoxicity

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Plain Language Synopsis: Drug-induced heart injury is a major concern to medicine. Protein and metabolite analysis technologies were used in this study to identify blood biomarkers that could predict heart injury induced by chemotherapy. The data has the potential to identify susceptible individuals and to identify opportunities to prevent heart injury.

Abstract:
Doxorubicin (DOX) is a commonly used anti-cancer agent known for its cardiotoxicity; its use may lead to irreversible cardiomyopathy and heart failure. Currently, there are no diagnostic tools for detection of pre-symptomatic DOX-induced cardiotoxicity; conventional blood-based biomarkers such as cardiac troponin T (cTnT) and I (cTnI) are limited to the identification of nascent drug-induced cardiotoxicity. To identify potential biomarkers for prediction of symptomatic cardiotoxicity, 34 breast cancer patients were enrolled for treatment with a combination of DOX (Adriamycin, 60 mg/m2) and cyclophosphamide (600 mg/m2). Cardiac function of all subjects was assessed by a multigated acquisition (MUGA) scan before the start of DOX treatment and at completion of four cycles of chemotherapy. MUGA scan analysis of breast cancer patients revealed that 24 maintained normal left ventricular ejection fraction (LVEF), 5 presented LVEF decline by 5–10% (moderate), and 5 presented LVEF decline by >10% (severe, or left ventricular dysfunction, LVD) at the completion of DOX treatment. SOMAscan-based proteomic analysis of subject plasma before (baseline), during and after DOX treatment identified 90 proteins from the group with moderately decreased LVEF that had differential baseline levels as compared with the normal patient group, while severely decreased LVEF (LVD group) had 132 proteins whose baseline abundance differed from normal subjects (fold change $\geq 1.2$ and $p < 0.05$). Compared with the normal LVEF group, 64 and 37 proteins changed abundance in the moderately decreased LVEF group, while 41 and 58 proteins changed in abundance in the LVD group, after the 1st and 2nd cycles of chemotherapy, respectively. Metabolomic analysis using LC-MS revealed that plasma pyroglutamate and lysophosphatidylcholine (16:0) increased in the group of patients with

LVD (p<0.05) while plasma docosahexaenoic acid and taurocholic acid decreased in both the moderate and LVD groups (p<0.05) after the second cycle of chemotherapy. The data suggest that differential abundance of proteins at the baseline levels or after early cycles of DOX treatment could be useful biomarkers to predict cardiotoxicity. Protein and metabolite changes after DOX treatment could also be associated with the mechanism of cardiotoxicity development, especially the change of proteins that were associated with LVD.

### 11. The Utility of Urinary Glycosaminoglycans as a Predictor of Clinical Response in Mucopolysaccharidosis Trials

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Abstract:
Urinary glycosaminoglycans (uGAG) is often measured as an initial diagnostic in mucopolysaccharidosis (MPS) disorders. Many sponsors frequently include uGAG as a biomarker of treatment response in clinical trials. In the development programs of 4 approved enzyme replacement therapies (ERT) for 4 distinct MPS types (I, II, IVA, and VI), substantial reduction in uGAG levels following ERT were observed in all treated patients. However, the relationship between uGAG reduction and clinical response to ERT in MPS is not established, making its validity as a surrogate endpoint uncertain. In this study, the relationship between uGAG reduction and an important clinical outcome measure, improvement in 6-minute walk distance (6MWD), was evaluated.

Exploratory analyses consisted of investigation of baseline patient characteristics including uGAG levels and 6MWD, and changes from baseline following ERT. Covariates evaluated through regression analyses were age, sex, geographical region, treatment arm, baseline uGAG, and baseline 6MWD. Specifically, logistic regression was used to assess the relationship between uGAG reduction and improvement in 6MWD. Concordance statistics were calculated to assess the discriminative ability of the change in uGAG in predicting 6MWD.

Subjects' age at enrollment was significantly associated with baseline uGAG levels in all the programs and the baseline uGAG levels strongly correlated with magnitude of uGAG reduction in all active treatment arms (p <0.001). In a 53-week trial of Elaprase in MPS II, the odds of achieving ≥ a 30-meter increase in 6MWD was significantly greater for subjects with ≥ 45% reduction in uGAG than those with < 45% reduction [OR=4.4; 95%CI:1.9-10.7]; the C-statistic was 0.68. In trials of Aldurazyme in MPS I (26 weeks) and Vimizim in MPS IVA (24 weeks), the relationship between the magnitude of uGAG reduction and improvement in 6MWD was weak (respectively, [OR=1.9; 95%CI: 0.5-6.6], and [OR=1.2; 95%CI: 0.6-2.3]).

In conclusion, baseline uGAG levels appear predictive of magnitude of uGAG reduction following ERT for MPS. A consistent relationship between uGAG reduction and 6MWD could not be established; however, the impact of length of ERT treatment on this relationship is unclear and should be among the factors to be evaluated in further studies.

### 12. Impact of imaging protocol and gender-associated anatomy on coronary calcium scoring with CT: an anthropomorphic phantom study

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Plain Language Synopsis: Computed tomography (CT) imaging protocols and patient characteristics can impact the accuracy and precision of the calcium score and differences from established standards may lead to

unreliable patient treatment recommendations. This work investigates the impact of CT imaging protocols and gender characteristics on coronary artery calcium scoring.

Abstract:
Computed tomography (CT) imaging protocols and patient characteristics can impact the accuracy and precision of the calcium score and differences from established standards may lead to unreliable patient treatment recommendations. This work investigates the impact of CT imaging protocols and gender characteristics on coronary artery calcium scoring.This work investigates the impact of scanning mode, reconstruction algorithm and gender characteristics on coronary artery calcium scoring based on a phantom study using CT. Four synthetic heart vessels of different sizes (representing sizes of average female/male left and left circumflex arteries) with calcification deposits of varying sizes and densities were inserted into an anthropomorphic thorax phantom, and were scanned with and without synthetic female breast plates. Ten repeat scans were acquired in both single- and dual-energy mode and were reconstructed using filtered-backprojection (FBP), iterative algorithm with medium and strong denoising options. Agatston and calcium volume scores were estimated for each vessel. Total calcium score (summation of the four vessels), and male/female calcium scores (summation of the two male/female-specific vessels in the appropriate male/female phantom scan) were calculated and compared accordingly. Results showed that Agatston score and calcium volume scores were consistent between single- and dual-energy scans. The calcium scores were significantly larger for FBP reconstruction. Both gender-based anatomical differences and vessel size were significant factors based on ANOVA analysis. The calcium scores tended to be underestimated when the vessels were smaller, scanned in female phantom or reconstructed using IR algorithms.

**13. Investigation of longitudinal electrophysiological, neuroinflammatory, and functional biomarkers for mild blast brain injury induced by high-intensity focused ultrasound in mice**

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Plain Language Synopsis: We developed a novel blast brain injury model using high-intensity focused ultrasound in mice. The injury was characterized by postmortem histology and behavioral tests. Longitudinal electrophysiological signal changes were identified following the injury. The electrophysiological signatures can potentially serve as biomarkers for the detection of mild brain injury.

Abstract:
Traumatic brain injury (TBI) is a complex and often chronic disease that affects a diverse population. Despite the high incidence of TBI, with 2.5 million TBI cases reported in 2010, there exist no widely-used devices or drugs for diagnosis or treatment. This is due, in part to a lack of reliable biomarkers to identify and monitor this multi-factorial chronic condition. Mild TBI (mTBI), such as concussion, accounts for about 80% of all TBI and remains particularly enigmatic due to the low sensitivity of CT imaging and neurologic clinical assessment. In this study, we identified and validated longitudinal electrophysiological, neuroinflammatory, and functional biomarkers of mTBI in a mouse model in which high-intensity focused ultrasound (HIFU) was used to induce blast-related, non-impact mTBI. We assessed the neuroimmune response to injury through immunohistochemical examination of astrocyte reactivity (GFAP) and microglial activation (Iba-1) throughout the brain at multiple post-injury time points. Locomotor impairments resultant from mTBI were assessed using rotarod and open field tests (OFT). Lastly, chronic periodic brain activity recordings were acquired from freely moving animals with a 16-channel epidural micro-

electrocorticography (μECoG, Neuronexus) array to investigate the effect of blast waves on resting state brain rhythms. Our data revealed variable neuroimmunological responses across animals, but consistent long-term alterations in OFT performance and impaired locomotor performance on the rotarod compared to sham injured animals. Phase-amplitude coupling analysis of brain waves demonstrated increased modulation of delta (1-4 Hz) phase on beta (13-30 Hz) amplitude within 2 days after injury. Resting state brain waves showed a chronic reduction in the absolute power in delta band and in the delta/gamma (30-100 Hz) ratio from 2 days to 4 months post-injury. This change is accompanied by an increase in the broadband (1-100 Hz) mean frequency. Taken together, our results suggest that although mTBI may not elicit strong neuroinflammation, it produces consistent impairments in motor behavior accompanied by acute and chronic disruptions in spontaneous brain activity. Thus, behavioral tests and monitoring quantitative electrophysiological indices (e.g. qEEG) may be more sensitive outcome measures for mTBI in non-clinical settings, and have the potential to serve as diagnostic biomarkers for mTBI.

### 14. Analysis of IL13 signaling through IL13R alpha2 in human brain tumor specimens in situ

*Authors: Rukmini Bhardwaj; Akiko Suzuki; Pamela Leland; Bharat H. Joshi; Raj K. Puri*

Abstract:
Previously, we demonstrated that IL-13 receptor alpha2 (IL-13Ralpha2), a high affinity receptor for Th2 cytokine IL-13, is overexpressed in glioblastoma multiforme (GBM) cell lines and the patient-derived tumors. We targeted IL-13Ralpha2 by many immunotherapeutic agents including chimeric antigen receptor modified T (CAR-T) cells, targeted lenti and adenoviral vectors, and a chimeric fusion immunotoxin consisting of IL-13 and truncated Pseudomonas exotoxin (IL-13-PE). However, the signaling mechanism by IL-13 through the IL13Ralpha2 in GBM is not well known. We recently observed that IL-13 signals through IL-13Ralpha2 by activating AP-1 transcription factors in human brain tumor cell lines. Herein, we examined IL-13Ralpha2 expression in human brain tumors and normal brain specimens, and the AP-1 signaling in situ. Using six human glioblastoma and three astrocytoma specimens, we studied the AP-1 transcription factors by IHC and compared with three normal brain specimens. GBM specimens showed a high degree of immunostaining for c-Fos, c-Jun, Jun D and Fra-1 (AP-1 family of transcription factors) and a high percentage of positive fields. These specimens also showed strong immunostaining for IL-13Ralpha2 in >70% fields ($P<0.001$). The three astrocytoma specimens showed less intense staining for IL-13Ralpha2 (>2+ and 32% fields), than GBM. Similar to IL-13Ralpha2 expression, the extent of staining and percentage of positive fields for AP-1 transcription factors were statistically significant in both GBM and astrocytoma compared to normal brain. The extent of immunostaining in GBM was highest for c-Fos (4+, 78% fields) followed by c-Jun (3+, 57% fields), Fra-1 (2+, 70% fields) and Jun-D (2+, 28% fields). Jun-B expression was the lowest among the AP-1 transcription factors (<1+, 7% fields) in GBM. Astrocytoma specimens showed less immunostaining for AP-1 members compared to GBM; c-Fos showed 2+ staining and 42% positive fields followed by c-Jun (2+, 12% fields), Fra-1 (2+, 48% fields) and Jun-D (<1+, 18% fields), while Jun-B intensity was <1+ in only 6% fields. In contrast, normal brain specimens showed no immunostaining for AP-1 family members. Thus, our results corroborate with data obtained from GBM cell lines and confirm that IL-13 can signal in IL-13Ralpha2 positive GBM tumors in-situ through the AP-1 pathway.

### 15. Ado-Trastuzumab Emtansine Targets Hepatocytes Via Human Epidermal Growth Factor Receptor 2 to Induce Hepatotoxicity

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Plain Language Synopsis: Ado-trastuzumab

emtansine (T-DM1) is an antibody-drug conjugate (ADC) approved for the treatment of HER2-positive metastatic breast cancer. T-DM1 consists of trastuzumab, a microtubule inhibitor DM1 and a thioether linker. In this study, we used the cellular and murine models to investigate the mechanisms by which T-DM1 induced hepatotoxicity.

Abstract:
Ado-trastuzumab emtansine (T-DM1) is an antibody-drug conjugate (ADC) approved for the treatment of HER2-positive metastatic breast cancer. It consists of trastuzumab, a humanized monoclonal antibody directed against human epidermal growth factor receptor 2 (HER2) and a microtubule inhibitor DM1 conjugated to trastuzumab via a thioether linker. Hepatotoxicity is one of the serious adverse events associated with T-DM1 therapy. Mechanisms underlying T-DM1-induced hepatotoxicity remain elusive. Here, we use hepatocytes and mouse as models to investigate the mechanisms of T-DM1-induced hepatotoxicity. We show that T-DM1 is internalized upon binding to cell surface HER2 and is co-localized with LAMP1, resulting in DM1-associated cytotoxicity, including disorganized microtubules, nuclear fragmentation/multiple nuclei, and cell growth inhibition. We further demonstrate that T-DM1 treatment significantly increases the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) in mice, and induces inflammation and necrosis in liver tissues and that T-DM1-induced hepatotoxicity is dose dependent. Moreover, the gene expression of TNFα in liver tissues is significantly increased in mice treated with T-DM1 as compared with that treated with trastuzumab or vehicle. We propose that T-DM1-induced upregulation of TNFα enhances the liver injury that may be initially caused by DM1-mediated intracellular damage. Our proposal is underscored by the fact that T-DM1 induces the outer mitochondrial membrane rupture, a typical morphological change in the mitochondrial-dependent apoptosis, and mitochondrial membrane potential dysfunction. Our work provides mechanistic insights into T-DM1-induced hepatotoxicity, which may yield novel strategies to manage liver injury induced by T-DM1 or other ADCs.

## 16. Analysis of Drug-induced ECG Changes from 70 Cardiac Safety Clinical Studies

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Plain Language Synopsis: A fatal heart arrhythmia is associated with a prolonged QT-interval on the electrocardiogram. New drugs undergo a clinical assessment of their potential to prolong QT. However, not all QT prolonging drugs cause arrhythmias. We built a map of drug-induced ECG changes that groups drugs in regions with different proarrhythmic mechanisms.

Abstract:
New drugs undergo an assessment of their potential to block the hERG potassium channel and prolong the QT-interval in the electrocardiogram (ECG) because these effects are associated with torsade de pointes (TdP), a potentially fatal arrhythmia. Recent studies have shown that a combined assessment of QT and J-Tpeak intervals can differentiate predominant hERG blockers with high TdP risk from multi-channel blockers (hERG plus late sodium or calcium current block) with low TdP risk. We analyzed drug-induced effects in QT and J-Tpeak intervals as indicators of ion channel effects associated with different TdP risk.

We used FDA ECG software to assess QT and J-Tpeak on ECGs with matched drug-concentration of 84 drugs from 70 studies. For each drug, linear prediction of placebo-corrected changes from baseline (ΔΔ) of QT and J-Tpeak at maximum drug-concentration (Cmax) was calculated. Drugs were labeled based on ion channel effects as

C1 "Predominant hERG block", C2 "Balanced hERG+late sodium/calcium block", C3 "Predominant late sodium/calcium block", C4 "No ion channel effects", and drugs with unavailable ion channel effects were labeled with C5 "unknown/unavailable". 84 drugs were categorized to 8 C1, 8 C2, 3 C3, 3 C4, and 62 C5. A two dimensional Cartesian space (map) of drugs was generated by predicted ΔΔQT and ΔΔJ-$T_{peak}$ (as X-Y axes) at Cmax.

By using a polygonal plot, C3 and C4 drugs have separate regions on the map. C1 drugs mostly fall around a positive diagonal line where ΔΔQT and ΔΔJ-Tpeak are greater-equal than 10 ms. C2 drugs generally reside under the line of ΔΔJ-Tpeak less than 10 ms, while sharing some space with C1 drugs. C4 drugs are in an area between |ΔΔQT| (absolute value) and |ΔΔJ-Tpeak| less than 10 ms. The C5 drugs are broadly distributed.

The map illustrates four distinct regions for C1, C2, C3, and C4 labels demonstrating different ion channel effects. Some drugs have a "misplaced" region which can be related to dosing regimen or unknown mechanisms of that drug. To improve the map, we are analyzing more studies, collecting ion channel data for C5-drugs and studying other ECG biomarkers to extract more information from each study.

**17. Targeting of Pyruvate Kinase M2 (PKM2) Gene Induces Subcellular Compartmentalization of PKM2 and Characterizes A Therapeutic Response in vitro and in vivo Mouse Model of Human Non-small-cell Lung Cancer**

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Plain Language Synopsis: Subcellular compartmentalization of PKM2 in cancer cells

Abstract:
Previously, we have demonstrated that the M2 isoform of pyruvate kinase (PKM2), an alternatively spliced variant, is one of the 97 genes over-expressed in patient-derived non-small- cell lung cancer (NSCLC) cell lines. Since PKM2 promotes cancer cell-specific glucose metabolism known as aerobic glycolysis or the Warburg effect, we studied the effect of targeting this glycolytic enzyme using gene silencing shRNA (shRNA-PKM2) or treatment with a small molecule inhibitor of PKM2 (SMI) and characterized the biological response.

Both methods of PKM2 targeting significantly reduced mRNA expression of PKM2, PKM2 activity, cell viability, and colony formation of NSCLC cells in a concentration-dependent manner ($P<0.001$ compared to controls). Interestingly, both methods of targeting resulted in a significant decrease in cytosolic localization of PKM2 and increase in nuclear compartment ($P<0.0012$) compared to untreated cell cultures in vitro.

Next, we developed human xenografts in athymic nude mice by implanting fast growing H1299 and slow growing H358 NSCLC cell lines and gave intratumoral injections of different doses of SMI and monitored tumor growth. We also developed xenografts from H1299 or H358 cell lines after shRNA-PKM2 knockdown. Both approaches showed a moderate anti-tumor effect in mice, which resulted in complete, partial or no response to PKM2 targeted therapy. By immunohistochemistry analysis, we observed > 70% reduction in cytoplasmic PKM2 with low or undetectable nuclear PKM2 expression in regressing tumors compared to placebo treated control tumors. In contrast, non-regressing tumors showed an opposite trend in which nuclear PKM2 expression was higher (~38%) compared to cytoplasmic PKM2 (~2.86%). Our results indicate a novel function of PKM2 in the cytoplasm and nuclei of NSCLC tumors, which may characterize the therapeutic resistance to anti-PKM2 therapy in pre-clinical models of human NSCLC.

Based on these data, we believe that subcellular localization of PKM2 may serve as a useful biomarker of therapeutic response in experimental and investigational studies involving NSCLC.

**18. In Silico Integration of Epidemiologic and Genetic Evidence Reveals Candidate**

### SNPs as Sex-dependent Modifiers of Hip Arthroplasty Outcomes

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Plain Language Synopsis: As part of CDRH's efforts for strengthening device evaluation, new evidentiary approaches are needed to individualize risk-benefit assessment and facilitate Precision Medicine. This project illustrates a novel in silico framework which is based on pre-existing epidemiologic/genetic data and is aimed at discovery of biomarkers indicative of hip arthroplasty-related adverse events.

Abstract:
Regulatory research in Translational Epidemiology and Genetics is critically important for enabling CDRH's vision to provide access to safe and effective medical devices. As part of the efforts for developing new evidentiary approaches for predictive evaluation of real-world device performance, we are developing an in silico framework that is based on re-utilization of pre-existing epidemiologic and genetic data and is aimed to identify device-related biomarker candidates.

Objectives: The current project is focused on: 1) characterizing sex/race-specific trends in the occurrence of hip arthroplasty adverse events (AE), and 2) identifying putative SNP biomarkers for improving the hip arthroplasty outcomes in sex/race-stratified subpopulations.

Methods: The Nationwide Inpatient Sample from Agency for Healthcare Research & Quality (NIS/AHRQ) was used for a retrospective analysis of hip arthroplasty related discharges identified by ICD9 codes. STATA14 was used to compare the hip arthroplasty related AE in sex/race-stratified discharges. The frequencies of these AE were then analyzed in juxtaposition to the allele distribution of some disease-related SNPs in hip arthroplasty patients, using pre-existing genetic data from PMRP/MCRF. HIVE analytics was applied for the analysis of SNP-AE associations and visualization of sex-dependent SNP/AE clusters.

Results: The NIS/AHRQ-derived epidemiologic evidence suggested a number of possible sex/race-related modifying effects, showing, for instance, that White Females have lower frequency of Osteolysis (OR=0.54 [0.50, 0.58]). The PMRP/MCRF-derived genetic evidence suggested that a higher frequency of Osteolysis in White Males vs. White Females may be associated with a corresponding 2-fold increase of the rs7121 C-allele. The HIVE clustering based heatmaps showed distinct SNP-AE subclusters in Females vs. Males with hip arthroplasty.

Conclusion: In silico discovery of biomarkers can enhance predictive evaluation of device performance in patient subpopulations, thus paving the way for cost/time-efficient implementation of Precision Medicine.

### 19. H-Ras is a potential biomarker if tumor resistance to the death receptor targeted therapy

*Authors: Kim, Su-Ryun, FDA/OBP; Zhang, Baolin, FDA/OBP*

Plain Language Synopsis: Death receptors are attractive targets for the development of novel cancer therapy. This study aim is to identify biomarkers to predict tumor response to the death receptor-targeted therapies.

Abstract:
TNF-related apoptosis inducing ligand (TRAIL) induces apoptosis through its death receptors (DRs) 4 and/or 5 expressed on the surface of target cells. Despite its selectivity in killing cancer cells over most normal cells, recombinant human TRAIL or its receptor agonists (monoclonal antibodies against DR4 or DR5) encountered resistance in many tumor cells while the underlying mechanisms remain partially understood. Here, we show that wild-type H-Ras GTPase is systemically upregulated in TRAIL-resistant cells compared to TRAIL-sensitive cells. The elevated H-Ras expression correlated with a deficiency of DR4 and DR5 on plasma membrane in TRAIL-resistant cell lines. Notably, knockdown of H-Ras in

TRAIL-resistant cells successfully restored the surface expression of DR4 and DR5, thereby sensitizing the cells to TRAIL-induced apoptosis. Consistently, ectopic expression of H-Ras in TRAIL-sensitive cells reduced surface DR4 and DR5 which was associated with a loss of TRAIL sensitivity. By contrast, the status of K-Ras or its mutations was not casually linked to TRAIL receptor expression or TRAIL sensitivity across the panel of cancer cell lines tested. These data suggest that H-Ras may play a distinct role to negatively regulate TRAIL receptors and apoptosis. The upregulated H-Ras could be a predictor of tumor resistance to DR-targeted agents and a potential therapeutic target for combinational therapy to achieve better treatment outcomes.

### 20. Identifying biomarkers to predict cardiac risk in individual patients prior to administration of anthracyclines

*Authors: Zhao, Liqun, FDA/CDER; Zhang, Baolin, FDA/CDER*

Plain Language Synopsis: Drug-induced cardiotoxicity is a major safety concern in cancer treatment. This project aims to develop innovative assays and biomarkers for assessing drug-induced cardiotoxicity.

Abstract:
Anthracyclines are highly effective anticancer agents but cause severe cardiotoxicity in many patients. Dr. Baolin Zhang's laboratory at CDER investigates doxorubicin-induced cytotoxicity using human induced pluripotent stem cells-derived cardiomyocytes (iPS-CMs) mimicking primary cardiomyocytes. We have recently found that doxorubicin and related anthracycline agents (e.g., daunorubicin, idarubicin, and epirubicin) significantly upregulated the expression of death receptors (DRs) (TNFR1, Fas, DR4 and DR5) in iPS-derived cardiomyocytes at both protein and mRNA levels. The resulting iPS-CMs cells underwent spontaneous apoptosis which was further enhanced by physiologically relevant death ligands including TNF-related apoptosis inducing ligand (TRAIL). Furthermore, TRAIL potentiated doxorubicin-induced decrease in beating rate and beating amplitude of iPS-derived cardiomyocytes. These data demonstrate that the induction of death receptors in cardiomyocytes is a critical mechanism by which anthracyclines cause cardiotoxicity. Our article describing these results has received accelerated publication in Scientific Reports. Based on these data we propose that patients who are associated with high baseline serum levels of the predefined TNF cytokines would be at high cardiac risk to anthracycline treatment. We are currently working to validate the cardiotoxicity biomarkers through collaborations with outside cancer institutions.

### 21. Design of a Novel Loop-Mediated Isothermal Amplification (LAMP) Assay for Detecting Salmonella ser. Typhimurium

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Plain Language Synopsis: Loop-Mediated Isothermal Amplification Assay for Salmonella ser. Typhimurium Detection

Abstract:
Loop-mediated isothermal amplification (LAMP) has been widely investigated for the detection of microbial pathogens in many fields, due to its advantages of specificity, sensitivity, speed, accuracy, simplicity, and low cost. The objective of this study was to develop a new LAMP assay for the detection of Salmonella ser. Typhimurium. The primers were designed by using PrimerExplorer V4 software, based on Salmonella ser. Typhimurium str. LT2 chromosome, complete genome: STM3845 (NCBI Reference Sequence: NC_003197.1). Six sets of primers ranked top by the software were selected and evaluated for their effectiveness in detecting Salmonella ser. Typhimurium using isothermal master mix (OptiGene, UK), with 2 strains of Salmonella ser. Typhimurium (Sal 0723、Sal 0728), 3 strains of Salmonella ser. Enteritidis isolates (SE12、18579、CDC_2010K_1441), and 2 strains of Salmonella ser. Heidelberg (607310-1、579082-8). The following set of primers was determined to be effective in detecting Salmonella ser.

Typhimurium: F3 5'-TCTCCTTTTCGTGTGTGG-3', B3 5'- GATGAAATACTGGCTATATCATCT-3', FIP 5'- GCATTTTTTGCTGTGTAAGTGAGTACG ATGTACGTGCACCAAT-3', BIP 5'-CTTCACGAA CATTTCATTCTAGCTGCAAACACCAGAAGGTC CG-3', LF 5'- CTATCCCTAAAACTGGGGGGA-3'. The ratio of outer and inner primers and the amount of DNA template per reaction for the assay were optimized for this set of primers. Results indicated that our newly designed assay can differentiate Salmonella ser. Typhimurium from Salmonella ser. Enteritidis, Salmonella ser. Heidelberg, other Salmonella serotypes, and some non-Salmonella bacteria of pure cultures. Its effectiveness in detecting Salmonella ser. Typhimurium in food products is being investigated. This new LAMP method could be another quick molecular tool for detecting Salmonella ser. Typhimurium in food products.

### 22. Characterization of microRNA expression in acinar, islet, and ductal cells of the canine pancreas using laser capture microdissection

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Plain Language Synopsis: Pancreatic microRNAs are showing promise as serum biomarkers of pancreatic injury with greater sensitivity and specificity than traditional pancreatic injury biomarkers, amylase and lipase. Herein, we have evaluated the expression of 5 pancreas-enriched microRNAs in dog and rat serum from RNA recovered from formalin-fixed, paraffin-embedded pancreatic tissue.

Abstract:
microRNAs with enriched expression in the pancreas are showing promise as serum biomarkers of pancreatic injury in laboratory animals and in clinical studies. In a previous study, we observed time- and dose-dependent elevations in 5 pancreas-enriched microRNAs (miR-216a, miR-216b, miR-217, miR-375, and miR-148a) in the serum of dogs treated with caerulein to induce reversible injury to the exocrine pancreas. In the current study, the cell type expression of these 5 microRNAs was evaluated by droplet digital PCR (ddPCR) of RNA recovered from formalin-fixed, paraffin-embedded (FFPE) pancreas using laser capture microdissection (LCM). Acinar and ductal cells (exocrine), along with islet cells (endocrine) were each isolated using LCM from FFPE samples from 4 dogs at the end of a four week recovery period following caerulein treatment. In agreement with published results, we found that miR-375 was most highly expressed in islet cells and that miR-216a, miR-216b, miR-217 were most abundant in acinar cells. However, none of the 5 microRNAs was exclusively expressed in any one of the 3 cell types. miR-216a, miR-216b, and miR-217 were also present at lower levels in islets and at very low or absent levels in ductal cells. miR-375 and miR-148a were detected in all 3 pancreatic cell types. The finding that the 5 microRNAs observed to increase in serum with caerulein-induced pancreatic injury are all expressed in acinar cells is consistent with the histopathology observations for the study, that found caerulein-induced injury confined to the exocrine pancreas. As a comparison to validate the ddPCR methodology for RNA recovery from FFPE pancreas using LCM, the same microRNAs are being evaluated in control rat pancreases that have not been treated with caerulein. Preliminary results show similar microRNA trends in islet and ductal cells, however, acinar cell miRs appear lower in rats than in dogs. Additional rat pancreatic samples will be evaluated to further examine the distribution of these miRs.

### 23. Characterizing Novel MicroRNA Biomarkers of Acute Pancreatic Injury

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Plain Language Synopsis: Candidate microRNA biomarkers were characterized in mice, rats, and dogs during acute exocrine pancreatic injury. The microRNAs equaled or surpassed the traditional biomarkers, serum amylase and lipase, in sensitivity, specificity, and range of response. This work supports these microRNAs as enhanced safety tools for drug development.

Abstract:
Scientific and medical consensus suggest that the traditional pancreatic injury biomarkers, serum amylase and lipase, are relatively insensitive and non-specific. Practically this has made them unsuitable for use in drug development and prompted a demand for improved biomarkers of acute pancreatic injury clinically and non-clinically. The Division of Applied Regulatory Science (DARS) with support from CDER Critical Path and RSR grants conducted studies in mice (miR-216a, miR-217), rats (miR-216a, miR-217), and dogs (miR-216a, miR-216b, miR-217, miR-375, miR-148a) to assess these miRNAs enriched in pancreatic tissue as candidate serum biomarkers of acute pancreatic injury. Assay development and optimization were essential to exploring this relatively new area of science. During assay development and optimization, the critical relevance of isomirs (small sequence variants) in design of appropriate assays was revealed. Following assay optimization, multiple pancreatic injury models (caerulein, L-arginine, pancreatic ductal ligation) were utilized in mice and rats to demonstrate an excellent correlation of serum levels of candidate microRNA biomarker to histopathology defined acute injury in the pancreas. In these models, the candidate microRNA biomarkers equaled or exceeded the traditional biomarkers in sensitivity, demonstrated specificity, and had a much larger dynamic range in response. The caerulein model was subsequently used in dogs, a common second non-clinical drug development species, yielding similar results and the same conclusions in dogs as those identified in rodents. DARS research demonstrated translatability across mice, rats, and dogs and supports clinical investigation of these biomarkers that in several cases have identical sequences in humans. Additional DARS research (described in a separate poster) used laser capture microscopy (LCM) to acquire data on cell specificity of the miRNAs in pancreas and next generation sequencing (NGS) to posit a mechanistic anchor for these miRNAs (also described in a separate poster) The Predictive Safety Testing Consortium of the Critical Path Institute has formed a working group to collect data on these microRNAs and submit a non-clinical biomarker qualification package for review by the FDA. This work is an example of FDA research to develop new tools for safety assessment in drug review.

### 24. A microRNA and mRNA Co-Sequencing Approach Provides a Functional Genomic Mechanistic Anchor for miR-216a and miR-217 as Biomarkers of Pancreatic Injury in Rats

*Authors: Rouse, Rodney, FDA/CDER/OTS/OCP/DARS; Li, Zhihua, FDA/CDER/OTS/OCP/DARS*

Plain Language Synopsis: Sophisticated analytics and bioinformatics linked microRNA biomarkers of acute pancreatic injury with genes they modulate. The identified genes had previously reported roles in autophagy and apoptosis, critical processes in survival and death during cellular stress. This study provides a mechanistic anchor for these microRNAs as biomarkers of acute pancreatic injury.

Abstract:
MicroRNAs (miRNAs) have been implicated in modulation of gene expression by interfering with translation of messenger RNA (mRNA) to protein. DARS experiments characterizing the change in serum levels of specific candidate miRNA biomarkers (miR-216a and miR-217) in response to acute pancreatic injury provided pancreas tissue for histopathology evaluation but also yielded concurrent samples for molecular analysis. Co-sequencing of miRNA and mRNA across a time series (1, 3, 6, 24, and 48 hours) was used to identify all potential miRNA-mRNA and gene interactions during pancreatic injury, to specifically associate serum and tissue levels

of miR-216a and miR-217, and to functionally link these candidate biomarkers to observed histopathology. RNAs were derived from pancreatic tissue obtained during experiments describing changes in these miRNAs during acute pancreatic injury. Next Generation Sequencing (NGS) and complex bioinformatics were used to generate differential expression lists between injured and control rats for both mRNA and miRNA. The most up to date miRNA target database was then queried to determine which differentially expressed mRNAs would be targets for those differentially expressed miRNAs. Since miRNAs are posited to suppress gene expression, the resultant miRNA-mRNA pairs were filtered for negative correlation. This much smaller list of negatively correlated miRNA and target mRNA pairs was then examined for previously reported relationships in the literature. This combination of bioinformatics and literature validation identified mRNAs and miRNAs that were differentially expressed, negatively correlated, and experimentally associated. This process produced a complex signaling network for future investigation and linked candidate miRNA biomarkers to cellular processes representing the prominent histopathology observations in those same experimental samples. This functional genomic approach suggests a mechanistic anchor for the use of these biomarkers in pancreatic injury. During the study, RNA quality bias by treatment was observed and a statistical correction applied. The impact of that correction on results are reported and briefly discussed.

### 25. Induced Pluripotent Stem Cells: Response to low oxygen tension culture

*Authors: Osborn, Cindy, FDA/CBER; Bellayr, Ian, FDA/CBER; Hursh, Deborah, FDA/CBER*

Plain Language Synopsis: Pluripotent stem cells (IPSCs) are a promising cell source for regenerative medicine products. We seek to identify manufacturing conditions that improve stem cell quality. Counterintuitively, we found IPSCs grown under low (physiological) oxygen conditions increase their mitochondrial number. We are investigating the impact of this on IPSC growth and differentiation.

Abstract:
Induced pluripotent stem cells (iPSCs) are being widely investigated as a source of cells for regenerative medicine, but methods are needed to assess their quality during the reprogramming and extensive differentiation procedures required to make cellular products. Mitochondria, a critical participant in cell function, can respond to developmental signals and are vulnerable to environmental changes like external toxins and amounts or types of carbon sources; thus, the state of mitochondria can serve as a predictive indicator of cellular history and future behavior. We propose to examine mitochondrial characteristics in iPSCs to assess if they are predictive of cell quality. Specifically, we are interested in one of the most common variability in stem cells' culture which is the level of oxygen during cell culture. Oxygen is an important component of the cellular microenvironment, serving as metabolic substrate and signaling molecule. As reported in literature, reprogramming somatic cells in low oxygen tension improved the reprogramming efficiency and potentially decreased any reprogramming-associated mutation. However, the effect of oxygen level on stem cell self-renewal and differentiation is incompletely understood. We sought to explore the effect of low oxygen tension on iPSCs with the emphasis on assessing if there are correlations between mitochondrial characteristics and performance of iPSCs. We found that there is an increase in mitochondrial mass when iPSCs are grown under low oxygen tension. This mass increase is accompanied by an increase in mtDNA copy number and the level of proteins of its replication machinery. Surprisingly, we do not observe an increase in the level of oxidative phosphorylation or the level of respiration. As a follow up study, we are using microarray to systematically identify differential gene expression in iPSCs grown under low oxygen tension with the goal of identifying the cause of increased mitochondrial mass. Any results will be validated using gene knock-down experiments.

## 26. Impaired BCR-induced NFAT1 nuclear translocation is implicated in low TACI expression in newborn B lymphocytes

*Authors: Sakai, Jiro, FDA/CBER; Coleman, Adam, FDA/CBER; Akkoyunlu, Mustafa, FDA/CBER*

Plain Language Synopsis: An explanation of the newborn vulnerability to bacterial infection

Abstract:
Neonates and infants have impaired immune responses to immunization compared to adults, resulting in high susceptibility to microbial infection. B lymphocytes have a central role in humoral immunity to immunization and infection, and signals via B cell receptor (BCR) are essential for the development and maintenance of B lymphocytes. T cell-independent type 2 (TI-II) antigens activate B lymphocytes through BCR and transmembrane activator and CAML interactor (TACI). Newborns, however, are unable to generate TI-II responses, partly because TACI expression is severely reduced in newborn B lymphocytes. Since BCR signaling regulates TACI expression, we hypothesized that suboptimal BCR activation may be responsible for the reduced TACI expression on newborn B cells. We found that engagement of murine newborn BCR with anti-mouse IgM f(ab')2 results in ablated Nuclear Factor of Activated T-cells 1 (NFAT1) nuclear translocation as compared to adult BCR. Neither anti-IgM stimulation nor the calcium influx inducer, Ionomycin stimulation elicits a significantly different calcium influx profile between newborn and adult B cells, suggesting that the factors downstream of calcium influx are responsible for the suppression of NFAT1 activation. Diminished NFAT1 translocation is likely responsible for the reduced TACI expression in newborn B lymphocytes because blocking NFAT1 pathway with the selective NFAT inhibitor, FK506 prevents the upregulation of TACI in anti-IgM-stimulated adult B lymphocytes. We are currently investigating the activities of calcium-dependent NFAT1 activators calmodulin and calcineurin in newborn B lymphocytes to assess whether insufficiencies in these molecules are responsible for the ablated translocation of NFAT1.

## 27. Detection of Chagas Disease Biomarkers in Mouse Models for Drug Discovery

*Authors: Nagarkatti, Rana, FDA/CBER; Acosta, David, FDA/CBER; de Araujo, Fernanda, FIOCRUZ/ Centro de Pesquisas René Rachou; Debrabant, Alain, FDA/CBER*

Plain Language Synopsis: Chagas disease drug discovery has been hampered by the lack of validated pre-clinical assays to establish cure in animal models. Detecting parasite antigens (PAs) circulating in the blood of a drug treated Trypanosoma cruzi infected host could indicate the presence of residual parasitemia and thus, treatment failure.

Abstract:
Chagas disease is caused by the parasite Trypanosoma cruzi (T. cruzi). The majority of infected individuals have lifelong asymptomatic infection; however, 30% of them develop severe cardiac or gastrointestinal symptoms. There is currently no vaccine available to prevent Chagas disease. Two drugs, Nifurtimox and Benznidazole, are effective at controlling blood parasitemia but treatment rarely results in parasitological cure. Thus, new drugs and vaccines need to be developed. However, development of new therapeutics for Chagas disease has been hampered by the lack of validated pre-clinical assays to establish cure in animal models. We have previously developed animal models to assess treatment efficacy using parasite antigen (PA) based biomarker detection assays. In this study, 6 PA candidate biomarkers were selected from the proteomic profile of the T. cruzi excreted secreted antigens (TESA). For each PA an immunodominant peptide was selected and used to generate specific antibodies. ELISAs with these anti-peptide antibodies detected the 6 PAs in TESA, trypomastigote and epimastigote extracts obtained from various strains of T. cruzi. Mouse models to evaluate drug treatment efficacy during the acute and the chronic phase of Chagas disease were used to validate the PA ELISAs. Blood levels of biomarker #192 and #120, were found to be significantly elevated in the infected group compared to the non-infected control group at 55 (acute) and 130 (chronic) days post infection.

In both the acute and the chronic phase models 100% of the infected mice treated with benznidazole showed #192 and #120 biomarker levels above the assay cutoff. These results demonstrated that cure was not achieved in these animals even though blood parasitemia was reduced or not detected by microscopy after benznidazole treatment. Further, the ELISAs were specific as serum of mice infected with other parasites such as Leishmania was not positive for these biomarkers. In this study, we validated a number of novel biomarkers of Chagas disease. Our results showed that the biomarker assays provide an overall picture of the infection in the host. They represent useful tools to evaluate vaccine and drug efficacy in animal models, and could facilitate development of new therapeutics for Chagas disease.

### 28. Cellular and Molecular Markers of Radiation Induced Attenuation in Intrahepatic Plasmodium falciparum Parasites

*Authors: Oakley, Miranda, CBER, FDA; Zheng, CBER, FDA; Takeda, Kazuyo, CBER, FDA; Gao, Yamei, CBER, FDA; Tripathi, Abhai, Bloomberg School of Public Health, Johns Hopkins University; Mlambo, Godfree, Bloomberg School of Public Health, Johns Hopkins University; Aravind, L., NLM, NIH; Kumar, Sanjai, CBER, FDA*

Abstract:
The parasite biomarkers of growth attenuation and enhanced immune protection induced by radiation attenuated Plasmodium falciparum sporozoite vaccine remain poorly understood. By microarrays, we have identified over 200 novel biomarkers that associated with growth and attenuation of Plasmodium falciparum sporozoites growing in liver cells in response to y-irradiation.

Abstract:
Radiation attenuated sporozoite (RAS) vaccination has proved to be a promising approach for malaria vaccine development. While the host requirements for sterile immunity induced by RAS vaccination have been studied, the molecular events that cause attenuation of sporozoites in response to radiation remain poorly understood. Electron microscopy and immunofluorescence microscopy were performed to determine the cellular markers of attenuation in Plasmodium falciparum sporozoites (PfSPZ) and genome-wide transcriptional profiling in P. falciparum liver stage parasites and bioinformatics analyses were performed to identify the biomarkers of growth attenuation and enhanced immune protection. y-irradiation treated PfSPZ were vacuous with a partially disrupted plasma membrane and inner membrane complex; growth of day 3 intrahepatic parasites was not affected. In microarray studies, 180 intrahepatic parasite genes were significantly transcriptionally altered. Among the transcriptionally altered biomarkers, we identified a signature of seven candidate parasite genes that associated with functionally diverse pathways that may regulate radiation induced cell cycle arrest of the parasite within the hepatocyte. A repertoire of 14 genes associated with protein translation is transcriptionally overexpressed within the parasite by radiation. Additionally, 37 genes encode proteins expressed on the cell surface or exported into the host cell, 4 encode membrane associated transporters, and 10 encode proteins related to misfolding and stress-related protein processing. These results have significantly increased the repertoire of novel targets for 1) generating genetically attenuated parasite vaccine candidates 2) subunit vaccines against the hepatic stage cycle and 3) biomarkers of safety to define proper attenuation.

### 29. Learning EEG: Identification of novel electroencephalogram classifications and baseline features in a large clinical dataset

*Authors: Nahmias, David, UMD FDA/CDRH; Kontson, Kimberly, FDA/CDRH; Civillico, Eugene, FDA/CDRH NIH/OD*

Plain Language Synopsis: This study aims to determine whether electroencephalographic (EEG) features can be used as biomarkers for patient characteristics and clinical observations including demographics, medication, epilepsy, and traumatic brain injury (TBI) status. Additionally, we seek to further understand the consistency and variability of baseline EEG

measurements in both healthy and patient populations.

Abstract:
Medical devices that interface with the nervous system for diagnostic, therapeutic, or research purposes are a major area of innovation in the medical products industry. Electroencephalogram (EEG), which measures brain electrical signals from the scalp, is a common neuro-monitoring technique used in both clinical and research settings. EEG can be non-invasive, is relatively inexpensive, and could theoretically contain biomarkers for certain neurological disorders. However, EEG has only recently begun to move into quantitative applications beyond visual inspection by trained professionals. Further, despite the increasing use and public health importance, very little is known about the consistency and variability of baseline EEG measurements in healthy individuals and in patient populations. This has led in certain cases to the inability to distinguish between baseline variability and clinically meaningful differences. In the same way that individual variation in gene sequences makes certain drugs more or less effective for certain people, giving rise to the need for pharmacogenomics, individual variation in EEG could strongly affect the safety and efficacy of therapeutic medical devices. Across disciplines, the use of new machine learning techniques and large data-sets (i.e. "Big Data") has improved the ability to understand and accurately classify data. The goal of applying these methods to EEG is to determine how quantitative characteristics might be distributed differently across normal and clinical populations. The Neural Engineering Data Consortium EEG corpus from Temple University currently has over 15,000 EEG recording sessions (~500GB) with an expanded set of 30,000 sessions to be released this year. Each session is accompanied by the physician's notes containing clinical impressions and patient characteristics. Though the text of these notes is relatively unstructured, in preliminary analysis we have successfully extracted gender, age, medication, epilepsy, traumatic brain injury (TBI) status and other clinical data from the majority of these notes and are proceeding to use this information to train and test novel machine learning methods on the EEG data. Results from this study will provide better insight to what EEG is capable of predicting in terms of a patient's demographics, medications, neurological disorders and establishing the baseline variability of EEG features across populations.

### 30. An ion pair reverse-phase liquid chromatography method coupled to tandem mass spectrometry for analysis of glyphosate and related compounds


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Plain Language Synopsis: Long term and wide usage of Glyphosate raises concerns about public health. A robust, sensitive testing method is required. This method will replace instrument portion of LIB4595 and allow the harmonization of LIB4595, 4596, 4604 and 4613 into one overall method. ORA has organized a multi-lab validation based on it.


Abstract:
An ion pair reverse-phase high performance liquid chromatographic method is developed and optimized for determination of glyphosate, N-acetyl glyphosate, glufosinate, and aminomethylphosphonic acid (AMPA). Tetrabutylammonium formide is used as the ion-pairing reagent at pH 2.8. A triple-quadrupole tandem mass spectrometer is coupled to the liquid chromatographic system to detect the compounds in negative electro-spray ionization (ESI) mode. The stable-isotope dilution methodology in combination with LC-MS/MS in this study provides high analytical specificity for quantitative analysis of the four compounds at the regulatory limit of 10 ng/g in food products for the FDA Pesticides Program. This LC-MS/MS method was paired with the extraction method stated in LIB 4596 and applied to carrot, spinach, corn and avocado. The resulting data showed good sensitivity, precision, accuracy and linear range. This successful pairing of the LIB 4596 extraction with this new LC-MS/MS method will allow the harmonization of LIB 4595, 4596, 4604 and 4613 into one overall method for most food matrices. ORA and CFSAN has organized a multi-lab validation based on this instrument method.


### 31. Determining Worst-Case Ni Release in Benchtop Studies of Nitinol


*Authors: Sussman, Eric, FDA/CDRH; Loh, Sulvia, FDA/CDRH; Chandrasekar, Vaishnavi, FDA/CDRH; Mannuel, Priscilla, FDA/CDRH; Sivan, Shiril, FDA/CDRH; Weaver, Jason, FDA/CDRH; Di Prima, Matthew, FDA/CDRH; Saylor, David, FDA/CDRH*


Plain Language Synopsis: Some medical devices are made of a nickel/titanium alloy (called nitinol), which is useful for its material properties, but has been implicated in causing nickel-related adverse reactions. This study investigates whether conditions exist where nickel release from nitinol is worse than currently predicted under established testing protocols.


Abstract:
Nitinol is a commonly used alloy composed of nickel and titanium found in various medical devices with few adverse effects reported. Recently, however, reports of allergy symptoms attributed to nickel have been made, highlighting some uncertainty in the usefulness of in vitro assays for predicting Ni exposure in vivo. Current in vitro assays examine nickel release under static conditions in phosphate buffered saline. However the implant environment includes complex biological media, dynamic mechanical conditions, and the presence of activated leukocytes, all which could potentially enhance nickel release and cellular uptake. The goal of this study is to determine whether benchtop models that more closely mimic the in vivo environment provide a more physiologic pattern of Ni release compared to currently used approaches.

Specifically, this poster reports on methods that were developed to study Ni release due to activated leukocytes seeded directly on Ni alloys, a model system for cells involved in the foreign body reaction. Fifty-thousand activated Thp-1 cells were cultured for up to 7 days on 6.4 mm square coupons of various alloys (200 Ni alloy, 625 Ni alloy, and 316L stainless steel) or on tissue culture plastic surfaces (TCPS; controls). Ni release and cell viability were monitored with ICP-MS and Alamar Blue, respectively.

After 7 days of cell culture, Ni release was not

detected from 316L, was 0.5 µg/cm2 for 200 Ni, and 7 µg/cm2 for 625 Ni, indicating alloy-specific Ni release rates. Moreover, Ni release was associated with a reduction in cell viability. After 7 days of culture, relative to TCPS controls (normalized to 1.0), viability of Thp-1 cells on Ni 625 was 0.2 ($p<0.05$), while it was unchanged for cells cultured on the other alloys ($p>0.05$).

The results indicate a potential correlation between Ni release and reduced viability of cells in direct contact, which has been reported in other studies. This data represents an initial method development stage. Continuing studies are underway to understand the effects of extract media, surface finish, and dynamic mechanical conditions on Ni release rates. The results will be used to inform updated toxicological risk assessments of Ni exposure locally and systemically.

### 32. Monitoring for Zika Virus Occurrence Among the U.S. Elderly, 65 and Older, Using Large Medicare Databases

*Authors: Mikhail Menis1, Richard A. Forshee1, Hector S. Izurieta1, Zebulin Kessler2, Stephen McKean2, Rob Warnock2, Sumit Verma2, Bo Kim2, Christopher M. Worrall3, Jeffrey A. Kelman3, Steven A. Anderson1*

*1. FDA/CBER, Silver Spring, MD, United States.*

*2. Acumen LLC, Burlingame, CA, United States.*

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Abstract:

Zika is a flavivirus transmitted to humans primarily by Aedes species mosquitos. Although it can be asymptomatic, Zika infection may result in acute onset of fever, rash, arthralgia, conjunctivitis, myalgia and headache. Zika infection has been associated with Guillain-Barré syndrome as well as with other complications. As Zika infection may be more severe in the elderly due to underlying comorbidities and lower immunity, the objective of our investigation is to establish monitoring for Zika virus infection in the U.S. Medicare beneficiaries ages 65 and older.

Methods: Our claims-based study utilized large Medicare databases to monitor for Zika occurrence in the elderly starting from October 2016. Potential Zika cases were ascertained via the recorded diagnosis-specific code introduced in October 2016. Our study evaluated Zika occurrence among the elderly, overall and by diagnosis month, age, sex, race, and state of residence.

Results: Our investigation identified 68 Zika cases among the elderly Medicare beneficiaries during the period of October 2016 through March 2017. Of them, 37 were females and 31 males; and majority of recorded cases were identified in whites (N=56) versus 12 in non-whites. About 84% of Zika cases were younger elderly ages 65-79. The case distribution by age categories 65-69, 70-74, 75-79, 80-84, 85 and over were 27, 16, 14, 5, and 6, respectively. The monthly numbers of recorded Zika cases in the six-month monitoring period were 20, 20, 13, 5, 5, and 5, respectively. About 60% (N=41) of the recorded cases were diagnosed in Puerto Rico (N=21), Florida (N=9), New York (N=6), and Texas (N=5).

Conclusions: Overall, our investigation is in the process of evaluating capability of Medicare databases for the near-real time monitoring of Zika occurrence among the U.S. elderly. Preliminary results show variations in Zika occurrence by state, month of diagnosis, and demographic characteristics. Continued monitoring will help FDA to better understand possible spread of Zika in continental U.S., its severity, and to ascertain risk of transfusion-transmission in elderly. The study was based on claims data and thus limitations include potential under- or mis-recording of Zika cases. Also, delayed uptake of new Zika coding is a potential limitation that will be ascertained as monitoring continues.

### 33. CARDIOVASCULAR OUTCOMES AND ALL-CAUSE MORTALITY STRATIFIED BY DEGREE OF HDL-C RAISING AND/OR TRIGLYCERIDE LOWERING IN THE AIM-HIGH TRIAL

*Authors: Tejas Patel1, Ahmed Hasan2, Eileen Navarro1, for the Meta-AnalyTical Interagency Group (MATIG)*

*1 OCS/OTS/CDER/FDA, 2 NHLBI/NIH*

Abstract:

Serum HDL-C raising and/or triglycerides (TG) lowering is believed to provide cardiovascular (CV) benefit, but the levels that provide optimum protection are not known.

Methods: Four groups, based on percent change from baseline to 6 months in HDL-C and TG due to antidyslipidemic therapy in the AIM-HIGH trial, were compared. Time-to-first event analyses for major adverse CV events (MACE: composite of MI, stroke, and CV death) were performed.

Results: Surprisingly, greater benefit was observed with less HDL-C and TG change, alone or in combination, whereas an increased risk of MACE was seen in the group with the greatest drug response. Subjects at increased risk were older, male, and had an eGFR ⩽60 mL/min. No difference was observed among 4 groups for all-cause mortality.

Conclusions: Post hoc analyses showed lower MACE risk with more modest reductions in HDL-C and TG. Meta-analyses may reveal lipid lowering targets that optimize MACE reduction.

**34. Alerting physicians to waterpipe-induced acute eosinophilic pneumonia—a cause of respiratory failure in young adults**

*Authors: Retzky, Sandra, FDA/CTP*

Plain language synopsis: Waterpipe (WP) smoking, also known as hookah, is becoming a common method of tobacco smoking in young adults. A rare and serious lung disease has been reported in these smokers. Early diagnosis and treatment is critical. We describe how we identified these cases and how we made physicians aware of it.

Objective:

To increase awareness of:

a. Acute eosinophilic pneumonia (AEP) and the connection to WP smoking; and

b. Our safety reporting portal for reporting adverse tobacco experiences to FDA.

Method: FDA's WP Workgroup performs routine medical literature scans semi-annually. We identified five conference meeting abstracts reporting AEP in WP smokers. Of these, four cases were associated with tobacco WP use. All were serious events. Three patients were placed on respirators required intubation and mechanical ventilation. A fourth case required extracorporeal membrane oxygenation for cardiac and respiratory support after mechanical ventilation failed. Although not specifically tobacco-related, a fifth case of AEP in a marijuana WP user was also reported.

In response, we wrote a letter to CHEST—the most commonly cited respiratory journal—alerting physicians to this problem and encouraging voluntary reporting into FDA's SRP. This enables FDA to identify and investigate population-based safety signals that may otherwise go unnoticed due to low frequency.

Results: Our document was accepted and elevated to editorial status because due to the critical nature of the finding. The acceptance came with an invitation for an expanded word count to further explain AEP and how to report tobacco-related events to FDA.

Conclusion: Safety surveillance plays a vital role in protecting public health—FDA's mandate. We highlight the importance of routine literature reviews in safety surveillance for tobacco adverse events.

Abstract:
Waterpipe-induced acute eosinophilic pneumonia (AEP): A cause of respiratory failure in young adults

Background: Waterpipe (WP) smoking, also known as hookah, is a centuries old method of smoking. Recently, WP smoking has made a resurgence especially among youth because of its perception of reduced harm. WP, also known as hookah, has been used for hundreds of years; however, over the past decade this form of smoking has gained significant popularity in the United States, especially among youth and young adults. Based on the 2011–2015 National Youth Tobacco Survey, approximately 7.2% of high school students currently smoke hookah—almost as many as those who smoke cigarettes.

AEP is an extremely rare, non-infectious lung disease that can be life-threatening. Since

its original description in 1989, roughly two hundred cases have been reported. While AEP's cause remains unknown, it is thought to be an acute allergic reaction to inhaled particles. For good outcomes, early diagnosis and appropriate treatment is critical. We report on an AEP cluster in young WP smokers and describe: (1) how we identified this population; and (2) how we made physicians aware of it.

Objective:

To increase awareness of:

c. AEP and the nexus with WP smoking; and

d. Our safety reporting portal (SRP) for reporting adverse tobacco experiences to FDA.

Method: FDA's WP Workgroup performs routine medical literature scans semi-annually. We identified five conference meeting abstracts reporting AEP in WP smokers. Of these, four cases were associated with tobacco WP use. All were serious events. Three cases required intubation and mechanical ventilation. A fourth case required extracorporeal membrane oxygenation for cardiac and respiratory support after mechanical ventilation failed. Although not specifically tobacco-related, a fifth case of AEP in a marijuana WP user was also reported.

In response, we wrote a letter to CHEST—the most commonly cited respiratory journal—alerting physicians to this problem and encouraging voluntary reporting into FDA's SRP. This enables FDA to identify and investigate population-based safety signals that may otherwise go unnoticed due to low frequency.

Results: Our document was accepted and elevated to editorial status due to the critical nature of the finding. The acceptance came with an invitation for an expanded word count to further explain AEP and how to report tobacco-related events to FDA.

Conclusion: Safety surveillance plays a vital role in protecting public health—FDA's mandate. We highlight the importance of routine literature reviews in safety surveillance for tobacco adverse events.

## 35. Chemical analysis of ENDS aerosol by LC/MS/MS-based methodology

*Authors: Oktem, Berk FDA/CDRH; Moran, Charles FDA/CDRH; Wickramasekara, Samanthi FDA/CDRH*

Plain Language Synopsis: Electronic nicotine delivery systems (ENDS) have emerged as an alternative form of nicotine delivery. Research has been conducted on aerosol generated from ENDS, however the industry is rapidly evolving. This project aims to develop an improved method to analyze ENDS aerosols using state-of-the-art chemical analysis instruments.

Abstract:
CORESTA recommended methods (CRM) 74 and 75 describe methods for LC-UV and LC/MS/MS to analyze mainstream cigarette smoke targeting carbonyl-containing species and tobacco specific nitrosamines (TSNAs). These compounds have been found in ENDS aerosol and are relevant to analyze due to their potential toxicity. These methods have also been employed to analyze aerosol created by ENDS. Current technology in high resolution mass spectrometry is capable of combining CORESTA methods 74 and 75 as well as improving the method's capabilities. This poster discusses development of a combined method using UHPLC and a high resolution Q-TOF mass spectrometer that can be used for analyzing aerosols generated from temperature controlled ENDS using a tank atomizer.

Carbonyl-containing species generated by ENDS are derivatized by 2,4-Dinitrophenylhydrazine (DNPH) in a solution while the aerosol is captured in a series of 2 to 4 impingers (Midget impinger SKC Inc). The samples are then introduced to three different instruments: an HPLC with a high sensitivity flow cell (SPD-20A-Shimadzu Corp.) where the 360 nm response is recorded and a UHPLC (Accela 1250 - Thermo Scientific) equipped with a photodiode array (PDA) as well as an ion trap mass spectrometer (LTQ XL) and an LC-QTOF-MS (Infinity 1260 with 6540 UHD- Agilent Technologies) system.

Standards of 2,4-DNPH derivatives were prepared and analyzed by an LC-PDA-MS system where the UV absorbance and

MS data are acquired together. The MS/MS fragmentation ions are used to confirm the identity of co-eluting ions. The LOD is approximately 10 ng/mL for the targeted analytes.

The coil temperature of the ENDS was regulated by the built in controller of the ENDS device. A puff profile of 3 second puff lengths and puff volume of 55 ml are used as described in CORESTA recommended method 81. Aerosol generated from ENDS is trapped using impingers containing recrystallized 2,4-DNPH solutions in acetonitrile, water, and phosphoric acid, prepared according to CRM 74. Preliminary work shows detectable quantities (>10 ng per sample) of formaldehyde in the entrapped aerosols. Current work includes the use of Q-TOF MS with higher resolution (~40,000) for accurate identification of unknown vapor components as well as TSNAs.

### 36. Nicotine Content and Physical Properties Of Large Cigars and Cigarillos in the United States

*Authors: Hull, Lynn, FDA/CTP; Schroeder, Megan FDA/CTP; Taylor, Kenneth FDA/CTP ; Thanner, Meridith Battelle Public Health Center for Tobacco Research; Koszowski, Bartosz, Battelle Public Health Center for Tobacco Research*

Plain Language Synopsis: The objective of this study was to understand the variability of cigar characteristics, a critical aspect in conducting and interpreting clinical study results. The study found the following results:

- There are wide variations in product size and nicotine content, including within cigar classes, although pH was similar across all cigars.
- Secondary analyses of four products found considerable variation in the nicotine content and concentration.
- There is a wide variation in product size and nicotine content within the domestic cigar market.
- Because cigar size and tobacco weight do not necessarily correspond to the amount of free nicotine, a basic analysis of cigar products may be essential before cigar use in clinical studies.
- Consumers smoking the same brand of cigar may unintentionally be exposed to varying doses of nicotine and potentially other smoke constituents.

Abstract:
Cigars are combustible tobacco products consisting of filler, binder, and wrapper, which are derived from tobacco. Despite the abundance of literature on the composition of traditional combusted cigarettes, research is limited on the physical and chemical properties of cigars. Therefore, research on cigar properties may be useful to better understand these properties. In this study, twenty large cigar and cigarillo products were characterized for physical properties (i.e., weight, length, diameter), filler nicotine content, and tobacco pH. Measured tobacco pH, free nicotine content, free nicotine concentration, and percent free nicotine were calculated for all cigars using the Henderson-Hasselbach equation. An additional analysis was performed on a second batch of two large cigar and two cigarillo brands to determine within-brand consistency. All analyses were performed in triplicate.

### 37. Expression and Characterization of a Codon-Optimized Blood Coagulation Factor VIII

*Authors: Svetlana A. Shestopal[1], James H. Kurasawa[1], Jian-Jiang Hao[2], Elena Karnaukhova[1], Yideng Liang[1], Min Lin[1], Mikhail V. Ovanesov[1], Timothy K. Lee[1], John H. McVey[3] and Andrey G. Sarafanov[1]*

*[1]Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring MD;*

*[2]Poochon Scientific, Frederick MD, U.S.A.;*

*[3]School of Biosciences and Medicine, University of Surrey, Surrey, UK*

Plain Language Synopsis: FVIII products are used to treat its deficiency (Hemophilia A). However, production of recombinant FVIII is challenging due to its low expression level. In our work, we used codon optimization of FVIII, which resulted in about 7-fold increase of the protein yield while did not affect the biochemical properties.

Abstract:
The deficiency in blood coagulation factor VIII (FVIII) results in excessive bleeding known as Hemophilia A. This disease is treated by FVIII products, and the gene therapy approach is under development. These applications are limited by low expression level of FVIII, which correlates with its very low level in plasma. In our work, we utilized codon optimization of FVIII cDNA to increase the protein expression. However, the synonymous coding of a protein may affect its primary sequence, post-translation modifications, conformation, functions and stability. The concerns about clinical use of such protein include its altered pharmacokinetics and pharmacodynamics, and increase of its immunogenicity. Therefore, the major focus of our work was verification of the codon-optimized FVIII characteristics.

Objective. To express and characterize biochemical properties of a B-domain deleted FVIII encoded by either a codon-optimized or wild-type cDNA sequence (CO and WT, respectively).

Experimental Approach. The WT and CO were expressed in CHO cells using a lentivirus-based platform and purified using affinity chromatography. Several independent preparations of each FVIII variant, produced from different cell lines, were analyzed by PAGE, Western-blot, ELISA, mass-spectrometry, circular dichroism, FVIII activity assays and by surface plasmon resonance.

Results. The average yield of CO was approximately 7-fold higher than that of WT. The proteins were identical in the amino acid sequences and very similar in patterns of the molecular fragments (including those produced upon thrombin cleavage), glycosylation and tyrosine sulfation, secondary structures and binding to von Willebrand factor (a physiological career of FVIII in plasma) and to a fragment of the low-density lipoprotein receptor-related protein 1 (FVIII clearance receptor). Unexpectedly, the CO was found to have about 1.5-fold higher FVIII specific activity (activity normalized to protein mass) than WT.

Conclusions. The higher specific activity of CO was attributed to better preservation of its structure due to consistently higher concentrations than WT at all steps of the production. We concluded that the codon optimization of FVIII resulted in significant increase of its expression, while did not affect the protein's properties.

## 38. Assessing the Biological Impact of Degradants Evolving from Biodegradable Polymeric Medical Devices

*Authors: Skoog, Shelby, FDA/CDRH; Guo, Ji, FDA/CDRH; Malinauskas, Richard, FDA/CDRH; Lu, Qijin, FDA/CDRH; Casey, Brendan, FDA/CDRH*

Plain Language Synopsis: Biodegradable polymers are being used in high-risk emerging technologies, such as Class III cardiovascular devices, which introduce new risks to patients as these polymers degrade. To assess the safety of these devices, we have developed a research paradigm to evaluate biological responses to polymer degradants, including cytotoxicity and hemocompatibility.

Abstract:
Novel use of biodegradable polymers in medical devices, including Class III cardiovascular devices, has resulted in new risks as these polymers degrade in the human body and could potentially release toxic compounds. To address these risks, it is critical to develop an enhanced, quantitative understanding of how these materials affect local and systemic tissue responses. Unfortunately, assessing biocompatibility and the risks they pose to patients is quite complex since these materials are constantly changing.

The goal of this study was to assess the biocompatibility of degradants released from biodegradable polymers widely used in medical products. Polymeric materials, such as lactide- and glycolide-based polyesters (PLGA, PDLA, PLLA) as well as polycaprolactone (PCL), of low and high molecular weights (MW) were degraded in vitro in a physiologically relevant process (phosphate buffered saline, 37°C). Over an 8-month degradation period, we systematically characterized polymer degradation and assessed the in vitro biocompatibility of the degradants. Degradant species were identified and quantified

using electrospray ionization (ESI) liquid chromatography – mass spectrometry (LC-MS). The influence of degradants on cytotoxicity in various cell types (mouse fibroblasts, mouse osteoblasts, human coronary artery endothelial cells, and human monocytes) was assessed by evaluating cell viability, inflammation, oxidative stress, and morphology. The effects on human blood compatibility were also investigated, including hemolysis, coagulation cascade activation, and platelet activation/aggregation.

The results showed that degradation products caused adverse biological responses at certain time points for the fast degrading polymers (PLGA 50:50). The polymer degradants from low MW PLGA 50:50 resulted in significant cell death in all cell types as early as four weeks. By contrast, the high MW PLGA 50:50 with less degradation resulted in only moderate cell death at the corresponding early time points. Decreases in pH due to the acidic degradation products assessed under these conditions might have contributed to the adverse biological responses. Minimal to no cytotoxicity was observed for PDLA, PLLA, and PCL, which exhibited no measured degradation at the early time points.

### 39. Multiplex Real-Time PCR Assay for Detection and Differentiation of Escherichia coli O157:H7 and Non-O157 Shiga Toxin-Producing E. coli

*Authors: Li, Baoguang, FDA/CFSAN; Liu, Huanli, FDA/CFSAN; Wang, Weimin, FDA/CFSAN*

Plain Language Synopsis: We developed a multiplex real-time PCR that can rapidly and simultaneously detect and differentiate E. coli O157:H7 and non-O157 STEC strains. This assay demonstrated high sensitivity and specificity. Also, we have applied this assay to detect E. coli O157:H7 and non-O157 STEC strains from two food matrices (beef and spinach).

Abstract:
Shiga toxin-producing Escherichia coli (STEC), including E. coli O157:H7, are responsible for numerous foodborne outbreaks annually worldwide. E. coli O157:H7, as well as pathogenic non-O157:H7 STECs, can cause life-threating complications, such as bloody diarrhea and hemolytic-uremic syndrome (HUS). Previously, we developed a real-time PCR assay to detect E. coli O157:H7 in foods by targeting a unique putative fimbriae protein ORF Z3276. To extend the detection spectrum of the assay, we report a multiplex real-time PCR assay to specifically detect E. coli O157:H7 and non-O157 STEC by targeting Z3276 and Shiga toxin genes (stx1 and stx2). Also, an internal amplification control (IAC) was incorporated into the assay to monitor the amplification efficiency. The multiplex assay was optimized for amplification conditions. The limit of detection (LOD) for the multiplex assay was determined to be 200 fg of bacterial DNA, which is equivalent to 40 CFU per reaction which is similar to the LOD generated in single targeted PCRs. Inclusivity and exclusivity determinants were performed with 182 bacterial strains. All E. coli O157:H7 (n = 135) were detected as positive and all STEC strains were positive for stx1, or stx2, or stx1 and stx2. No cross reactivity was detected with Salmonella enterica, Shigella strains, or any other pathogenic strains tested. Additionally, the profiles of Shiga toxins of E. coli O157:H7 strains determined by the multiplex assay matched exactly with those assessed by different schemes. Furthermore, we have successfully applied this multiplex assay to detect E. coli O157:H7 from spiked spinach and beef samples, suggesting this may also be applied to other food matrices for detection of E. coli O157:H7 and non-O157 STEC strains.

### 40. How is FDA addressing public health concerns regarding Breast Implant (BI) Associated Anaplastic Large Cell Lymphoma (BIA-ALCL)?

*Authors: Jiang, Hongying (Helen), FDA/CDRH; Lin, Yu, FDA/CDRH; Nast, Karen, FDA/CDRH; Kurtz, Peter, FDA/CDRH; Yoon, Sung, FDA/CDRH; Eloff, Benjamin FDA/CDRH; Loyo-Berríos, Nilsa, FDA/CDRH* (* corresponding author)*

Plain Language Synopsis: In recent years, BIA-ALCL cases have been reported in the US and around the world. To address this urgent public health need, we established a surveillance system, integrating disparate data

sources. Surveillance findings will inform FDA regulation of BI and any public communications (e.g. webpage, public, press or congressional inquiries).

Abstract:
In 2011, FDA became aware of ALCL cases among women with BI, and issued a safety communication. The PROFILE Registry was established in collaboration between FDA, the American Society of Plastic Surgeons and the Plastic Surgeons Foundation. Recently, the World Health Organization recognized BIA-ALCL as a new rare T-cell lymphoma; and the French, New Zealand and Australian governments released public safety alerts.

Methods: We established the BIA-ALCL Surveillance System to develop methodologies to integrate and analyze data from disparate sources (literature, PMA Annual Reports, medical device adverse events reports (MDRs), and registry) for a better understanding of BI role in ALCL etiology. This will also support FDA public communications (e.g. ALCL public webpage and public, press or Congress inquiries).

The conceptual framework for data integration and analysis will be presented along with description of BIA-ALCL reports, by data source, regarding: (1) implant fill (silicone gel, saline), (2) implant surface (textured, smooth), (3) time to diagnosis, and (4) selected clinical data. Preliminary results are presented for MDRs as of February 1, 2017, and for the systematic literature review as of January 28, 2017. PMA Annual Reports and registry data will be available in early March and will be included in the final analysis.

Results: MDR analysis shows that among 359 reports (9 deaths), 203 (57%) reported textured and 28 (8%) reported smooth implants (the rest are not specified); 186 (52%) and 126 (35%) reported silicone and saline filled implants, respectively. The estimated median time to diagnosis is 7 years. Among reports with biomarkers data, all had CD30 positive (100%) and ALK negative (100%). Similar findings were observed from the literature (based on 41 original clinical studies), with 90% of reports on textured and 56% on silicone implants, with median time to diagnosis estimated as 8 years. All patients had CD30 positive (100%) and mostly ALK negative (95%). Based on literature, treatment may involve explant/capsulectomy, and single or combined chemo/radiation therapies.

The findings confirm a potential correlation with textured implants. Reports of cases with saline implants are worth noting. We demonstrated that integration and analysis of disparate data sources is a valid methodology to use when dealing with rare adverse event outcomes.

### 41. Absorbable Polymer Degradation Responding to Artificial Plaque Compositions

*Authors: Aleer M Yol, Ji Guo, FDA/CDRH*

Plain Language Synopsis: FDA is facing regulatory challenges to assess the safety and efficacy of new generation of drug eluting stents made of absorbable polymers. Our study used artificial plaques to assess potential differences in degradation of absorbable polymers with respect to known physiological differences in the composition and chemistry of atherosclerotic plaque.

Abstract:
Drug eluting stents (DES) are combination products that consist of a composite coating of drug and polymer on a stent scaffold to treat atherosclerosis. The novel generation of DES incorporates abrogable polymers that completely degrade in-vivo. In 2016, CDRH granted marketing approval to the first fully absorbable stent for treating coronary artery disease, thereby increasing access to an innovative technology that patients have requested for years. The degradation behavior of these absorbable implants is extremely complex and impacted by a myriad of factors, including polymer properties and chemistry of the implant location. It is well established that the composition of atherosclerotic vessels can vary dramatically with patients' age and gender. However, these factors are not considered in the design of DES devices, i.e. all patients are treated with the same device. If the local effect is significant, treatment with the same device,

either a DES with an absorbable coating or an absorbable scaffold, may lead to different clinical outcomes.

To address this regulatory challenge, we assessed potential differences in degradation of absorbable polymers with respect to known physiological differences in the composition and chemistry of atherosclerotic plaque. In previous work, we developed an artificial tissue model to elucidate the impacts of sex-based differences in plaque compositions on the drug release and transport behavior of DES. In current project, we exposed absorbable polymer coatings used in DES to the same artificial tissue model, which better emulate the composition of atherosclerotic plaque. To determine the identity and quantities of the intermediate degradation products for polymers degrading in contact with different artificial tissue, we used analytical methods such as Liquid Chromatography Mass Spectrometer (LCMS). In conclusion, we found that plaque composition significantly influenced the degradation behavior of absorbable polymers. The results showed that not only polymer mass loss, but also chemical structure and amounts of degradation products responded to plaque compositions. This research strongly supports the evaluation of the safety and efficacy of DES containing absorbable polymers, and encourages FDA to address the specific needs of women with cardiovascular disease.

### 42. Identifying cellular factors affecting Ebola virus entry in primary macrophages by using virus-like particles under biosafety level 2 conditions

*Authors: Stantchev, Tzanko, FDA/CDER/OBP; Zack-Taylor, Autumn, FDA/CDER; Mattson, Nicholas, FDA/CDER/OBP; Wunderlin, Grant, FDA/CDER/OBP; Clouse, Kathleen A., FDA/CDER/OBP*

Plain Language Synopsis: We have optimized the use of beta-lactamase containing virus-like particles (VLP), pseudotyped with different filovirus surface glycoproteins (GP1,2), to evaluate the impact of various cytokines on filovirus entry into primary human monocyte-derived macrophages (MDM).

Abstract:
Filoviruses, particularly members of the Ebolavirus and Marburg genera, cause severe disease in humans with a very high mortality rate. Macrophages play a critical role during filovirus infection by being one of the first and major sites of virus replication, as well as a source of multiple cytokines. Although presumed to be important, the precise role of cytokines in filovirus pathogenesis has not been adequately studied. A significant obstacle has been the requirement for the highest biosafety level 4 (BSL-4) containment to perform work on wild type (wt) filoviruses. By employing replication incompetent, filovirus GP1,2 pseudotyped, β-lactamase or green fluorescent protein (GFP)-containing VLPs, we have been able to assess the effects of specific cytokines, elevated during Ebola virus disease (EVD), on filovirus-cell fusion under BSL-2 conditions. Of the various cytokines tested, we found that pre-incubation of primary human MDM with interleukin-10 (IL-10) significantly enhanced filovirus entry, an effect consistently observed in cells derived from multiple healthy donors. In contrast, fusion of IL-10-treated macrophages with influenza hemagglutinin/neuraminidase pseudotyped VLPs in parallel was unchanged or slightly reduced relative to mock treated cells. We then demonstrated that the IL-10 effect on filovirus entry is due to increased virus-cell binding. Intriguingly, earlier studies have reported a correlation between elevated serum IL-10 and increased mortality in filovirus infected patients, although the underlying mechanism has not been established. More recently (Panchal, et al.), it was observed that suppression of circulating IL-10 was associated with increased survival in an Ebola virus animal model due to modulation of Natural Killer (NK) cell function and/or interferon γ levels. In summary, our studies have identified a novel mechanism that may account for the IL-10-associated increase in filovirus pathogenicity. In addition, the expertise acquired during these studies will be useful in establishing less hazardous systems for development and evaluation of new filovirus countermeasures.

## 43. Method Development for the Measurement of Cold Flow (CF) in Pressure Sensitive Transdermal Drug Delivery Systems (TDS)

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Plain Synopsis: Recently, a warning letter was issued due to adhesive transfer from a commercial estradiol TDS to the removable release liner (resulted from CF). This type of product quality issue played a significant role in product recalls. Here we developed a method to better determine CF behavior for product quality evaluations.

Abstract:
In TDS, excessive cold flow (CF) is a critical quality issue with pressure sensitive adhesives (PSA). Recently a warning letter was issued due to adhesive transfer from a commercial Estradiol TDS to the removable release liner (resulted from CF). Although the degree of CF is required to be reported in the TDS product specifications (i.e. measured as distance, area change or weight loss), there is no consensus over the appropriate acceptance criterion. In the current study, we studied the CF phenomenon based on intrinsic material physical properties such as viscoelastic behavior. More specifically, the CF was evaluated using rheological testing techniques and expressed as creep compliance (J). In rheological terms, CF of the adhesive should be nominal at low stress, e.g., gravitational effects; and yet, it should be able to maintain its tackiness, and ability to flow and adhere on the skin with a low pressure applied. Here we report a new method to determine CF in Climara® (estradiol) TDS products using Dynamic Mechanical Analysis (DMA). Using a frequency sweep (1 – 200 Hz) at a constant stress of 0.05 MPa, followed by a strain sweep (amplitude 5 to 50 μm) at 1 Hz, the viscoelastic region (LVR) of the TDS was identified under tension mode. Based on the results, we performed a time sweep at 0.15% of strain and 1 Hz for 1 hour. Results showed that the stress increased from 0.035 to 0.060 MP, indicating the rigidity of the sample as a function of time. From this proof-of-concept study, we infer that stress increase was due to the rigid backing membrane, because both elastic and loss modulus remained constant. In addition, scanning electronic microscopy (SEM) revealed a sharp contrast between the rough surface of a time-sweeped sample and a smooth surface of a fresh sample. In house TDS will be prepared to further evaluate the CF behavior of the drug-in-adhesive matrix without interference from the backing membrane in future studies.

## 44. Comprehensive assessment of hepatotoxicity induced by Herbal and dietary supplement

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Plain Language Synopsis: We collected the information about liver injury caused by 42 herbal and dietary supplements (HDS), and more liver injury cases were found in women than in men for some HDS.

Abstract:
Cases of herbal and dietary supplements (HDS) associated liver injury (i.e., HDS-induced liver injury) in clinics have been widely reported. However, HDS are generally not studied and approved as drugs and their regulation is less strict in many countries. The rise in use of HDS in the U.S. market increases the risk of liver injury in certain populations. Specifically, the use of HDS is more popular in women and minority population that consequently leads a higher risk of hepatotoxicity in these groups.

In this study, we conducted a comprehensive assessment of hepatotoxicity of HDS to better understand its impact on public health, especially for women health. First, unique Latin and common names of 42 HDS popularly used in the U.S. market were identified based on the European, U.S. or Chinese pharmacopoedia; and then information related to these HDS

including their application, hepatotoxicity, mechanisms, HDS-drug interaction and sex differences, was collected from monographs, web databases and literature data, using the Latin names of HDS as keywords. We found that 26 HDS (62.0%) were associated with liver injuries, and the clinical patterns of hepatotoxicity vary. Besides, 20 HDS (47.6%), such as Black Cohosh and kava kava, are potentially interacted with other drugs. Importantly, 18 HDS (42.9%) more frequently exhibited hepatotoxicity in women than in men. In summary, the HDS hepatotoxicity dataset collected from diverse sources provides a single entry point for better studying HDS-induced liver injury, which should benefit public health, especially women's health.
This study was supported by Food and Drug Administration Office of Women's Health (OWH).

### 45. Likelihood ratio test based approaches for safety signal detection in large safety databases for drugs/devices

*Authors: Huang, Lan, FDA/CDRH; Zalkikar, Jyoti, FDA/CDER;Xu, Zhiheng, FDA/CDRH;*

*Yao, Zhihao, FDA/CDRH; Chang, Isaac, FDA/CDRH;Tiwari, Ram, FDA/CDRH*

Plain Language Synopsis: This poster provides likelihood ratio test (LRT) based methods for safety signal detection in large drug/device datasets collected from varying sources.

Abstract:
Post-market safety evaluation and safety signal monitoring is very important to the public health and it is urgent to provide the right analytical tools for analyzing the large safety data collected from different studies, sites, and sources. This poster provides likelihood ratio test (LRT) methods for safety signal detection with focus on identifying signals of adverse events (AEs) from a large number of AEs associated with a particular drug/device or inversely for signals of drugs/devices associated with a particular AE, which differ from the traditional meta-analysis with focus on estimation of a parameter such as odds ratio, risk ratio, or risk difference. The methods discussed include regular LRT method for single study or pooled studies and its variations such as the weighted LRT that incorporates the total exposure information by study/site for multiple studies. For illustration, the methods are applied to a hypothetical clinical data for Proton Pump Inhibitors (PPIs) for the effect of concomitant use of PPIs in treating patients with osteoporosis, to Lipiodol (a contrast agent) data obtained from literature for evaluating its safety profiles, and to a class of cardiovascular left ventricular assistance devices (LVAD) using post-market data obtained from Medical Device Reporting (MDR) system. The adverse event information from patient (i.e., patient problem codes) will be used in modeling device-AE event reporting rates in the device exploration. The methods can be extended to analyze longitudinal data collected over time, with exposure to drug/device information, to monitor safety signals over time, and controlling the family-wise Type-I error rate.

### 46. Performance Testing of Tissue Containment Bags for Power Morcellation

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Plain Language Synopsis: The use of laparoscopic power morcellators has come under scrutiny lately as there is a risk for the spread of cancer. To alleviate this, some investigators recommend using the device with a tissue containment bag. Our goal is to develop methods to adequately test the performance of these bags.

Abstract:
Laparoscopic power morcellators are medical devices used to divide tissue into smaller fragments to facilitate removal using minimally invasive techniques that rely on small incisions. In some cases, tissue fragments can be left behind leading to significant complications. A recently published FDA safety communication highlighted the risk to patients undergoing treatment of uterine fibroids with these devices during a hysterectomy or myomectomy. In some cases women undergoing treatment

for fibroids may have an unsuspected uterine sarcoma. Use of laparoscopic power morcellators in these patients carries the risk of spreading cancer cells within the abdomen and pelvic region which may worsen survival. To minimize the risk of spreading cancerous cells, some investigators have recommended the use of tissue containment bags deployed inside the body to isolate the extirpated tissue and morcellator from surrounding tissue/ organs. However, these commercially available specimen bags are not indicated for this type of use.

Device manufacturers evaluate the performance of these devices using standard tensile, burst, dye penetration, and puncture tests. However, these tests are not adequate to evaluate the physiological forces experienced by the tissue containment bags during power morcellation. Additionally, investigators have advocated insufflating the bag with CO2 gas to create a working space for the morcellator and a laparoscope for visualization, adding more force to the system. As a part of this study, we are developing new performance test methods that evaluate the propensity of tissue containment bags to leak when subjected to all of the forces imparted during a power morcellation procedure.

Currently, we tested three different legally marketed tissue containment bag materials in a burst test. This experiment enabled us to estimate the safety factor for each bag by comparing the threshold pressure at failure to the insufflation pressure exerted on the bag during the morcellation procedure.

Ongoing studies with the new performance test methods, will examine potential failure modes for these bags when used with power morcellators.. The results from this study may aid in the development of FDA guidance documents and new testing standards for pre-clinical testing of tissue containment bags used for power morcellation.

**47. Development of a BSL-2 animal model to evaluate safety and potency of Ebola therapeutics and vaccines**

*Authors: McWilliams, Ian, FDA/CDER; Manangeeswaran, Mohan, FDA/CDER; Verthelyi, Daniela, FDA/CDER*

Plain Language Synopsis: New Ebola virus (EBOV) interventions are needed, but EBOV studies require high containment BSL-4 facilities that are expensive and are not widely accessible to researchers. This leads to significant delays in therapeutic development. Therefore, we developed a BSL-2 infection model system to test EBOV therapies for efficacy and safety.

Abstract:
Ebola virus (EBOV) is a BSL-4 pathogen that can cause life-threatening hemorrhagic fever, with mortality rates as high as 90% of cases. The recent 2013-2016 Ebola virus disease (EVD) epidemic was the largest EVD outbreak in history and exposed the absence of therapeutic options and the need for new interventions. A critical hurdle to developing new Ebola therapies is the requirement for scarce high containment BSL-4 laboratories, which can significantly delay GLP studies. The EBOV glycoprotein (eGP) is the only virally expressed protein on the EBOV surface and is essential for viral tropism, attachment, and fusion to host target cells. Additionally, anti-eGP immune responses can protect rodents and non-human primates from lethal EBOV infection, highlighting the importance of eGP. In order to streamline eGP therapy development, we developed a lethal BSL-2 infection model using recombinant Vesicular Stomatitis Virus expressing Zaire eGP (rVSV-EBOVgp). We found that adult wild-type (WT) mice are resistant to rVSV-EBOVgp, while similar infectious doses administered to interferon receptor deficient (IFNAR-/-) mice caused 100% mortality. These data demonstrate that rVSV-EBOVgp can cause lethal infection in immunodeficient mice and that interferon is protective. Because immunomodulatory product testing requires an immunocompetent mouse model, we tested WT neonatal mice susceptibility. Upon infection, ~100% of mice succumbed to disease 15 days post-infection (DPI). Furthermore, rVSV-EBOVgp was present in the brain and eye (a natural reservoir for Ebola in humans) by 9 DPI. Together these data demonstrate that WT neonatal mice can serve as an

immunocompetent BSL-2 model system to test anti-eGP therapeutics.

### 48. Development of a New Generation Microarray Assay for the Detection and Identification of Foodborne Pathogens

*Authors: Christine Yu, Mark Mammel, Jayanthi Gangiredla, Michael Kulka. FDA/CFSAN/OARSA/DMB*

Plain Language Synopsis: We demonstrate the development and application of a new custom DNA microarray for detection of foodborne pathogens. This method is being developed in order to address multi-virus detection and identification in FDA surveillance and outbreak investigations.

Abstract:
The detection and identification of microbial contaminants in food are essential for prevention and investigation of foodborne illnesses. There is increasing demand to develop methods for the rapid and reliable detection of foodborne pathogens. Currently, nucleic acid-based detection remains the method of choice. Microarray analysis, one such nucleic acid-based detection, has been applied to simultaneously detect and genotype multiple foodborne pathogens. The purpose of this study was to develop a new generation custom DNA microarray for detecting and identifying foodborne pathogens from multiple sources of samples. The new array is aimed to achieve better sensitivity and broader pathogen coverage than the previous tiling array with Affymetrix GeneChip design. The new, high-density peg format design is based on individual gene sets of selected virus strain containing a central nucleotide mismatch for paired-complimentary probe set. In addition to common foodborne viruses, the design of the new array specifically increased the sequence density for human norovirus and selected surrogates detection, as well as internal (RNA) controls for food processing.

Hepatitis A virus (HAV) strain HM175/18f and norovirus (NoV) strain MD145 are used as viral targets for protocol optimization and performance evaluation. Viral RNA is either extracted from culture supernatant or synthesized by in vitro transcription. Microarray analysis is performed following the modified Affymetrix GeneAtlas protocol. The new microarray can detect and identify HAV HM175/18f as a subtype IB strain and NoV MD145 as GII.4 genogroup. Multiple approaches to data analysis were applied and assay specificity was confirmed by the complete absence of cross reactivity observed between and/or among unrelated viral species. This method will help expedite FDA response to urgent public health needs in food safety, particularly in the areas of FDA surveillance and outbreak investigations.

### 49. Label-free optical biosensing of single Ebola virions: evaluation with nanofabricated virus-mimicking structures

*Authors: Agrawal, Anant, FDA/CDRH; Stantchev, Tzanko, FDA/CDER; Clouse, Kathleen, FDA/CDER*

Plain Language Synopsis: Optical biosensors can provide rapid and simple point-of-care detection of viruses in biological fluids. To evaluate this new in vitro diagnostic device technology conveniently and comprehensively, we have developed a new type of biohazard-free reference material by fabricating nanoscale structures resembling individual Ebola viruses.

Abstract:
To facilitate the response to biological threats such as the recent Ebola virus disease outbreak, optical biosensors based on scattered light measurement can provide rapid point-of-care detection of unlabeled single virus particles (virions) captured from biological fluids. As in vitro diagnostic devices, these biosensors require CDRH premarket review. In light of the regulatory science needs of both sponsors and FDA staff, highly controlled and stable reference materials producing virus-like signals without exposure to any biohazard are valuable tools to calibrate and evaluate the performance of such biosensors. To date, spherical polymer nanoparticles have been the only non-biological reference material employed with optical biosensing.

Using electron beam lithography, we designed and fabricated nanostructures as reference materials resembling individual Ebola

filamentous virions attached to a biosensing substrate (silicon wafer overlaid with silicon oxide film). To assess the relevance of these nanostructures, we compared their scattered light signals across the visible spectrum to signals recorded from replication-incompetent Ebola virus-like particles (VLPs), which can be studied under Biosafety Level 2 (BSL2) conditions (versus BSL4 required for the wild type virus). We used the nanostructures to examine the relationship of virion geometry and key biosensor parameters (e.g., substrate focal position, polarization state of light) to biosensor signal. Our results independently provide insights into the capabilities and limits of this biosensing method for Ebola virus detection and demonstrate our virus-mimicking nanostructures as an effective approach to calibrate and evaluate in vitro diagnostic devices based on optical biosensing.

### 50. Risk or Protective Factor? An Exploration of Peer Crowd Influence and Youth Cigarette Smoking Risk

*Authors: Walker, Matthew, FDA/CTP; Navarro, Mario, FDA/CTP; Hoffman, Leah, FDA/CTP*

Plain Language Synopsis: Peer crowds, or peer groups with macro-level connections that transcend geography and race/ethnicity can influence health behaviors, and can be a useful strategy for health communication campaigns seeking to target youth. This study investigates the role of peer crowd influence, including dual peer crowd influence, on youth smoking risk.

Abstract:
Background: Peer crowds, defined as peer groups with macro-level connections that transcend geography and race/ethnicity, share a culture with similar interests, lifestyles, and social norms. Examples include Popular, Mainstream, Hip Hop, Alternative, and Country. Peer crowds can influence health behaviors, and can be a useful strategy for health communication campaigns seeking to target youth. The current study investigates the role of peer crowd influence, including dual peer crowd influence, on youth smoking risk.

Methods: Data were collected from 15,831 youth ages 12-17 recruited via social media as part of a study in support of FDA's tobacco education campaign targeting Hip Hop youth. A photo-based peer crowd identification tool (I-Base Survey) was used to measure peer crowd influence. Smoking risk was assed using Mowery's progression to established smoking model and Pierce's susceptibility measures. Five groups of peer crowd influence were analyzed (Mainstream, Hip Hop, Alternative, and Popular), and three mixed groups (Hip Hop/Mainstream, Hip Hop/Popular, and Hip Hop/Alternative) to determine the association between Hip Hop peer crowd influence and smoking risk.

Results:

- Preceded by the Alternative group, more of the Hip Hop group members were experimenters compared to the Popular or the Mainstream groups.
- Smoking risk was larger when combined with Alternative. More of the Hip Hop/Alternative group members were experimenters compared to the Hip Hop group or any other co-influenced group.
- Smoking risk was smaller when combined with mainstream or popular. Fewer members of the Hip Hop/Mainstream and Hip Hop/Popular groups were experimenters compared to the Hip Hop or Hip Hop/ Alternative groups.

Conclusions: Hip Hop peer crowd influence is associated with smoking risk among youth, and other peer crowd influences can increase or diminish this effect. This study provides evidence to support the peer crowd targeting of interventions and investigates the effect of dual peer crowd influence on smoking risk.

### 51. Optimizing pre-analytical parameters of liquid biopsy based in vitro diagnostic assays for cancer disease monitoring and therapy decision making

*Authors: Chikkaveeraiah, Bhaskara, FDA/CDRH; Bijwaard, Karen, FDA/CDRH; Tang, Xing, FDA/CDRH; Goering, Peter, FDA/CDRH*

Plain Language Synopsis: Liquid biopsy involves isolating and enhancing signals of circulating cell-free DNA (cfDNA) or circulating tumor DNA

(ctDNA) fragments from blood that can reveal information related to cancer and prenatal abnormalities. In this project, we will optimize the pre-analytical parameters from blood draw to storage of cfDNA that influence assay performance.

Abstract:
Liquid biopsy represents a breakthrough technology to provide clinicians with current, accurate and minimally invasive detection of their patients' diseases. However, the development of reliable methods to detect ctDNA from plasma continues to be a major challenge. FDA is attempting to ensure rapid and successful expansion of this technology into the clinical setting by proposing to define the critical factors that impact the greatest limitation to its implementation, the availability of cfDNA from patient specimens. This technology offers many advantages compared to current biopsy of tissue such as (1) providing an accurate and current reflection of the tumor genotype, (2) minimizing patient risk from invasiveness procedures and (3) enabling tracking disease progression over time. Currently, two cfDNA based PMAs have been approved and more submissions are expected in the near future. However, major obstacles still remain in translating the cfDNA analyses to clinical practice. These include limitations in isolating sufficient cfDNA from the blood, limitations in specimen availability for assay performance validation, lack of uniform protocols and controls, and differences in the biological behavior of different tumors (e.g., shedding rate of cells). Furthermore, there is an incomplete understanding of the impact of pre-analytical handling steps from blood draw to storage of cfDNA on cfDNA quality/quantity, and ultimately on the precision and accuracy of assay results.

The specific goal of this project is to 1) provide unbiased, peer-reviewed data relevant to assessing the performance of liquid biopsies, and 2) understand the pre-analytical factors that impact performance parameters for use with future regulatory submissions. In early feasibility experiments, we identified gene sequences from the literature and gene databases for the epidermal growth factor receptor (EGFR) gene deletion mutation (exon 19 deletion, p.E746_A750delELREA) and a exon 21 point mutation (L858R) for non-small cell lung cancer (NSCLC), as a model cancer based on one of the PMAs that has been approved. We optimized steps involved in the PCR assay to synthesize the best quality mutant fragments.

## 52. A Suite of Phantom-Based Image Quality Test Methods for Photoacoustic Imaging Systems

*Authors: William C. Vogt, Congxian Jia, Keith A. Wear, Brian S. Garra, Joshua Pfefer*

Plain language synopsis: Photoacoustic Imaging (PAI) is an emerging technique that combines lasers and ultrasound for applications including deep vascular imaging and mammography. However, robust image quality test methods are needed for PAI device evaluation and intercomparison. We have developed tests incorporating breast-mimicking phantoms and evaluated performance of a custom PAI system.

Abstract:
Photoacoustic Imaging (PAI) is a rapidly emerging technology that combines pulsed laser irradiation with acoustic detection to produces images with optical absorption-based contrast, spatial resolution similar to ultrasound, and penetration to several centimeters. Clinical applications include vascular imaging, tissue oximetry, cancer detection, and surgical guidance. One of the strongest potential applications is mammography, where PAI can provide deep tissue oxygenation mapping that may aid in diagnosing suspicious lesions. Thus, PAI may address a critical public health need for improved early breast cancer detection. While PAI is quickly maturing, there are no standardized performance test methods for assessing system performance (i.e., image quality). Objective, quantitative, and least-burdensome test methods are needed to facilitate device evaluation, intercomparison, and quality assurance. For mature medical imaging modalities such as CT, MRI, and ultrasound, tissue-mimicking phantoms are frequently incorporated into consensus

standards for image quality characterization and performance testing. A well-validated set of phantom-based test methods is needed for evaluating performance characteristics of PAI systems. To this end, we have constructed a group of tissue-mimicking phantoms using a custom PVC plastisol material with biologically relevant optical and acoustic properties. Each phantom enables quantitative assessment of one or more image quality characteristics including 3D spatial resolution, spatial measurement accuracy, ultrasound/PAI co-registration, uniformity, penetration depth, sensitivity, and linearity. Phantoms contained targets including high-intensity point source targets and dye-filled tubes. This suite of phantoms was used to measure the dependence of performance of a custom PAI system (equipped with four interchangeable linear array transducers) on system design parameters. Phantoms also allowed comparison of image artifacts, including surface-generated clutter. Phantom test results were compared to data acquired with targets embedded in layered stacks of chicken tissue. Results showed that transducer design parameters create strong variations in performance including a trade-off between resolution and penetration depth, which could be quantified with our method. This study demonstrates the utility of phantom-based image quality testing in device performance assessment, which may guide development of consensus test methods for PAI systems.

## 53. Preliminary Data on the Effectiveness of Non-Contact Infrared Thermometers (NCITs)

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*Affiliations: Center for Devices and Radiological Health, U. S. Food & Drug Administration, Silver Spring, MD and University of Maryland, Baltimore County, Baltimore, MD*

Plain Language Synopsis: NCITs are used as an early fever detection tool for screening and isolating sick individuals in healthcare settings and in ports of entry during a disease outbreak. The temperature measurement accuracy of NCITs was validated using a black body emitter and is being assessed with subjects using real-world clinical conditions.

Abstract:

Previous studies using clinical data from hospitals and transit centers have been inconclusive regarding the effectiveness of NCITs as a screening method during severe acute respiratory syndrome (SARS) and other influenza outbreaks [1-3]. When people were screened for fevers caused by the SARS virus, the sensitivity of NCITs varied from 4% to 90% in comparative studies [1-3]. As a result of this measurement variability, there is an urgent need to ensure the manufacturers' labeled temperature values are accurate in real-life situations. The primary concern is that NCITs might underestimate body temperature resulting in false negatives, which would significantly undermine efforts to counter the threat of spreading pandemics.

Methods: Ten samples each of six different commercially available NCIT models were tested using a black body calibration method adapted from the ASTM E1965-98(2009) Standard Specification for Infrared Thermometers for Intermittent Determination of Patient Temperature procedure for NCITs. Device accuracy was determined based on comparisons to a reference thermometer and on the manufacturers' labeling specifications. The NCIT samples will also be used to obtain temperature measurements on both febrile and non-febrile subjects in a clinical setting.

Preliminary Results: Preliminary uncorrected black body results revealed all NCIT models displayed temperatures above the reference from 0.8 to 6.0 °C with errors from 0.2 to 1.0 °C due to a combination of measurement error and the built-in algorithm offset error. Continuing efforts are underway to correct the data. An interim analysis of the NCIT measurements obtained for the initial subjects in the clinical study will be compared to the reference thermometer for accuracy and performance. This work will reveal current limitations

of NCITs to produce accurate temperature measurements and may identify the root causes of those inaccuracies.


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**54. Title: Optimization of Zika Virus Inactivation in Whole Blood via UV Irradiation**


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Plain Language Synopsis: Transmission of Zika Virus (ZIKV) via blood transfusion presents a serious risk for pregnant women and other highly susceptible patients. These studies demonstrate for the first time successful UV-mediated inactivation of ZIKV in whole blood samples, with and without photosensitizer. Potential means for further optimization of virus inactivation are highlighted.


Abstract:
Transmission of Zika Virus (ZIKV) via blood transfusion presents a threat to pregnant women, their infants, and others at high risk of severe infection outcomes. Even in asymptomatic patients, ZIKV infection can produce viremia, with viral burdens in the blood sometimes exceeding 106 copies/ml. Several cases of ZIKV transmission linked to blood donations from asymptomatic donors have been reported in Brazil, and in areas with active infection, ZIKV has been detected in approximately 1 to 3% of locally collected blood donations. Inactivation of ZIKV in transfusion products remains a possible avenue for limiting risk of transmission to patients. Pathogen reduction (PR) by licensed methods can reduce ZIKV load in plasma and platelet preparations. However these licensed methods have not been shown effective in whole blood, which is often transfused in lieu of purified blood components in trauma and combat settings, and in developing nations. Therefore a method that can inactivate ZIKV in whole blood is of great interest. The Cambodia and Puerto Rico strains of ZIKV were cultured in A. albopictus C6/36 mosquito cells to prepare high-titer stocks, which were spiked into whole blood samples. ZIKV-spiked blood samples were treated with UV-A or UV-B at doses of 3 to 155 J/cm2. In some experiments, UV treatment was combined with the photosensitizing compounds psoralen or vitamin B2. Remaining ZIKV post-UV treatment was quantified by TCID50 assay and by nucleic acid testing. Log reduction of infectious ZIKV was confirmed in a susceptible mouse model of infection. In spiked whole blood samples, ZIKV reduction of 4 to 5 logs was achieved with either UV-A plus psoralen photosensitizer, or with UV-B treatment, when the inactivation was carried out in minimal depth containers made of plastic that permits UV transmission. Standard blood bag plastics and sample depth limited ZIKV inactivation efficiency. Red cell integrity was enhanced during pathogen reduction with the addition of a licensed stabilizer. Our ongoing studies aim to further optimize UV irradiation and photosensitizer parameters and blood container materials to maximize ZIKV inactivation. This information may accelerate use of UV-mediated pathogen reduction technologies in whole blood preparations, to increase patient safety during transfusion in high-risk settings. These contributions are an informal communication and represent the authors' best judgment. These comments do not bind or obligate FDA.


**55. An inexpensive, portable, and user friendly method to test for inorganic arsenic in rice**


*Authors: Gray, Patrick, FDA/CFSAN*


Plain language synopsis: Rice contains ten times more arsenic than other grains. Rapid

analytical methods are needed to ensure compliance with FDA's proposed action limit of 100 ppb inorganic arsenic in infant rice cereal. We present an inexpensive and portable testing method suitable for rice mills wishing to pre-screen rice for inorganic arsenic.

Abstract:
Arsenic is found in rice in two forms: organic arsenic which is non-toxic, and inorganic arsenic which is carcinogenic. Testing for inorganic arsenic in rice is usually performed using complex equipment that costs hundreds of thousands of dollars. This expensive instrumentation is a barrier to expanded testing and monitoring. Rapid analytical methods for determining inorganic arsenic analysis are needed to ensure compliance with FDA's proposed action limit of 100 ppb inorganic arsenic in infant rice cereal.

We present a new method that measures inorganic arsenic in rice. The new method does not require expensive instrumentation, lab space or even highly trained personnel. Instead, it uses simple and targeted chemical reactions using pre-weighed and portioned reagents for a simple and cost-effective analysis. We first grind dried rice in a coffee grinder, weigh out a portion of rice into a flask and then boil the rice in very dilute swimming pool muriatic acid. This step extracts arsenic from the rice into solution. After the rice solution cools, we add a commercially available pre-portioned sachet of sulfamic acid and a premade tablet of sodium borohydride. The reagents combine to form arsine gas which bubbles from the solution. We catch the arsine gas on an indicator filter paper. The filter paper darkens as arsine gas is trapped, and then a battery powered color comparator displays the inorganic arsenic concentration.

The new inorganic arsenic testing method costs less than $5 per test including all equipment and reagents. There is no calibration, no expensive laboratory space and no extensive training required. From beginning to end the testing procedure is less than one hour per sample. We show ±15% accuracy at the FDA's proposed limit of 100 part per billion inorganic arsenic in infant rice cereal.

## 56. Assessment of Common Portable Automated External Defibrillator's (AED's) for Sensitivity, Specificity and Defibrillation Waveform Parameter Performance

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Plain Language Synopsis: Automated External Defibrillator's (AED's) potentially save lives during cardiac arrest. From 2005 to 2009, FDA received over 23,000 reports of adverse events for AED's; 5% of these may have contributed to patient harm or death. We propose to develop a method to evaluate AED essential functions and critical parameters.

Abstract:
In the United States there are 300,000-400,000 deaths per year from sudden cardiac arrest. Most cardiac arrest deaths occur outside of the hospital. Out-of-hospital survival rates are 1 to 5 percent. Treatment, namely Cardiopulmonary resuscitation (CPR) and defibrillation, is very time sensitive.

Abnormal heart rhythms, with ventricular fibrillation (VF) being the most common, cause cardiac arrest. Treatment of VF with immediate electronic defibrillation increases survival by 90 percent. Every minute of delay in defibrillation causes a 10 percent increase in mortality. Effective defibrillation depends on applying a sum of energy, which is represented as a function of current and voltage over time. While the therapeutic agent is current, the therapeutic dose is best described in terms of energy (joules). In ventricular and atrial defibrillation, cardioversion energy is a good descriptor of therapeutic dose.

The aim of this study is to develop a test method that can be validated for Automated External Defibrillator's (AED's). Our WEAC team has created an integrated plan for the evaluation of AED's essential functions and critical parameters. This consists of inputting simulated Electrocardiogram (ECG) waveforms and acquiring output data for variables including the energy delivered by the AED to a

patient.

These input waveforms are from selected AED models sold in the U.S., they include ECG waveform, heart rate (a waveform type), ECG voltage amplitude, and rhythm classifications such as VT (poly), VT (Mono), VF (coarse), VF (fine), Asystole, Normal Sinus, Energy levels, shockable and non-shockable rhythms. If the ECG changes from a non-shockable rhythm to a shockable rhythm, the algorithm's response may vary by manufacturer. Shockable rhythm and nature of the rhythm is included in the analyzed data. Using a commercially available test simulator, WEAC has automated data acquisition for sensitivity, specificity and other variables. The WEAC method combines the simulator software with a spreadsheet matching the AED input ECG waveforms with respective output parameters like energy. AED rhythm algorithms have unique input waveforms with significant variations among different models that trigger a shock event.

**57. Identification of viral reservoirs in Zika virus infected macaques to inform polices for human cells, tissues, and cellular and tissue-based products**

*Authors: Michelle McClure, Tracy MacGill, Richard McFarland, Robert Orr (FDA)*

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Plain Language Synopsis: The FDA and University of California, Davis partnered to investigate which tissues Zika virus targets in non-human primates. Information from these studies will inform FDA policies by increasing our understanding of the risk of Zika virus transmission through infected tissue products and ultimately improving product safety.

Abstract:
As part of its mission to protect public health, FDA creates policies to minimize the risk of transmission of infectious diseases through human cells, tissues, and cellular and tissue-based products (HCT/Ps). This effort includes methods designed to ensure that donors are not infected with certain infectious diseases that could be transmitted through an HCT/P resulting in harm to the recipient. An emerging infectious disease, such as Zika virus (ZIKV), presents potential new policy considerations due to lack of knowledge regarding tissue tropism. For example, prolonged persistence of ZIKV has been observed in tissues such as placenta and semen. However, the presence and persistence of ZIKV, and therefore its potential for transmission, in other tissues are largely unknown. In an effort to better understand this risk, FDA partnered with the California National Primate Center at the University of California, Davis to perform studies to investigate ZIKV tissue tropism using a nonhuman primate model. For this study, reverse transcription polymerase chain reaction (RT-PCR) was first used to detect and quantify levels of ZIKV RNA in 38 different tissues from 4 pregnant rhesus macaques inoculated intravenously with a Brazilian strain of ZIKV during the 1st or 2nd trimester. ZIKV RNA was detected in selected tissues from pregnant macaques 7, 65, 87, or 105 days post-inoculation. The acutely infected animal sampled 7 days post-inoculation had the highest rate of ZIKV RNA positive tissues (with levels up to 8.8 log10 RNA copies/gram tissue), while the other 3 animals had fewer positive tissues with lower ZIKV RNA levels (up to 6.4 log10 genome copies/gram tissue). Lymphatic and genitourinary tract tissues were primary reservoirs, as evidenced by ZIKV RNA in all four animals. Some macaques also contained detectable ZIKV RNA in skin, cardiopulmonary, musculoskeletal, and digestive tract tissues. Despite the presence of ZIKV RNA in many tissues, infectious ZIKV was rarely detected via plaque assay. In situ hybridization labeling ZIKV RNA in tissues was also performed. This information, together with additional ongoing studies, will inform FDA with regard to the risk of transmission of ZIKV through HCT/Ps and development of appropriate policies to minimize risk.

## 58. Portable Devices for Developing Rapid Vibrational Spectroscopic and Chemometric Methods for the Analysis of Omega-3 Dietary Supplements

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Plain Language Synopsis: This research focused on using portable spectroscopy devices to analyze omega-3 dietary supplements and compare label declarations with product compositions.

Abstract:
Dietary supplements containing omega-3 polyunsaturated fatty acids (FA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are among the most commonly used non-vitamin, non-mineral dietary supplements in United States because of their potential health benefits. Increased demand for omega-3 products has required rapid and accurate analytical techniques for quality assurance. We recently developed an attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopic method combined with partial least squares regression (PLSR) analysis for the rapid quantitative prediction of FAs in marine oil omega-3 dietary supplements (Karunathilaka et al. J. Agric. Food Chem. 2017; 65:224). For this method, a calibration library was generated using 174 mixtures of marine oils, and test samples that included neat marine oil dietary supplement products. We sought to improve upon this procedure by building a robust calibration library comprised of a wider variety of marine oils and larger concentration ranges for FAs and FA classes. Using portable devices, ongoing research includes the development of ATR-FTIR, near infrared (NIR), and Raman spectroscopic methods combined with PLSR for predicting full fatty acid compositional information. This simple, rapid approach has the potential to be used in the analysis of omega-3 products for verifying label declarations and also in quality control and monitoring during production.

## 59. Leveraging Genomics to Measure Antimicrobial Resistance: Development of Genotypic Cutoff Values

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Plain Language Synopsis: Antimicrobial resistance is one of the greatest threats to public health in the 21st century, as it has complicated the treatment of serious bacterial infections. Genomics has enhanced our understanding of how antimicrobial resistance arises, which led us to develop genotypic cutoff values (GCVs) as an improved measure of resistance.

Abstract:
Whole-genome sequencing has transformed our understanding of antimicrobial resistance, helping us to better track and identify the genetic mechanisms underlying resistance. However, epidemiological cutoff values (ECOFFs), which are designed to measure emerging resistance in bacterial populations, do not take advantage of the robust genomic datasets that are already publicly available. Instead, ECOFFs are based only on phenotypic susceptibility data determined by in vitro susceptibility testing. While these data are valuable, in the absence of underlying resistance mechanisms, it can be unclear how to differentiate isolates with and without resistance mechanisms based on phenotype alone. As a result, from combined genomic and phenotypic data for 1,738 Salmonella isolates, we established what we term genotypic cutoff values (GCVs) for 13 antimicrobials for Salmonella. These GCVs refer to the highest susceptibility value at which an isolate is expected to have no acquired resistance mechanism. This definition of GCV is distinct from ECOFFs, which currently differentiate 'wild-type' from 'non-wild-type' strains based on susceptibility data in the absence of knowledge regarding genetic information. We believe that GCVs are an improved measure of resistance, encapsulating all the prevailing knowledge of resistance mechanisms while

utilizing the existing phenotypic resistance data.

### 60. Development of a Physiological Screening Strategy that Improves the Post-Enrichment Recovery of Shigella and Enteroinvasive Escherichia coli from Mixed Bacterial Populations

*Authors: Kim, Jina, University of Maryland / JIFSAN; Pirone-Davies, Cary, FDA / CFSAN; Duvall, Robert, FDA / CFSAN; Agbaje, Oluwaseun, FDA / CFSAN; Hall, Sherwood, FDA / CFSAN; Binet, Rachel, FDA / CFSAN*

Plain Language Synopsis: Shigella and enteroinvasive Escherichia coli cause bacillary dysentery, and are difficult to isolate from contaminated foods due to the lack of specific discriminating growth medium. Here we developed a selective step based on their inherent tolerance to pH 2.2, improving their recovery yield by up to 1,000 fold.

Abstract:
While most diagnostic tools rely today on molecular methods for pathogen detection and/or identification, regulatory agencies prefer to isolate the adulterant before taking enforcement action. In the absence of a specific selective enrichment medium for Shigella and the phylogenetically related enteroinvasive Escherichia coli (EIEC), the recovery of these pathogens from food samples is challenged by the concomitant growth of the food background flora. To improve detection, we evaluated incubation in a low pH environment to enrich for Shigella and EIEC by killing the background flora prior to isolation. Initially the acid tolerance of 63 EIEC, 78 Shigella and 28 "background" isolates was assessed in pure culture. While a 15-min incubation in Tryptic Soy Broth (TSB) pH 2.2 caused little harm to most of the bacterial strains tested to date, a 5-minute incubation in acid lacking nutrients (i.e 0.2 M KCl pH 2.2) appeared optimal in killing the "background" strains, with little effect, if any, on the Shigella and EIEC isolates. Subsequently, the bacterial populations from six different food types (cilantro, romaine lettuce, alfalfa sprouts, green onions, spinach and salmon), and six different isolates of Shigella or EIEC, obtained from 24-hours growth in Shigella broth, in presence or absence of oxygen, were mixed to various ratios prior to acid treatment and recovery on selective MacConkey agar (MAC), used in the FDA Bacteriological Analytical Manual's (BAM) method for Shigella, or additional chromogenic agars designed to enhance the differentiation of Shigella [Biolog Shigella/ Aeromonas Rainbow, and Hardy diagnostic HardyCHROM SS]. We consistently observed an enrichment in the Shigella/EIEC – like colonies following 5-minute incubation in 0.2 M KCl pH 2.2, compared to incubation in TSB pH 2.2 for up to 30 minutes, producing up to a three-log improvement in the recovery yield of the pathogens when a fluorescent Shigella strain was used for quantification. While neutralization with monopotassium phosphate after the 5-minute acid treatment allowed some of the background cells to resuscitate, the recovery yield of the pathogens remained better than after 30 minutes treatment with TSB pH 2.2. Amending the BAM will improve our ability to detect foods contaminated by Shigella or EIEC.

# Poster Session 2 (Day 1, PM)

Scientific Topic: Additive Manufacturing and 3D Printing

1. **Evaluation on Raman Spectroscopy as a PAT Method for Monitoring the Continuous Crystallization of Carbamazepine**

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Plain Language Synopsis: Continuous Manufacturing of pharmaceuticals has become popular due to its potential greater assurance of product quality and economic savings. Process Analytical Technology (PAT) tools can significantly enhance process monitoring abilities in continuous manufacturing. Here, we developed and validated a Raman Spectroscopy PAT method to monitor a continuous crystallization process.

Abstract:
Implementation of a well-justified process monitoring approach including Process Analytical Technology (PAT) tools is a key component of the control strategy in continuous manufacturing. PAT tools are continuously evolving which facilitates their adoption in a variety of unit operations. Crystallization has a significant impact on the properties of the active pharmaceutical ingredient (API) and the downstream operations, since it is the final step in the manufacturing of the drug substance and determines particle size distribution, purity, polymorphs, etc. Many publications have focused on the implementation of PAT tools for monitoring batch and continuous operation; however, a comprehensive evaluation of the development of Raman spectroscopy to monitor continuous crystallization has not been presented. Specifically, a calibration model to monitor the solute concentration of a specific API (Carbamazepine) was developed and validated here; the limit of detection for a metastable polymorphic form was quantified. The study was based on the cooling crystallization of Carbamazepine to produce the most stable form (Form III). The impact of multiple factors, such as solid content, and temperature, on the method was studied. The method was validated following the principles described in USP <1225> Validation of Compendial Procedures. The results demonstrated that a linear model can predict the solute concentration with less than 10 percent error. The repeatability and intermediate precision was evaluated and the relative standard deviation was below 5 percent. The limit of detection and quantification were 3.8 and 7.4 mg/ml, respectively. A robustness analysis through design of experiment showed that changes in laser power and agitation speed can affect the model prediction. The limit of detection for the metastable form (form II) was determined by monitoring the ratio of characteristic peaks when increasing the percentage of the metastable form in the total amount of crystals in the solution. A significant change on the peak ratio was observed after 22.9 % g form II/g total solid was present. The knowledge developed in this project will enhance the Agency's understanding towards similar processes and aid future regulatory reviews of PAT for continuous manufacturing.

**Disclaimer: This abstract only reflects the views of the authors and should not be construed to represent FDA's views or policies.*

2. **Evaluation of Quantitative Imaging Biomarkers with Customized 3D-Printed Phantoms: Application to Liver Tumor Volumetry with Computed Tomography**

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Plain Language Synopsis: Quantitative information, for example tumor volume, is routinely extracted from medical images to characterize disease status. The creation of objects with available ground truth for assessment of quantitative biomarkers is a new application of 3D printing. We present our work

on a 3D-printed liver phantom for evaluating tumor volume measurements.

Abstract:
Imaging phantoms enable the evaluation of repeated quantitative measurements with respect to known ground-truth (reference standard). 3D printing has become increasingly available in a variety of biomedical applications. In this study, we created a modular, anthropomorphic liver phantom to analyze tumor sizing across computed tomography (CT) imaging protocols. This liver phantom was printed on the Stratasys Objet 260 3D printer in the Additive Manufacturing of Medical Products Laboratory at FDA. The phantom was tailored for evaluating CT tumor volume measurement error across specific tumor sizes and radiodensities.

In order to design a phantom with a range of tumor-to-background contrast, we first developed a method for dithering two printing materials (Tango Black Plus and Vero Clear). Rather than mixing the materials directly, they are placed adjacent to each other on a sub-voxel scale. By adjusting the fraction of each material, the tumor CT values varied linearly between 85 and 125 Hounsfield units (HU, a standard CT imaging radiodensity scale), with background value of 80 HU. The resulting phantom has low cost relative to commercially available products, and is highly customizable. Tumors can be printed over very small size increments in order to determine the minimum detectable change, and the liver background can be uniform, complex, or based on clinical CT images.

The 3D-printed phantom was used to assess the effects of CT dose, and reconstruction method on the volume measurement bias and repeatability. The findings of the study will complement clinical data and will inform guidelines and standards toward the reliable and least-burdensome use of quantitative imaging biomarkers.

**3. 3D-printed cerebrovascular phantoms for performance testing of near-infrared optical diagnostic devices**

*Authors: Liu, Yi, FDA/CDRH; Ghassemi, Pejhman, FDA/CDRH; Wang, Jianting, FDA/CDRH; Pfefer, Joshua, FDA/CDRH*

Plain Language Synopsis: We made 3D printed models of the human brain for testing of diagnostic devices based on near-infrared light. One model has cylindrical blood vessels, and another with a realistic brain shape is generated from MRI images. Our results indicated that 3D printing's promise as test tools for innovative light-based technologies.

Abstract:
Innovative near infrared (NIR) imaging and spectroscopy devices for noninvasive cerebral oximetry and intraoperative tumor visualization hold promise for improving patient care. However, there are no well-validated test methods for system development, standardized preclinical testing and quality control. 3D printing represents a novel approach for fabricating phantom-based test methods that are reproducible, easily disseminated and can provide customized morphology and optical properties.

We have developed and evaluated two preliminary 3D-printed cerebrovascular phantoms for NIR optical diagnostics. The first is a non-biomimetic turbid phantom with an array of linear cylindrical channels representing blood vessels. By injecting hemoglobin with a range of CO-oximeter referenced saturation levels into the channels, it was possible to test the accuracy of two commercial cerebral oximeters. We also designed a biomimetic cerebrovascular phantom by modifying an existing segmented human cerebral MRI image volume into a printable morphology. Polymer printing materials were customized with similar optical properties as white matter, gray matter and dura matter. A mixture of indocyanine green fluorescent dye, human serum albumin and hemoglobin were then injected into the vascular network and imaged with a custom NIR fluorescence system. Finally, the effect of 3D printing parameters (e.g., print orientation) on phantom quality was evaluated using micro-CT imaging.

Our results indicated that 3D-printed cerebrovascular phantoms have significant

promise for evaluation of NIR oximetry and fluorescence imaging systems, and may serve as effective medical device development tools (MDDTs) for emerging biophotonic technologies.

**4. Design and Evaluation of a Clinically Relevant Challenge Device for Cleaning of Additive Manufacturing Residues**

*Authors: Hernandez, Jorge, FDA/CDRH; Coburn, James, FDA/CDRH*

Plain Language Synopsis: This work addresses the need for a clinically-relevant cleaning challenge device for use with cleaning protocols for manufacturing residues in AM devices. It may serve as a starting point for helping evaluate cleaning protocols or establish a worst case standard for cleaning of AM residues in medical devices.

Abstract:
Additive Manufacturing (AM) has enabled medical device manufacturers to produce highly intricate and patient-matched devices more easily than in the past, leading to more AM medical products and increased research interest over the last decade. The ability to produce intricate internal and porous features particularly increases considerations for cleaning processes. To be suitable for implantation, manufacturing residues and debris must be sufficiently removed using pre-determined acceptance criteria for cleanliness of that AM device. These challenges are specific to the intended use, geometries, and AM technique. While test devices have been produced to characterize capabilities of 3D printers, none has been designed to challenge cleaning protocols with complex geometries relevant to AM medical devices.

Researchers created a challenge device representing possible worst case cleaning scenarios. It contained six circular wedges assembled over a circular base. Each wedge contained lattice structures simulating macro-porosity typical to AM implants. Assembled wedges alternately had open porosity (two exposed faces) and blind porosity (one exposed face). The device can be assembled immediately after manufacturing and prior to post-processing, thereby comparing various cleanable sizes and thicknesses with open and blind porous structures in a single test assembly.

Evaluation of the challenge device was performed with powder-based AM techniques, using cleaning protocols representative of industrial methods. Quantitative and qualitative cleaning assessments were performed, with preliminary results showing the device may represent a challenging cleaning test piece. Residual powder from the AM process was observed in all wedges with 750 µm pores, upon completion of both tested protocols. Visual inspection of disassembled wedges showed distinct debris patterns for wedges cleaned with blind or open porosity, indicating both a directional nature of the steps making up the cleaning protocols, and the need to tailor protocols to the geometrical features of the device to be cleaned.

The designed device effectively represents a gradation of hard-to-clean porous medical device features. Its modular nature allows non-destructive evaluation and visualization of internal portions of the individual wedges after completion of cleaning protocols. Future testing with the cleaning challenge device will involve additional AM techniques and lattice types.

**5. Dimensional Deviation of Additive Manufactured Test Specimens**

*Authors: Hernandez, Jorge, FDA/CDRH; Coburn, James, FDA/CDRH*

Plain Language Synopsis: This work examines dimensional discrepancies between modeled 3D objects and their AM products, as well as variability introduced by powder-based AM technologies. It explores simple and complex test devices and metrics that may be used to identify points of variability within an AM process or measure variability in complex parts.

Abstract:
Additive Manufacturing (AM) technologies that can make complex and personalized parts have found increasing numbers of applications to medical products. The design freedoms brought by AM come with additional technical

challenges due to the manufacturing method. Some of these are possible dimensional discrepancies between the 3D digital designed model and the built product, as well as dimensional variability of parts from different builds and different locations and orientation within the build volume. The current work aims to identify points of variability with specific AM techniques and determine metrics to detect them.

Parts were built from the same 3D model using industry-grade, powder-based metal and polymer AM systems. Simple geometries were modeled as hollow cubes and half cubes, while complex geometries were modeled as macro-porous lattice structures. Cube/half cube wall width, height and thickness measurements were taken relative to the build direction using a digital caliper. Complex test parts were scanned with a micro-CT unit to extract measurements of lattice strut diameter.

Deviations of feature dimensions were observed between designed and built models for all parts and manufacturing methods tested. Most of the deviations measured on the simple parts were within the expected ranges based on the machine specifications, although some were larger in magnitude. Features of metal parts fabricated along the build direction (z axis) were generally smaller than designed, both for 10 mm and 1 mm scales. For polymer parts, features on the x-y plane were generally smaller than designed. Scanned, complex parts showed deviations from the designed strut diameters between builds and at different locations. Additionally, powder residues inside complex parts could be segmented from sintered material in the micro-CT imaging, increasing knowledge about the inner structures and residual powder remaining inside the parts.

Preliminary results indicate the need to closely monitor system performance and to develop process metrics that can identify parameters causing dimensional deviations and variability. Complexity can alter the curing, melting, or sintering profile of the printer and may create a new worst case condition. Therefore, increasingly complex parts may represent a more challenging test of dimensional deviation from CAD designs and of manufacturing variability.

### 6. Evaluating Cell and Materials Effects on the Quality of 3D Printed Cell-Laden Hydrogels

*Authors: Baker, Hannah B., FDA/CDRH and U. of Maryland; Jain, Tanmay, FDA/CDRH and U. of Akron; Fisher, John, U. of Maryland; Joy, Abraham, U. of Akron; Kaplan, David S., FDA/CDRH; and Isayeva, Irada, FDA/CDRH.*

Plain Language Summary: 3D printing benefits tissue engineering field by enabling fabrication of customized scaffolds with precise architecture to mimic a native tissue. Our goal is to evaluate the quality of 3D printed cell-containing soft scaffolds, in which cells can maintain their viability and function, to develop into a desired tissue or organ construct.

Abstract Text:
3DP benefits tissue engineering (TE) applications by enabling fabrication of customized scaffolds with precision architecture that can mimic a native tissue. The investigation of TE applications involving 3DP of multipotent stem cell-laden hydrogels has become increasingly popular. In order to translate these technologies into viable therapies it is necessary to understand how the cells and 3DP processes impact one another. In particular, the addition of a cellular component can alter physicochemical and viscoelastic properties of a printable resin, which in turn may influence the homogeneity of the cell suspension, by either impeding or inducing cell sedimentation. Moreover, the shear stress imparted during the 3D printing process can influence cell viability and behavior. This study sought to investigate these considerations in efforts to gather better information which can be used to develop standards and regulations pertaining to 3DP of cells and natural biomaterials.

Gelatin methacrylate (GelMA), a naturally-derived, photo-crosslinkable hydrophilic biopolymer, was used for cell encapsulation and co-printing in an extrusion based 3D printer (Bioplotter, EnvisionTec). Two cell types: immortal mouse fibroblasts (L929) and

human bone marrow derived mesenchymal stem cells (hMSC) were utilized. Cells were suspended in GelMA solution (10%w/v in PBS) at 1, 5, or 10 x106 cells/mL concentrations prior to printing and assessment. A rheology study was performed to evaluate the impact of cells on material properties, consisting of viscosity, gelation time, and temperature hysteresis. Alterations of cell properties in the cell-laden GelMA solutions were evaluated for sedimentation within the printer cartridges via DNA quantification of extruded volume fractions.

Rheological data indicated the addition of cells reduced viscosity of GelMA at high shear rates (>102 1/s) while temperature hysteresis and gelation kinetics were not affected. Sedimentation was not apparent for any cell concentration examined. These results illustrate 3DP resin properties are altered through the addition of cells with exacerbated changes occurring at shear rates associated with 3DP, which in turn necessitate changes in printing parameters to maintain print quality. Herein, we have established a useful system for further evaluation of the relationship between cells and scaffolds to better inform translation of 3DP applications.

**7. Effect of Bioactives on Extrusion Based 3D Printing of Soft Degradable Polyesters**

*Authors: Jain, Tanmay, FDA/CDRH and U of Akron, OH; Saylor, David, FDA/CDRH; Patel, Viraj, U of Maryland; Kaushal, Rahul, U of Maryland; Joy, Abraham, U of Akron, OH; Isayeva, Irada, FDA/CDRH.*

Plain Language Synopsis: 3D printing is an emerging technology that will allow for personalized approaches to medical device implants to be designed for specific patients. Our goal is to develop and evaluate 3D-printed scaffolds that can release drugs and break down over time, to enhance patient outcomes.

Abstract:
3D printing has enabled bench-top fabrication of customized constructs with intricate architectures. However, the printing of most biomaterials requires the addition of photoinitiators, plasticizers, solvents as well as high temperatures to achieve optimal print quality. Such processing conditions raise regulatory questions related to material stability and device biocompatibility. These questions are amplified when it comes to processing of bioactive materials, i.e., materials containing bioactive molecules, such as pharmaceuticals and biologics designed to elicit desired biological response when implanted.

We recently reported the synthesis and 3D printing of biodegradable soft polyesters without solvent, monomer, initiator, and at room temperature.1 The ability to print these polymers under benign conditions made these materials especially attractive for printing of bioactive polymer scaffolds. Though addition of bioactive fillers may cause the desired biological response, these fillers can significantly alter the physical properties of a baseline polymer and consequently, the printing process.

In this study we examine the effect of adding pharmaceutical, such as dexamethasone, on the printability of low modulus polyesters. Specifically, we investigate the effect of drug loading on polymer flow properties and drug/polymer interactions in order to better understand printing of combination products. We, 1) characterize drug-polymer interaction via binary phase diagrams constructed by combining the experimental melting point depression data with Flory-Huggins theory; 2) measure viscoelastic properties of drug/polymer formulations to understand the influence of a drug on polymer flow behavior and 3D printing processing parameters, and 3) show the effects of drug/polymer composition on the degradation and the drug release rate of the 3D printed scaffolds.

*References: 1. Macromolecules, 2016, 49 (7), pp 2429-2437.*

**8. Assessing the correlation between shape characteristics of spheroids formed from mesenchymal stromal cells and their capacity for chondrogenic differentiation using high-throughput methods**

*Authors: Lam, Johnny, FDA/CBER; Sung, Kyung,*

*FDA/CBER*

Plain Language Synopsis: We evaluate the aggregation of stromal cells using aggregate cell culture techniques, where it is hypothesized that their ability to aggregate as well as their resultant aggregate shape characteristics will be informative of their ability to form cartilage.

Abstract:
Multipotent stromal cells (MSCs), otherwise referred to as mesenchymal stem cells, are often coveted as a candidate cell source in regenerative medicine strategies due to their ability to undergo osteogenic, adipogenic, and chondrogenic differentiation in vitro. Unlike the osteogenic and adipogenic differentiation of MSCs, both of which can be induced to occur in two-dimensional monolayer culture, MSC chondrogenesis typically requires a three-dimensional (3D) culture platform that allows for the adequate aggregation of cells. Indeed, it is well-recognized that the condensation of progenitor cells represents a key event that precedes chondrogenesis in early limb bud development. However, tissue engineering strategies have yet to fully capitalize on this phenomenon for the biomimetic formation of robust cartilage constructs. Recently, advances in microfabrication techniques have enabled the creation of devices for the high-throughput formation of 3D cell spheroids. Using a combination of such microfabricated tools and conventional aggregate culture techniques, we evaluate the micro- and macroaggregation of MSCs, where it is hypothesized that their ability to aggregate as well as their resultant aggregate shape characteristics will be informative of their chondrogenic capacity. Specifically, we characterize the shape characteristics of MSC micro-/macroaggregates over time using high-dimensional morphological analysis and evaluate their correlation to chondrogenic matrix production. It is envisioned that the new knowledge gained would lead to the development of a robust and high-throughput assay for predicting the chondrongenic differentiation potential of MSCs.

**9. Development of a microphysiological system comprising an endothelium-lined lumen and mesenchymal stromal cells to investigate paracrine interactions between endothelial and mesenchymal stromal cells**

*Authors: Lam, Johnny, FDA/CBER; Sung, Kyung, FDA/CBER*

Plain Language Synopsis: Here, we adapt microscale tools and improved microfabrication techniques to generate hydrogel-encapsulated stromal cells surrounding microvascular lumens that are lined with vascular endothelial cells as a biomimetic co-culture model for investigating cell-cell interactions.

Abstract:
Bone-marrow derived multipotent stromal cells (MSCs), otherwise called mesenchymal stem cells, are well-known for their capacity to undergo musculoskeletal differentiation and can be easily harvested from the human body with minimal morbidity. In addition to their trilineage potential, MSCs are being increasingly recognized for their ability to indirectly stimulate tissue repair via the secretion of trophic and anti-inflammatory factors. Moreover, many developing regenerative medicine strategies are being designed to preferentially take advantage of this trophic phenomenon over the actual grafting of implanted MSCs as the primary mechanism for stimulating tissue repair. The observation that MSCs naturally reside in the perivascular niche of the bone marrow heavily suggests that there may be significant crosstalk between MSCs and vascular endothelial cells. Here, we adapt viscous finger patterning and improved microfabrication techniques such as LumeNEXT to generate hydrogel-encapsulated MSCs surrounding microvascular lumens that are lined with vascular endothelial cells as a biomimetic co-culture model for evaluating cell-cell paracrine interactions. It is hypothesized the recapitulation of the organotypic three-dimensional (3D) lumen structure for the culture of vascular endothelial cells will yield more physiologically relevant cell-cell interactions between the MSCs and vascular endothelial cells over traditional 3D cell co-culture models.

## 10. A method for the quantification of levels of phosphorylation and phosphorylated sugar moieties in the glycans of recombinant proteins

*Authors: Ketcham, Stephanie, FDA/CDER; Ashraf, Muhammad, FDA/CDER; Madhavarao, Chikkathur, FDA/CDER*

Plain Language Synopsis: The addition of phosphate to human proteins is commonly necessary for activity. Lysosmal enzyme proteins contain sugar chains as antennae and a subset of these sugars have phosphate, necessary for enzyme's potency. We report simple methods to measure relative levels of phosphate in three proteins using a phosphate binding dye.

Abstract:
Phosphorylation of proteins is an important post translational modification and a critical quality attribute especially if it is required in the mode of action of that protein. In this regard for lysosomal enzyme proteins, phosphorylation of the mannose residues on the glycans is critical for its precise targeting to the lysosomes and its action there, for clearing the accumulated metabolic waste. However, currently methods are time consuming, indirect or require specific instrumentation. Here we report the adaptation of a "phosphate specific binding dye" for quantification of relative amounts of phosphorylation of recombinant proteins in an SDS-PAGE assay. This method allows for quantification of phosphorylated sugar moieties on the glycans as well, as shown for Mannose-6-Phosphate glycosylation. In this case of Chinese hamster ovary cell secreted human recombinant ß-glucuronidase. Here we have developed two efficient methods to quantify the number of phosphate moieties per molecule. The first method utilizes SDS-PAGE, and can easily be applied to proteins at various levels of purity during the production of a recombinant protein. The second method immobilizes protein in a 96 well plate, making it more appropriate for purified proteins. Specificity of this method for phosphorylation was verified by enzymatic removal of phosphate groups, N-linked glycans or both. In summary, it was determined thatthe resulting quantification is in agreement with published data regarding the number of phosphorylation sites and Mannose-6-Phosphate glycosylation sites for three example proteins. This methodology will be widely applicable to characterize a broad array of proteins.

## 11. Influence of bioreactor process variables on production and quality of ß-glucuronidase

*Authors: Hamideh, Parhiz Bibi, FDA/CDER; Fratz-Berilla, Erica J., FDA/CDER; Ketcham, Stephanie, FDA/CDER; Ashraf, Muhammad, FDA/CDER; Madhavarao, Chikkathur, FDA/CDER*

Plain Language Synopsis: Therapeutic proteins are produced in large scale bioreactors while controlling a number of production parameters. Changes in these parameters affect production, protein chemistry or structure and influence potency. ß-Glucuronidase is a model therapeutic protein. In bench scale bioreactors we found changing production parameters affected its production and chemistry.

Abstract

ß-Glucuronidase is a lysosomal enzyme and a molecular model of a class of therapeutics approved as enzyme replacement therapies (ERT) for lysosomal storage diseases (LSDs). Understanding the effect of bioreactor process variables on production and quality of the biologics is critical for maintaining quality and efficacy of the bio-therapeutics. Here, we have investigated human recombinant ß-glucuronidase production by a Chinese hamster ovary (CHO) cell line in a parallel bioreactor system (n=8) as affected by three process variables, namely, 0.25 mM butyrate treatment, a temperature shift treatment (37 to 32 °C), a pH shift treatment (pH 7.0 to 6.8) along with a control (pH 7, Temperature 37 °C and no additive). The run was repeated one more time to increase the sample size to 4 per treatment and to confirm the results. Although statistically not significant (n=4), protein production was increased in both 0.25 mM butyrate treated (13%) and pH shift treated (7%) bioreactors and the mannose-6-phosphate contents (M6P, a CQA) in their glycans were comparable to that of control. But temperature shift decreased production

(12%) and M6P content. The CHO cell line and the Dasgip parallel bioreactor system were found suitable for these studies. These results demonstrate that it may be possible to increase the production of ß-glucuronidase enzyme in CHO cells without affecting its CQA, namely, the M6P content in the glycans of the enzyme.

### 12. Collagen I Hydrogels as an in vitro Model for 3D Culturing

*Authors: Mirdamadi, Eman, FDA/CBER; Oh, Gloria, FDA/CBER, Degheidy, Heba, FDA/ CBER; Bauer R. Steven, FDA/CBER*

Plain Language Synopsis: 3D collagen I hydrogels were developed in our lab to study cellular viability and proliferation within a 3D environment. Collagen I concentration, cell density, and the addition of fibrinogen are all parameters explored to assess their individual effects on MSC culture in 3D.

Abstract:
Collagen is a structural extracellular matrix (ECM) protein located within connective tissue and makes up about 30% of all proteins in the human body. As an abundant protein, collagen I has been ubiquitously used for developing 3D cultures in vitro. In this present study, we made collagen I hydrogels to study viability and proliferative capacity of bone marrow derived human mesenchymal stromal cells (hMSC); varying three parameters within the construct; collagen I protein concentration, cell density, and the addition of Fibrinogen. We optimized calcein and ethidium homodimer-1 stains for the developed hydrogels to assess cellular viability. To quantify cell proliferation over time, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) is used as a colorimetric assay. Finally, diameter and depth of the hydrogel were measured to determine the magnitude of shrinkage over time in terms of percent original volume. Our hMSCs showed above 90% viability in all developed hydrogels for up to 14 days. Our data showed that the optical densities obtained from MTS readings correlated with increased cell counts incorporated inside the hydrogel. In addition, we observed various effects on cellular proliferation using different collagen I concentrations with or without fibrinogen. Finally, we found that higher cell counts, lower collagen I concentrations and the absence of fibrinogen all contribute to accelerated hydrogel shrinkage. Given these findings about our collagen hydrogels, we determined that 5.0E4 cells/ml, 7mg/ml fibrinogen, and 4mg/ml collagen I protein makes a compatible in vitro model which maintains its structural integrity while promoting cellular proliferation. Further studies are being initiated to utilize this hydrogel for 3D bioprinting.

### 13. Cytotoxicity of Water Soluble Type-2 Photoinitiators for Two-Photon Polymerization

*Authors: Nguyen, Alexander, North Carolina State University and FDA/CDRH; Goering, Peter, FDA/CDRH; Narayan, Roger, North Carolina State University*

Plain Language Synopsis: Two-photon polymerization is a sterolithographic technique drawing upon the same photopolymers mainly used in the microelectronics industry where biological applications are not considered. This study correlates the efficacies of photoinitiator formulations at initiating polymerization to the doses where cytotoxicity is first observed to gauge its potential for medical device fabrication.

Abstract:
Two photon polymerization (2PP) can be used to create medically relevant structures with sub-micrometer features. This approach draws upon conventional photopolymers used with UV stereolithography which utilize toxic, non-water soluble photoinitiators requiring extensive washing processes to extract these compounds. Alternatively, biological compounds or compounds historically used with human tissues can be used within the paradigm of a Type-2 photoinitiator where a UV excitable chromophore excites a co-initiator to form the long-lived free radical initiating polymerization. The objectives of this study are to 1) determine the minimum chromophore and co-initiator concentrations required for photopolymerization and 2) evaluate their

cytotoxic effects at these concentrations.

Three chromophores were investigated: flavin mononucleotide (FMN), Rose Bengal (RB), and Crystal Violet (CV). Each chromophore was mixed with either triethanolamine (TEA) or L-arginine(ARG) as co-initiators. The minimum concentrations required for polymerization of polyethyleneglycol diacrylate were determined using 500mJ/cm2 exposure to 254nm UV light. L929 murine fibroblasts were exposed to six different concentrations of each compound individually corresponding to one-half to one-2048th the saturation concentration of each. Cell viability and proliferation were evaluated using the Alamar Blue and PicoGreen assays, respectively.

Polymerization was observed with a CV-ARG mixture. ARG at 6 mg/mL, orders of magnitude larger than what is needed for polymerization, showed no change in viability or proliferation. However, CV at 5 µg/mL, much less than required for polymerization, caused a drop in viability to 73%. 1 mg/mL TEA, a sub-cytotoxic concentration, was an appropriate co-initiator for FMN and RB. Polymerization was observed at concentrations down to 1.5 mg/mL and 8 µg/mL for FMN and RB, respectively; these concentrations are lower than the 25mg/mL FMN and 30 µg/mL RB concentrations at which statistically significant reductions in viability were observed.

Three of the six possible combinations of chromophores and co-initiators were found to initiate polymerization. Of these, FMN and RB with TEA as a co-initiator were also found to be non-cytotoxic to L929 fibroblasts at workable concentrations. These two formulations show significant promise as 2PP photoinitiators and should be further investigated for use in medical implant fabrication.

### 14. Quantifying Adipogenic Potential in Mouse Bone Marrow-Derived Mesenchymal Stem Cells

*Authors: Oh, Gloria, FDA/CBER; Mirdamadi, Eman, FDA/CBER; Degheidy, Heba M.D, PhD, FDA/CBER; Bauer, Steven, PhD, FDA/CBER*

Plain Language Synopsis: Our previously published data showed that dlk-1 -/- mice have greater tendency to develop fat compared to age-matched Wildtype control. Thereby, we developed quantitative imaging analysis to measure adipogenic potential of bone-marrow derived mouse mesenchymal stem cells, which could be applied to 2D and 3D in vitro culture systems.

Abstract:
Mesenchymal Stromal Cells (MSCs) are multipotent in nature and can differentiate to bone, cartilage, and fat under certain conditions. DLK-1(delta-like 1) has been previously reported to influence differentiation of MSCs to fat. Lack of DLK-1 is known to increase MSC differentiation to adipocytes, which may explain why the dlk-1 -/- mice have more fatty tissue than the dlk-1 +/+ mice. The objective of this study is to develop a quantitative method to assess the adipogenic potential of bone marrow derived mouse MSCs (mMSCs) isolated from both dlk-1 -/- and wildtype mice. We harvested MSCs from the bone marrow of 6 to 8-week old dlk-1 -/- mice and wild type control. After reaching 85% confluence, growth media is replaced with adipogenic differentiation media and changed every 3 days for 10 to 14 days. We optimized an imaging method to quantify adipocytes from mMSCs, by staining the induced mMSCs with two different florescent dyes. Nile Red staining is used for fat droplet recognition and DAPI is used for cell nuclei recognition. Images were taken for both stains and analyzed using the Cellprofiler software. The overlaid images allow us to easily analyze the percentage of MSCs that differentiated into fat cells. Our output data from Cellprofiler shows percent of differentiated cells, as well as the number of fat droplets which can allow us to effectively compare the adipogenic potential between dlk-1 -/- and wildtype mice. Our method effectively quantifies adipogenic differentiation of mMSCs. This method is currently being optimized to quantify adipogenic potential within 3D cultures.

### 15. Physical stressors of the bioprinting process

*Authors: Maria J. Rodriguez, Poulomi Nandy, Jon W. Weeks, Anant Agrawal, Irada S. Isayeva, Katherine Vorvolakos*

Plain Language Synopsis: Our project focuses on understanding the physical stresses that cells experience during 3D bioprinting, especially when they are co-printed with polymers. Ultimately we want determine the optimal conditions in terms of material and printing parameters that can help mitigate these physical stresses.

Abstract:
During bioprinting, cells are exposed to a range of stresses created by changes in both the chemical and physical environment. Our project focuses on identifying, characterizing and mitigating physical stresses on cells in the context of 3D bioprinting, especially where cells and polymers are co-printed. We are developing a method for evaluating how cells are physically affected during the printing process. In order to study a wide variety of stresses we are exploring an exhaustive parameter space based on material dependent parameters (viscoelastic and Newtonian fluid behavior) and printing parameters (speed, pressure and nozzle geometries). We are using polyethylene glycol as a model material whose rheological properties can vary widely with molecular weight and concentration. We have developed methods for assessing sterility, endotoxin content and biocompatibility of these materials. Using their wide range of rheological properties we will then probe the stresses cells experience within Newtonian to non-Newtonian fluid flows through a range of nozzles sizes. We have established our printing and material parameter space and using these inputs have created a macro level fluid flow model of the stresses and strains encountered during printing. We will then use Optical Coherence Tomography (OCT) as a means to image and characterize this fluid flow on a micro level and construct a model to understand the stresses that cells will experience during printing. Using this model our aim is to create an optimal material and printing parameter space that can help mitigate the physical stresses experienced during printing.

**16. Additive Manufacturing Solutions to Enable Durability Studies of Metallic Wire**

*Authors: Weaver, Jason; Di Prima, Matthew*

Plain Language Synopsis: Additively manufactured, also known as 3D printed, components are critical for the completion of our studies of medical device durability. With additive manufacturing, we are able to quickly design and conduct experiments which help us to create effective pre-clinical tests that can protect the public health.

Abstract:
Durability testing is an important component of pre-clinical evaluation of medical devices and manufacturers typically conduct studies of fatigue, corrosion, and ion release to demonstrate device safety. In the Solid Mechanics Laboratory we have conducted and continue to conduct research to improve existing test methods and better understand durability test conditions (e.g., test speed and environment) by studying the durability of different metal alloy wires commonly used in medical devices. Additive manufacturing is playing an increasingly important role in the development of our test methods because of the ability to create test fixtures with complicated geometrical features quickly. Examples include:

(1) a baffle system to eliminate air bubbles and reduce false break detection events

(2) sliding arm components to allow more precise break detection location and remove metallic components from the test environment

(3) a glass tubing holder to enable dynamic nickel release testing in various test solutions at a controlled temperature

These additively manufactured components are robust and integrate well with our existing equipment. The ability to quickly iterate designs has allowed our laboratory to conduct research on new durability test methods in a short amount of time which overall allows us to more quickly and responsively work towards protecting the public health.

### 17. Assessing the Influence of Gut Microbiota on TNF and TNF Antagonists by Using Germ Free Animals: A Model for Testing Therapeutics

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Plain Language Synopsis: Auto-immune diseases continue to progress in the US. Biologic therapeutics have been used successfully to treat these diseases, but have also created new regulatory challenges with respect to variability in therapeutic responses. We speculated that the gut microbiome could play a role in this variable response to therapeutics.

Abstract:
Our objective was to set up an in vivo model to study the impact of the microbiome on biologic therapeutics, such as tumor necrosis factor TNF-antagonists, used in auto-immune diseases. We worked closely with the veterinarian services to set up a Germ Free animal facility in a room with sterile isolators maintained in a sterile environment. Biologic therapeutics could be injected under sterile conditions following a regimen used to to treat humans.

Germ Free (GF) mice colonies have been successfully used in this sterile environment. We observed a specific phenotype for these mice not previously described. The mice presented common features of GF mice, such as a five-fold enlarged cecum, absence of fat and smaller organs such as heart, liver, and spleen. The mice were very active and alert. We first checked for the presence of TNF in the gut of these animals and found a basal level of systemic and tissue TNF protein. The TNF could be inhibited by the use of several TNF-antagonists. We checked the basal status of GF mouse joints at 3 month of age and found a particular degradation at the knee and the hip, scored equivalent to that of 9 month-old arthritic mice. This phenotype has not been previously reported, but is consistent with observations that the gut microbiome is protective in arthritis. Based on published reports that this early joint degradation is driven by TNF and IL-1 beta, we then documented that basal levels of IL-1 beta are elevated in the gut of these mice.

We conclude that this is a practical model to assess microbiome involvement in potentially variable therapeutic responses, and to also assess variations in the mucosal immune system.

Our future direction is to test various TNF-antagonists in GF mice, evaluating the role of the microbiome relative to conventional control mice treated in parallel.

### 18. Sand fly gut microbiota augments virulence of vector-transmitted leishmaniasis and promotes parasite visceralization via inflammasome-derived IL1ß

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Plain Language Synopsis: For the first time, we establish that gut microbiota from the sand fly vector gets transmitted alongside the parasite and other vector-derived components where they hijack the host immune response through rapid activation of the inflammasome. This in turn drives a cascade of events directly responsible for enhancing parasite virulence and dictating disease outcome.

Abstract:
Leishmaniasis, caused by protozoan parasites of the genus Leishmania, is transmitted by the bite of infected phlebotomine sand flies. Sand fly transmission enhances virulence of leishmaniasis and can overcome vaccine-induced immunity that protects against needle challenge with parasites. This has

been attributed to a sustained recruitment of neutrophils to bite sites where they protect captured parasites early after transmission. The mechanism underlying this phenomenon remains unknown. Here, we establish that microbiota residing in the sand fly gut are egested during bites driving neutrophilic persistence following sand fly-transmission, and provide evidence of its importance as a determinant of L. donovani dissemination. Vector-transmission of L. donovani reproduces the intense accumulation of neutrophils at bite sites, and results in a 100-fold increased IL1ß induction compared to injected parasites. Diminishing the midgut microbiota of infected sand flies with antibiotic treatment causes a significant reduction in protein levels of NLRP3, caspase 1 as well as IL1ß, and leads to a loss of inflammasome complex formation. Importantly, treatment of flies with antibiotics, or mice with anakinra, an IL1ß blocker, abrogates neutrophil recruitment after infected bites and impairs parasite visceralization. This work demonstrates that midgut microbiota of the vector sand fly is a virulence factor in leishmaniasis. Importantly, the significance of vector microbiota may transcend sand flies and leishmaniasis and have analogous ramifications for other vector-borne diseases, particularly those that involve regurgitation of pathogens.

### 19. Detection and genomic analysis of Bacillus cereus strains isolated from cosmetics

*Authors: Tchagou, Irene, FDA/ ORISE; Smiley, James, FDA/ORISE, Huang Mei-Chuing Jo, FDA/ OCAC; Bell, Rebecca, FDA/CFSAN; Hammack, Thomas, FDA/CFSAN; Tall, Ben, FDA OARSA; Gopinathrao, Gopal, FDA OARSA; Carter, Laurenda, FDA/OARSA*

Plain Language Synopsis: Cosmetics intended to be applied on or around the eye area should be protected from pathogens because pathogenic microbes in products may lead to severe eye infection. We investigated the detection of Bacillus cereus member using a qPCR multiplex assay.

Abstract:
A qPCR multiplex assay was developed to rapidly detect Bacillus species in cosmetics. Primers of 16 S rDNA and phosphadylcholine-phospholipase (PC-PLC) were used for the rapid detection of members of the B. cereus group and for PC-PLC gene in 86 strains. In addition, the strains were screened for the presence of the HBL toxin genes hblDAC using the conventional PCR. Whole genome sequencing (WGS) of the strains was carried out on Illumina MISeq platform using the Nextera XT Library preparation kit. Comparative genomic analysis was carried out using CLC Genome Workbench 9.0, open source bioinformatic programs and in-house scripts.

qPCR multiplex results showed that the 16S rDNA (100%) and PC-PLC (99%) genes were detected in all isolates, except in the baby wipes: B. cereus strain 1-L which was PC-PLC qPCR-negative. Interestingly, hblDAC was detected in only 24 (32.43%) out of 74 strains isolated from baby wipes, and the presence of at least one gene of HBL complex (hblDAC) was detected in all 12 strains isolated from eye cream products. These results suggest that HBL, among these strains, is not as prevalent as PC-PLC. The genomes from the 86 strains were compared with representative genomes from food, feed and dietary supplement isolates, with 25 B. cereus genome groups as reference. The cosmetic isolates were found to be distributed across the different genome types providing a snapshot of emerging nucleotide divergence among B. cereus complex genomes.

The qPCR multiplex assay is an excellent tool for simultaneously detecting members of the B. cereus group and the presence of PC-PLC, which is a factor involved in tissue damage, especially in wound and also in ocular infections.

### 20. Vitamin K5 is an efficient UVA photosensitizer for bacteria inactivation

*Authors: Xu Fei; Li Ying Li; Jones Justen Jones; Vostal Jaroslav G,FDA/CBER/OBRR/DBCD/LCH*

Plain Language Synopsis: Platelets are stored in plasma at room temperature and can support bacterial growth during storage which can lead to transfusion transmitted sepsis. We developed a new pathogen reduction method

that involves ultraviolet light A irradiation and vitamin K5 which can reduce bacteria levels in plasma by seven logs.

Abstract:
Photodynamic inactivation of bacteria has been proven as an effective method to inactivate pathogenic bacteria. This study identified vitamin K5 as an efficient photosensitizer for ultraviolet light A (UVA) induced bacterial inactivation. Six species of bacteria, including Bacillus cereus (vegetative form), Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, and two species of antibiotic-resistant bacteria, Pseudomonas aeruginosa and Staphylococcus aureus, were suspended in aqueous solution with or without vitamin K5 and exposed to UVA irradiation. UVA irradiation (5.8 J cm-2) with vitamin K5 (1600 μmol l-1) reduced the colony forming units (CFU) of these bacteria by three to seven logs. Antibiotic resistant bacteria did not exhibit resistance to the bactericidal effects of UVA and vitamin K5 combination treatment. Inactivation of bacteria in human plasma required higher doses of UVA light and vitamin K5. UVA irradiation (30 J cm-2) with vitamin K5 (2000 μ mol l-1) reduced Escherichia coli and Staphylococcus aureus CFU spiked into human plasma by seven logs. Reactive oxygen species, hydroxyl radicals and superoxide anion radicals, were found generated in vitamin K5 aqueous solution after UVA irradiation, suggesting these oxygen species may mediate the inactivation of the bacteria.

### 21. HIVE CensuScope-HexaHedron: High resolution metagenomics analysis toolkit

*Authors: Karagiannis, Konstantinos FDA/CBER; Santana-Quintero Luis FDA/CBER; Simonyan, Vahan FDA/CBER*

Plain Language Synopsis: Web accessible tools for high resolution analysis of NGS metagenomic samples

Abstract

The taxonomic composition of a sample, whether from patient, food or environment, is important for several types of studies including pathogen diagnostics, epidemiological research, biodiversity analysis and food quality regulation. Furthermore, with the decreasing costs of sequencing, metagenomic data is quickly becoming the preferred type of data for such analysis. The high throughput of modern sequencing technologies has also enabled the study of the human gut microbiome in unprecedented resolution. Nevertheless, rapidly defining the taxonomic composition (both taxonomic profile and relative frequency) in a metagenomic sequence dataset is still a challenging task. This is mainly because the mapping of millions of sequence reads from a metagenomic study to a database such as the NCBI non-redundant nucleotide database (nt) is a computationally intensive task that could take several hours. We have developed a robust sampling-based algorithm, implemented within the High-performance Integrated Virtual Environment (HIVE) platform, called CensuScope, which allows a 'sneak peek' into the population distribution and estimates the taxonomic composition of the sample as if a census was taken of the metagenomic landscape. Additionally, we developed a diversity resolution algorithm called HexaHedron, which when coupled with CensuScope can restore the resolution and sensitivity on selected spectra of the resolved metagenomic landscape. HexaHedron takes advantage of existing alignment data and phased mutations, reported by any alignment algorithm. This deterministic algorithm makes no statistical assumption; it extracts information from the overlapping alignments in a step-wise fashion, using reference coordinates of aligned reads. The ability to accept input from alignments against a large number of reference sequences decreases the bias of reference selection and enables the identification of sequences in very broad metagenomic sub-spectra.

### 22. Synthesizing Scientific Knowledge to Uncover The Connection Between Drugs and the Human Gut Microbiome

*Authors: Mallory, Emily, Stanford University; Acharya, Ambika, Stanford University; Bright, Roselie, FDA/OC; Altman, Russ, Stanford*

*University*

Plain Language Synopsis: Advanced software developed at Stanford might be useful for helping FDA scientists understand complex information. Knowledge about how bacteria living in the gut interact with FDA-regulated products is complex and growing rapidly. We are trying the software to predict previously unknown drug metabolism by bacteria in the human gut.

Abstract:
Bacteria in the human gut have the ability to activate, deactivate, and reactivate drugs with both intended and unintended effects. For example, the anti-cancer drug irinotecan has an active metabolite, SN-38, that is converted into its de-active form, SN-38G, for elimination. Once SN-38G reaches the gut, it can be converted back into SN-38 by various bacterial enzymes, and this unintended conversion from SN-38G back to SN-38 can cause increased irinotecan toxicity. Understanding the complete space of drugs that are metabolized by the human gut microbiome is critical for predicting bacteria-drug relationships and their effects on individual patient response. As part of the UCSF-Stanford Center of Excellence in Regulatory Science and Innovation (CERSI), the Stanford/FDA team is applying data programming to publicly available structured data and unstructured text in the biomedical literature, at FDA and NIH, and other professional databases. This project has three primary goals: 1) to explore the use of existing open source natural language processors to assist FDA reviewers, 2) to build a prototype knowledgebase focused on the human gut microbiome, including biomedical relationships from literature and drug databases, and 3) to make prototype predictions of previously unknown drug metabolism caused by the microbes in the gut. To achieve these goals, we are developing prototype applications (called extractors) using DeepDive and Snorkel to extract relevant relationships between bacteria, drugs, and anatomical locations from unstructured text. In addition, we are using chemical similarity methods on extracted text and database relationships to build a system for predicting drug metabolism caused by gut microbes. Preliminary results for the task of extracting bacteria-anatomical location relationships are 58% precision and 94% recall on sentence-level relationships and 81% precision and 91% recall on document-level relationships. We are currently evaluating these relationship extractors, developing a prediction process, and forming an advisory group of FDA microbiome experts. The outcomes of this work will include open code, an openly available database of bacterial relationships with anatomy and drugs, and predicted similarities between drug metabolism and chemical reactions known to occur in the gut.

### 23. NGS Toolbox for Analysis of Live Microbial Ingredients in Dietary Supplements

*Authors: Tartera, Carmen, FDA/CFSAN; Gangiredla, Jayanthi, FDA/CFSAN; Barnaba, Tammy, FDA/CFSAN; Mammel, Mark, FDA/CFSAN; Elkins, Christopher, FDA/CFSAN*

Plain Language Synopsis: The application of cutting-edge science, specifically genomics and metagenomics, to the analysis of products containing live, beneficial microbes, can be used in post-market product surveillance. These analytical techniques will be leveraged and applied to the gut microbiome as a new safety endpoint for regulatory science development and application.

Abstract:
Live, beneficial microbes are added intentionally to many food products and dietary supplements which has led to increase production of these commodities to meet the demand for new health-related products. Furthermore, many of these strains used in these products are relevant to human microbiota constituents and are therefore intended to support gut health. Strain identification and subtyping are important characteristics to determine in order to assess product safety, proper labeling and can be used in post-market product surveillance. We have leveraged our expertise with pathogen subtyping to develop molecular tools that are built on a genomic-scale to analyze products with live beneficial microbes. Analysis of the shotgun genomic sequence-data was used to

identify product-specific microbial communities with unique clade-specific markers based on our in-house k-mer database. In parallel, using a culture-based approach, the microbial contents of the dietary products were grown under different temperatures and atmospheric conditions to allow for growth of each species listed on the product label. Purified isolates were sequenced with next generation Illumina platforms (NextSeq/MiSeq) and identified at a whole genome strain-specific level. A genome database has been established in NCBI under BioProject PRJNA336518 based on the sequences of single colony isolates associated with each product. To date, more than 25 products available in the US have been analyzed using metagenomics. In total, 619 isolates have been characterized, and over 22 distinct species identified. Phylogenic relationships of all microbial species sequenced were determined using SNP analysis from core genes specific for each species and subspecies. The application of cutting-edge science, specifically genomics and metagenomics, in product analysis have allowed for the development of a whole genome database, and a next-generation toolbox for quality control and routine monitoring of batch variation as part of "Good Manufacturing Practices" (GMP) for dietary supplements and other foods containing live microbes. Such efforts, including strain specific databasing and new metagenomic analysis techniques will be leveraged and applied to the gut microbiota as a new safety endpoint for regulatory science development and application.

### 24. The In-House Microbiomes of an Industrial Food Production Facility

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Plain Language Synopsis: The microbial communities in a food manufacturing facility were analyzed. The data outlines the composition and location of bacterial populations within the facility. It also provides insight into the influence of human activity in an industrial food manufacturing environment. This can help FDA target surveillance efforts and outbreak responses.

Abstract:
FDA regularly conducts environmental sampling of food manufacturers as part of outbreak investigations and routine surveillance. These samples are analyzed by cultivation methods targeting one organism at a time. Metagenomic sequencing is an emerging technology which can provide species-level identification of organisms in a mixed community without the need for cultivation. The technology is well suited for the analysis of the microbial communities or microbiomes that inhabit food production facilities. In this project, we investigate the microbiomes in several areas of an industrial fermented foods production facility. This facility is unique in that its product is manufactured solely by natural fermentation. The quality of the product is determined by how effectively lactic acid bacteria naturally present on vegetable skins can convert the vegetables to pickles and sauerkraut. This process has been used for several thousand years. However, the mechanisms by which human activity in an indoor environment influences the fermentation trajectory on an industrial scale remains largely unexplored. Our study seeks correlate the microbiomes of the factory environment, where employees work and raw vegetables pass through, to the beneficial content of the food product itself. Our results indicate that a distinct community of microbiota, dominated by species of lactic acid bacteria, establishes itself in areas of the factory where fermentation is taking place. Unsurprisingly, these bacteria are the active agents in a natural fermentation process. The fermentation room microbial community structure is significantly different from the communities in the factory's prep room, where raw vegetables are processed. Structural heterogeneity between factory environments suggests microbial transfer between humans and the food product. This

survey provides an outline of the composition and spatial location of bacterial populations within the food manufacturing environment. It also affords insight into the reciprocal interactions between humans and their microbial environment. This study serves as a proof of concept for the application of metagenomics to food environmental sampling. This type of data could assist FDA in drafting environmental sampling assignments by revealing potential pathogen reservoirs and uncovering the presence of pathogens not previously associated with a particular manufacturing process.

### 25. Development And Validation Of A LC-MS/MS Method For Quantitation of Ampicillin And Fosfomycin – Application to in Vitro Antibacterial Resistance Study

*Authors: Gandhi, Adarsh, FDA/CDER; Matta, Murali, FDA/CDER; Patel, Vikram, FDA/CDER; Weaver, James, FDA/CDER*

Plain Language Synopsis: Antimicrobial drug resistance is a serious health issue especially with gram negative bacterial infections Resistance susceptibility testing studies are time consuming. In an effort to optimize the dosing regimen for single or combination drug therapy, a mass spectrometry assay was validated to measure the drug concentrations in bacterial cultures.

Abstract:
Antibiotic resistance is considered as a global health concern among the society. More and more bacteria are becoming resistant to antibiotics than ever before due to overuse and misuse of clinical and chemotherapeutic agents. Thus, in order to effectively prevent the emergence of resistant (mutant) bacteria (especially gram negative bacteria) as a means to treat underlying bacterial infections, we have developed and validated a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) assay as per FDA guidelines for analysis of fosfomycin and ampicillin in lysogeny broth culture media. Fosfomycin and ampicillin were resolved on a Kinetex HILIC (2.1x 50 mm) 2.6 µm column with 2 mM Ammonium acetate (pH 4.76) and acetonitrile (with 0.2% formic acid) as mobile phase. The assay run time was 3.0 min. Multiple reaction monitoring (MRM) was used to acquire data on a triple quadrupole mass spectrometer with m/z 136.95à63.01 and 108.9à79.0 for fosfomycin and its internal standard (Ethylphosphonic acid) and m/z 349.9à105.9 and 354.9à110.9 for ampicillin and its internal standard (ampicillin D-5). The assay was found to be specific (devoid of matrix related interference) and selective for both fosfomycin and ampicillin. The assay was linear from 1-1000 µg/mL and 0.1-50 µg/mL for fosfomycin and ampicillin, respectively. Inter- and intra-assay precisions were less than 15% and inter- and intra-assay accuracies were within 85-115% for 3 different QC samples for both fosfomycin and ampicillin. No significant matrix effects were observed when corrected with the internal standard peak area ratios. Fosfomycin and ampicillin were stable for up to 24 h at room temperature, were stable for more than 3 freeze-thaw cycles and also stable for up to 72 h when stored at 40C in the autosampler. The validated LC-MS/MS assays for quantitative determination of fosfomycin and ampicillin in lysogeny broth will significantly help antimicrobial drug resistance studies which are conducted in vitro using hollow-fiber system to maintain the necessary concentration of the antibiotic(s) over the duration of the experiment.

### 26. Women's Health and Cosmetic Device Infections—A Combinatorial Approach to Interrupting The Pathogenesis Process: Prevention, Practice, and Preservation

*Authors: Wang, Yi, FDA/CDRH; Guan, Allan, FDA/CDRH; Phillips, Kenneth, FDA/CDRH*

Plain Language Synopsis: Our skin is colonized by a "microbiota" - community of microbes. Contamination of dermal fillers can happen during injection, by transferring microbes to sterile tissue/materials. The associated chronic infections may lead to tissue damage. We develop models testing filler materials, skin preparation and injection processes to minimize dermal filler-associated infections.

Abstract:
Dermal fillers are medical devices used to treat partial failure of skin elasticity caused

by factors such as sun exposure, free radicals and trauma. Compared to other solid biomaterials, fillers are mainly composed of ultrasoft hydrogels and can be placed in the body through injection. Although dermal filler injections are generally considered safe, contaminations with persistent, antibiotic resistant organisms can occur during injection1. The treatment for consequent biofilm development and associated infections may cause significant cost and pain to patients, among whom more than 90% are women.

Our research group is interested in understanding the pathogenesis process of dermal filler injection associated infection and how to prevent it. We previously developed flow chamber models to understand how dermal filler material properties might affect the pathogenesis of associated infections2. We then developed porcine skin explant models to assess injection techniques, skin preparation and practices to reduce contamination. We are also exploring the promising potential of using preservatives to further prevent injectable cosmetic device infections.

Reference:


1. DeLorenzi, C. Complications of injectable fillers, part I. Aesthet. Surg. J. 33, 561–575 (2013).
2. Wang, Y. et al. Interactions of Staphylococcus aureus with ultrasoft hydrogel biomaterials. Biomaterials 95, 74–85 (2016).


### 27. New Bactericidal Pathogen Reduction Method with Vitamin K5 As a Photosensitizer and UVA Light as an Activator


*Authors: Xu Fei; Li Ying Li; Jones Justen Jones; Vostal Jaroslav G, FDA/CBER/OBRR/DBCD/LCH*


Plain Language Synopsis: Transfusion products can become contaminated with bacteria during collection. Pathogen reduction method that utilizes vitamin K5 and UVA light can reduce bacteria by 7 logs and may be an effective way to reduce transfusion transmitted sepsis.


Abstract:
Photodynamic inactivation of bacteria has been proven as an effective method to inactivate pathogenic bacteria. This study identified vitamin K5 as an efficient photosensitizer for ultraviolet light A (UVA) induced bacterial inactivation. Six species of bacteria, including Bacillus cereus (vegetative form), Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, and two species of antibiotic-resistant bacteria, Pseudomonas aeruginosa and Staphylococcus aureus, were suspended in aqueous solution with or without vitamin K5 and exposed to UVA irradiation. UVA irradiation (5.8 J cm-2) with vitamin K5 (1600 μmol l-1) reduced the colony forming units (CFU) of these bacteria by three to seven logs. Antibiotic resistant bacteria did not exhibit resistance to the bactericidal effects of UVA and vitamin K5 combination treatment. Inactivation of bacteria in human plasma required higher doses of UVA light and vitamin K5. UVA irradiation (30 J cm-2) with vitamin K5 (2000 μ mol l-1) reduced Escherichia coli and Staphylococcus aureus CFU spiked into human plasma by seven logs. Reactive oxygen species, hydroxyl radicals and superoxide anion radicals, were found generated in vitamin K5 aqueous solution after UVA irradiation, suggesting these oxygen species may mediate the inactivation of the bacteria. This method may be an effective way to reduce transfusion transmitted septic reactions.


### 28. Characterization of Hepatitis C Virus E2 Protein Structural Dynamics for Antibody-Mediated Neutralization


*Authors: Lu Deng, Lilin Zhong, Hailing Yan, Maria Luisa Virata-Theimer, Yanqun Xu, Evi Struble, Pei Zhang*


Plain Language Synopsis: Virus proteins present themselves to our immune system in certain shapes to gain an evolutionary advantage for survival. By studying the three-dimensional structures of the HCV surface protein E2, we proposed a dynamic model for revealing the vulnerabilities in the E2 structures, as targets, to eliminate the virus by antibodies.


Abstract:
The structural basis for the antibody-mediated neutralization of hepatitis C virus

(HCV) is of great interest for the development of immunoglobulin therapies and future design of effective vaccines. The HCV envelope glycoprotein E2 is a major target for HCV neutralization. In this study, we have determined the X-ray crystal structures of two key epitopes on the E2 protein, which encompasses amino acid residues 427–446 (Epitope II) and 523-530 (Epitope III), respectively, in complex with a panel of neutralizing antibodies. Locally, the epitopes could adopt different conformations, e.g., the combination of the C-terminal α-helix with the N-terminal loop in Epitope II, and the side-chain rotations in the residues of Epitope III. We have shown experimentally that the former determines the outcomes of antibody binding, either neutralization or non-neutralization of HCV, while the latter appears to create a conformational equilibrium that could modulate the interface structure of the E2 protein used for interacting with antibodies or for engaging with host receptors, such as CD81 and SR-B1. The existence of various conformations demonstrates the structural flexibility of the E2 protein. In addition, we have systematically integrated the local structures in these key epitopes, and formulated a dynamic model to investigate the structural ensembles and coordinated motions of a multi-domain HCV E2 protein. These studies not only enabled us to illustrate the potential role of diverse conformations at the virus-host interfaces in eliciting effective neutralizing antibodies, but also revealed a possible mechanism for natural variants of HCV to evade immune responses that allows us to prevent from chronic HCV infections.

### 29. The Effect of Antibiotics on Gut Microbiome: A Metagenomics Analysis of Microbial Shift and Antibiotic Resistance in Antibiotic Treated Mice

*Authors: Xu, Lei; Chockalingam, Ashok; Stewart, Sharron; Li, Zhihua; Rouse, Rodney*

Plain Language Plain Language Synopsis:
Next generation sequencing (NGS) was used for mouse gut microbiome taxonomic compositional study and antibiotic resistance analysis in a murine model of E.coli urinary tract infection (UTI) subsequently treated with antibiotics

Abstract:
There are long standing concerns about the use of oral antibiotics and their impact on the normal gut microbiota/microbiome and the emergence of antibiotic resistance. More recently, drug-microbiota/microbiome interactions have been identified that have impacted drug efficacy and/or safety through altered drug metabolism. This project will characterize the effect of antibiotic treatment on the composition of the gut microbiota/microbiome and the prevalence of antibacterial resistance in the gut in the context of UTI treatment. While a larger project is determining the influence of antibiotics on emergent antibiotic resistance in an infection, the more focused objective of these NGS studies was to assess the influence of antibiotics on the gut microbiota/microbiome. This knowledge will provide a foundation for stratifying the risk potential of different antibiotic therapies to negatively impact health through a microbiota/microbiome mechanism and will support and inform FDA guidance that might be generated in this arena. Further, the framework for a more general microbiota/microbiome knowledge base will be created to support and inform future regulatory review. Standard NGS procedures were used with bio-informatics to conduct metagenome analysis on fecal samples from mice experimentally infected with inoculation of E.coli into the urinary bladder. Three antibiotics (ampicillin, ciprofloxacin, fosfomycin) commonly used for UTI were administered and fecal samples collected to assess treatment influence on the gut microbiota and microbiome composition. In addition, NGS and whole genome analysis of single colonies from infected urine, bladder, and kidney was used to identify factors contributing to emergent resistance and novel treatment regimens that might suppress or delay emergency.

### 30. A Proof of Concept Study for the Migration of Bacteria Through Condoms in the Presence of Personal Lubricants

*Authors: Zewdie, Abenezer and Sarkar Das, Srilekha FDA/CDRH*

Plain Language Synopsis: This report is about migration of various dye solutions as proof of concept for passage of small biological molecules through natural rubber latex condoms in the presence of commercial personal lubricants or major ingredients thereof.

Abstract:
In 1990s, results of several investigations confirmed the effectiveness of latex male condoms in obstructing the passage of small molecules and in effect, protecting consumers from sexually transmitted infections (STI). Effect of personal lubricants however, was not considered in these studies. Over–the–counter personal lubricants are assortments of various chemicals, where major components are usually water, glycerol, sorbitol, polyethylene or poly propylene glycol, and silicone oils. When in contact with the condom material, these lubricants may swell or shrink the cross-linked polymer and change the intermolecular space of the rubber network. The intermolecular interaction force may change the elasticity of the material also, during use. Enlarged pores of such thin films of rubber (from 30 to 50 μm in thickness), or decreased elasticity (which may soften the network), may promote the passage of viruses or small bacteria. Further, changes in the chemical potential gradient across the rubber membrane due to the application of a personal lubricant on one side of a condom may enhance transmission of infectious particles, even in the absence of swelling or elasticity reduction. Herein we report migration of various dye solutions as proof of concept. Future application of testing will focus on bacteria migration in the presence of personal lubricants. Initial results show that (i) hydrophilic dye or capture medium does not allow diffusion through condoms in 20 hours; (ii) hydrophobic dye diffuses through the latex membrane in the presence of hydrophobic capture medium; and (iii) the rate of diffusion increases with decreasing molecular weight.

## 31. Characterization of Pathways Involved in the Suppression of T Follicular Helper Cells in Newborns

*Authors: Jiyeon Yang, Shafiuddin Siddiqui, Kadriye Uslu, Robert C Lee, Derek DC Ireland, Windy R Allman1, Daniela Verthelyi2, and Mustafa Akkoyunlu1*

*US FDA/CBER/OVRR/DBPAP, and CDER/OPQ/OBP/DBRRIII, 10903 New Hampshire Ave., Silver Spring, Maryland, USA*

Plain Language Synopsis: Most pediatric vaccines need to be given 3-4 times while a single immunization induces protective immune response in adults. This delayed immune response exposes infants to life threatening infectious diseases. The study of understanding of the infant immune system provides important implication for devising improved vaccines for infants.

Abstract:
The immature immune system of newborns renders poor vaccine response. However, mechanisms of limited T follicular helper (Tfh)-mediated antibody production in newborns are largely unknown. We found significantly more expression of the Tfh inhibitory genes associated with regulatory T cell (Treg) cells and IL-2 signaling in immunized newborn mice CD4+CXCR5+PD-1+ Tfh cells than in adult mice. The IL-2-STAT5 signaling in CD4+CXCR5+PD-1+Foxp3+ T follicular regulatory cells (Tfr) was hyperactive in newborns as compared to adults. Also, in newborns IL-6-STAT3 signaling was lower in Tfh cells but higher in Tfr cells compared to adult mice. Immunization did not elicit IL-21 expressing Tfh cells but when provided sufficient IL-21, in vitro cultured newborn T cells differentiated into Tfh-like cells. These data indicate that in newborns, dendritic cell-derived IL-6 signaling preferentially induces Treg cells to differentiate into Tfr cells, which in turn suppresses Tfh generation. Supporting this hypothesis, co-injection of newborn mice with conjugate type 14 pneumococcal polysaccharide vaccine together with IL-21 or anti-CD25 blocking antibody reduced Tfr/Tfh ratio and enhanced Tfh formation. While IL-21 elicited both IgG and IgA antibodies against vaccine, anti-

CD25 antibody only improved IgA production, due in part to the elevated APRIL and TGF-b expression in T cells and fibroblast reticular cells. Since IL-21 acts both on T cells and B cells, improved IgG response in IL-21 injected mice is likely due to its direct effect on B cells.

These findings highlight the differences in the programming of IL-2 and IL-6 signaling in newborn and adult Tfh cells. These differences may be exploited in devising improved vaccines for infants.

### 32. Evaluation of the Metabolism of Azo Dyes and its Effects on the Staphylococcus aureus Metabolome

*Authors: Jinchun Sun; Jinshan Jin; Richard D. Beger; Carl E. Cerniglia; Huizhong Chen*

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Plain Language Synopsis:
This study examines the tattoo dye degradation by a skin bacteria and the effects of the dye on the skin bacteria.

Abstract:
Dyes containing one or more azo linkages are widely applied in cosmetics, tattooing, food and drinks, pharmaceuticals, printing inks, plastics, leather as well as paper industries. It has been reported that human bladder carcinogens may be produced from the reduction of the azo dyes by microbial and mammalian azoreductases. Previously, we reported that the skin bacterium Staphylococcus aureus (S.aureus) has the ability to reduce some azo dyes to genotoxic aromatic amines, which raises safety concerns regarding human dermal exposure to azo dyes such as those in tattoo ink and cosmetic colorant formulations. In order to comprehensively investigate azo dye-induced toxicity, it is very critical to understand the metabolic activation of azo dyes at the systems biology level. In this study, an LC/MS-based metabolomics approach was employed to globally investigate azo dye metabolism by S. aureus as well as its effects on the metabolome of the bacterium. S. aureus was inoculated into Brain Heart Infusion (BHI) medium containing DMSO (control vehicle), 10 μg/ml Sudan III (containing two azo bonds) or Orange II (with one azo bond) at 37oC for 18 h without agitation. The cell pellet (~1010 cells) and culture medium supernatants after protein precipitation were evaluated using UPLC/QTof-MS system (Waters, Milford, MA). Metabolomics results showed that Sudan III was metabolized to 4- (phenyldiazenyl) aniline (48%), 1-[(4-aminophenyl) diazenyl]-2-naphthol (4%) and eicosenoic acid Sudan III (0.9%). These findings indicated that the azo bond close to naphthalene group of Sudan III was preferentially cleaved compared with the other azo bond. The detection of the coupled eicosenoic acid with Sudan III could indicate Sudan III was transferred into the cells through hydrogen-bonding with eicosenoic acid. Much more Orange II (~90 x) were measured in the cell pellets from the active viable cells compared with those from boiled cells incubated with the same concentration of Orange II. This finding suggests that Orange II was transported into the S.aureus cells for metabolism, instead of the theory that the azo dye metabolism occurs extracellularly on the cell membrane or in the culture medium. In addition, the metabolomics results showed that Sudan III affected energy pathways of S. aureus cells, while Orange II had less noticeable effects on the cells. In summary, our metabolomics study provided novel information regarding azo dye metabolism by the skin bacterium, the effects of azo dyes on the bacterial cells and the important role on the toxicity and /or inactivation of these compounds due to microbial metabolism.

### 33. Drug Coated Balloon Manufacturing - The Evaluation of Coating Process Parameters and their Impact on Balloon Coating Integrity

*Authors: Steven Woolford, FDA/CDRH/OSEL/DBCMS; Agnes NguyenPho, FDA/CDER/OS/DQSA/QDAB; Samanthi Wickramasekara, FDA/CDRH/OSEL/DBCMS; Berk Oktem, FDA/CDRH/OSEL/DBCMS; Martin McDermott, FDA/CDRH/OSEL/DBCMS*

Plain Language Plain Language Synopsis: Drug coated balloons (DCB) are a popular treatment for blocked arteries. Our goal is to understand how manufacturing variables in the coating process impact the physical and chemical attributes of the coating and provide guidance for DCB industry in design, manufacture and standardize testing for DCB.

Abstract:
According to the Center for Disease Control and Prevention, approximately 8.5 million people in the United States have peripheral arterial disease (PAD), which is, the narrowing of vessels that carry blood from the heart to the extremities of our body's, and is primarily caused by the build-up of fatty plaque in the arteries (stenosis). Although balloon angioplasty and bare metal stents were the initial response for the treatment of stenosis, re-stenosis (re-blockage) became the rate limiting factor for these procedures. Drug coated balloons (DCB) have emerged as the superior treatment procedure for re-stenosis because of their ability to treat a variety of occlusion types with a uniform dose of anti-proliferative drugs. Clinical studies have shown that drug dosage between 2-3.5 µg/mm2 of paclitaxel on the balloon surface will result in inhibition of re-stenosis. Common balloon coating methods include dip-coating, spray-coating, and micro-pipetting. The micro-pipetting technique is the most popular coating method used by DCB manufacturing companies because it allows precise dispensing of a defined volume of drug coating solution onto the balloon surface. Currently, there is little information on how manufacturing parameters via micro-pipetting method impact coating integrity, uniformity, and drug release profile in biological conditions. Such factors are important for patients to receive reproducible full therapeutic benefits. Our study focuses on coating parameters, such as, coating flow rate, balloon revolution rate, solvent composition and drug to excipient ratio to determine how these parameters influence the balloon's coating attributes. Data collected through high performance liquid chromatography (HPLC-UV) analysis proves that our in-house micro-pipetting method has the ability to apply reproducible drug loads of 3 µg/mm2. However, the coating uniformity on the balloon surface has been highly variable from qualitative observation through scanning electron microscopy (SEM). Thus, we have devised a quantitative procedure, in which the application of HPLC-UV and SEM were used to understand how coating method variables have an impact on the optimization of the balloon coating process. Our data will be used to provide guidance in the DCB industry by highlighting and assessing the most optimal coating parameters to be used for DCB manufacturing via micro-pipetting method.

### 34. The Cardiotoxicity induced by Trastuzumab is Associated with the Dysregulation of Critical Gene Expression in Human Cardiomyocytes

*Authors: Jiangsong Jiang, Nishant Mohan, Wen Jin Wu*

Abstract:
Cardiotoxicity of chemotherapy and targeted therapy drugs has been a major risk for breast cancer patients and survivors. Combining drugs of different therapeutic strategies can better control the tumor growth and resistance, but may synergistically increases the risk of cardiotoxicity. Many studies have revealed that both anthracycline, (e.g. doxorubicin) and trastuzumab, a therapeutic monoclonal antibody approved for the treatment of HER2-positive breast cancer, can increase ROS/RNS stress level in cardiomyocytes. However, it is not clear how the adjuvant therapy with trastuzumab can affect the response of cardiomyocytes after anthracycline therapy. Our previous investigation has revealed

that trastuzumab treatment to mice can change the expression of genes related to cardiac function and significantly damage the ultrastructural compartments of myocardium. Further characterization linked trastuzumab's cardiotoxicity to phosphorylation dysregulation on the intracellular domain of HER2 protein, which in turn can signal through mTOR kinase to inhibit autophagy pathway and eventually cause the elevation of ROS/RNS production in the primary human cardiomyocytes. Based on these results, we carried out a comprehensive study with DNA microarray to identify the molecules responsible for trastuzumab-induced cardiotoxicity. We discovered that a class of genes are the target for trastuzumab in the primary human cardiomyocyte, including those related to apoptotic chromosome condensate pathway, mitotic G2 DNA damage signaling, Mitochondria function, DNA topoisomerase II related pathway and sarcoglycan complex. Further analysis showed that these genes can be significantly up- or down-regulated by trastuzumab compared to other anti-HER2 therapeutic monoclonal antibody. Although trastuzumab itself has only a minor effect on cardiomyocyte cell growth in vitro, treatment of cardiomyocytes with trastuzumab after doxorubicin treatment can significantly increase programed cell death. Cellular stress response markers indicating increased ROS/RNS level also present in the cells after doxorubicin-trastuzumab sequential treatment. Further investigation of mechanism of trastuzumab-induced cardiotoxicity is warranted.

Acknowledgement: This work was supported by FDA Office of Women's Health Research Science Program award (to W.J. Wu; Project ID: 750912CDR). Dr. Jiangsong Jiang and Nishant Mohan are ORISE research fellows supported by Food and Drug Administration Office of Women's Health.

**35. Vorinostat Re-Expressed Estrogen Receptor (ER) in Triple Negative Breast Cancer Cell Line Subtypes and Sensitized Cells To Tamoxifen And Indole-3-Carbinol In Vitro.**

*Authors: Lyn-Cook, Beverly, FDA/NCTR; Word, Beverly, FDA/NCTR; Moore, Rhonda, CTP/NCTR and Miranda-Carboni, University of Tennessee Health Science Center, Memphis, TN.*

Plain Language Plain Language Synopsis: Triple negative breast cancer is one of the most aggressive form of breast cancer. This cancer lacks targeted therapies and treatments are limited to cytotoxic chemotherapies with harsh side effects. This study show promising result for the use of epigenetic drugs in sensitizing breast cancer cells to FDA targeted therapies.

Abstract

Triple negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer. Although about 85% of breast cancers are estrogen positive, about 15% falls into this category. This subtype of cancer lacks targeted therapies receptors, such as the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor-2 (HER2). These patients are limited to cytotoxic chemotherapies with harsh side effects. Although mutations are involved in the initiation of TNBC, research has revealed that individuals are controlled by factors other than DNA sequences such as epigenetic mechanisms. Environmental and lifestyle related factors, such as the lack of population-based screening, and lack of access to care-factors, the urban environment, food deserts, social stress, diet, lack of exercise, alcohol intake, and tobacco use (i.e. cigarette smoking) have all been described as factors associated with breast cancer related disparities. This study investigated the role of epigenetic mechanisms in the re-expression of ER receptors in triple negative breast cancer (TNBC) cells by examining the effects of a FDA-approved epigenetic drug (vorinostat) and the dietary agent (indole-3-carbinol) on three subtypes of triple negative breast cancer. Basal-like 2 (HCC1806), mesenchymal stem cell-like (MSL) MDA-MB-231 and mesenchymal (BT-549) cell lines were treated with vorinostat for 6, 12, 24 and 48 hrs alone. The ER was expressed in HCC1806 (3-fold) and MDA-MB-231 (5-fold) at 6 hr. The ER was not expressed in BT-549 cells at any time point. MTS assay demonstrated a significant decreased in proliferation (60%)

in MDA-MB-231 when treated with vorinostat (10, 20 or 30 μM) and 10 μM of tamoxifen. Furthermore, a significant decrease (40%) in proliferation was also detected in MDA-MB-231 cells treated with I3C (200 μM) and vorinostat (10, 20 or 30 μM). Our preliminary results show that three triple-negative cell lines representing three subtypes responded different to treatment with vorinostat in re-expressing the estrogen receptor. However, these results showed promising result for the use of this drug in sensitizing triple negative breast cancer cells to tamoxifen and a dietary agent, indole-3-carbinol.

### 36. Morphological Features of Ifnγ-Stimulated Mesenchymal Stromal Cells Predict Overall Immunosuppressive Capacity

*Authors: Marklein, Ross, FDA/CBER; Klinker, Matthew, FDA/CBER; Lo Surdo, Jessica, FDA/CBER; Wei, Cheng-Hong, FDA/CBER; Bauer, Steven, FDA/CBER*

Plain Language Synopsis: Common markers of mesenchymal stromal cells (MSCs) do not adequately predict in vitro or in vivo responses. In this work, the morphological response of MSCs to the inflammatory cytokine IFNγ was demonstrated as a useful predictor of both MSC immunosuppression, as well as the effects of preconditioning on MSC immunosuppression.

Abstract:
Introduction: In many mesenchymal stromal cell (MSC)-based clinical trials, common markers of MSCs(1) do not adequately predict in vitro or in vivo responses. Therefore, improved methods to identify MSC quality attributes are needed. We previously established MSC morphology as a predictive marker for osteogenic capacity(2) and sought to apply this approach to MSC immunosuppressive capacity. Methods: High content imaging of MSC lines from multiple donors and passages was performed to correlate single cell morphological data with an overall quantitative immunosuppression score based on principal component analysis of high dimensional flow cytometric data from peripheral blood mononuclear cell (PBMC) co-culture assays. Results: Upon IFNγ stimulation, MSCs from all donors/passages became more elongated with increased cell perimeter and decreased nucleus:cytoplasm ratio relative to controls. MSC morphology upon IFNγ stimulation was highly correlated with the overall immunosuppression response and could be used to predict the immunosuppressive capacity of additional cell-lines. Morphological data was also predictive of the effects of pre-stimulation of MSCs with IFNγ, which resulted in enhanced immunosuppression of T Cells by all MSC lines. Discussion: The morphological response of MSCs to IFNγ highly correlated with immunosuppressive capacity and was validated using cell-lines from multiple MSC and PBMC donors. High content imaging of MSC morphology could be a useful tool for identifying MSC preparations with desired immunomodulatory properties, as well as a high throughput method for selecting cell manufacturing conditions or pre-stimulation regimens that enhance the immunosuppressive capacity.

References: 1)Krampera, et al. Cytotherapy. 2013. 2) Marklein, et al. Stem Cells. 2016.

### 37. Passive Immunoprophylaxis for the Protection of the Mother and Her Baby From Emerging Viral Diseases: Insights From In Vivo Models of Antibody Transport

*Authors: Authors: Xu, Yanqun, FDA/CBER/OTAT/DPPT; Mahmood, Iftekhar, FDA/CBER/OTAT/DCEPT; Zhong, Lilin, FDA/CBER/OTAT/DPPT; Zhang, Pei, FDA/CBER/OTAT/DPPT; Struble, Evi, FDA/CBER/OTAT/DPPT;*

Plain Language Synopsis: The goal of our research is to help determine a safe and effective antibody-based therapy of pregnant mothers who are infected with the existing and emerging viruses, such as hepatitis B (HBV), Zika (ZIKV), and Cytomegalovirus (CMV).

Abstract:
Pregnant women are at high risk for infection by pathogens. Vertical transmission of infectious agents, such as Zika, hepatitis B, and cytomegalovirus during pregnancy remains a public health problem, associated with dire outcomes for the neonate. Thus, a safe prophylactic and therapeutic approach for

protecting the mother and the neonate from infections remains a high priority.

Our work is focused on better understanding the safety and efficacy determinants of immune globulin G (IgG) preparations when used during pregnancy to benefit the mother and her baby. Using pregnant guinea pigs we demonstrated that bio-distribution of administered IgG to the fetus increases with gestation resulting in lower maternal and higher fetal antibody concentrations with progression of pregnancy. In our studies, fetal partition of the hepatitis B neutralizing antibodies resulted in fetal neutralizing activity that, depending on gestation age, reached and surpassed the accepted serological level of protection for children and adults.

Additional studies are needed to determine the level of neutralizing antibodies that can prevent fetal viral infections, including Zika, and what the effects of reduced maternal exposure to administered antibody therapy would be. Well-designed clinical studies and careful dosing considerations, especially in light of changes in biodistribution to the fetus at different gestation ages, are needed to assess the safety and efficacy of therapeutic antibody treatments during pregnancy.

### 38. Design of a Novel Therapeutic to Preserve Vaccine Memory Following Ionizing Radiation Exposure

*Authors: McFarland, Hugh, FDA/CDER, Surujdin, Ryan, FDA/CDER, Arankalayil, Joseph, FDA/CDER, Rosenberg, Amy, FDA/CDER*

Plain Language Synopsis: Lymphocytes are highly susceptible to ionizing radiation. We are developing a therapeutic that stimulates lymphocyte survival mechanisms to preserve vaccine memory and prevent infection, a leading cause of death following gamma radiation exposure.

Abstract:
Vaccine memory responses mediated by CD8+ T cells is eliminated by sublethal gamma irradiation of mice. We have recently published in two dissimilar models, that these memory responses can be rescued in the aftermath of such exposure by revaccination within 3-4 days following irradiation. This window of time after radiation exposure could provide time for treatment of victims, and suggests the possibility of developing a practical therapeutic for maintenance of vaccine memory. Full revaccination in a radiation mass casualty situation would be problematic. In this proposal, we seek to develop a single therapeutic or a cocktail of drugs, and biologics that can mimic the effects of revaccination. Revaccination rescue is antigen-specific, providing strong evidence that T cell activation is involved in the survival of vaccine memory after ionizing radiation exposure. We are basing our design of potential therapeutics by targeting survival pathways stimulated by T cell activation via the T cell receptor (TCR) and costimulation such as Akt and MAPK/ERK1/2 which upregulate or activate anti-apoptotic factors such as Bcl-2, Bcl-xL, Mcl-1, and inactivate pro-apoptotic factors such as BAD, BIM, and BAX, and inhibit pro-apoptotic pathways such as p53. Agonists and antagonists of these pathways include numerous approved drugs and biologics, as well as various peptides, Toll-like receptor agonists, and adjuvants which we are testing as candidate therapeutics to preserve vaccine memory. In addition a therapeutic, unlike revaccination, need not be antigen-specific and could preserve a broader range of memory cell types and specificities. We are currently optimizing a promising therapeutic composed of nicotine tartrate/TiterMax adjuvant emulsion which can reduce bacterial titers 5-fold in a mouse model of Listeriosis, as well as nicotine patches, which would provide an ideal delivery system in a radiological disaster scenario.

### 39. How FDA's Veterinary Laboratory Investigation and Response Network protects human and animal health by conducting in-depth complaint investigations

*Authors: Reimschuessel, Renate, FDA/CVM; Ceric, Olgica, FDA/CVM; Jones, Jennifer , FDA/CVM; Tkachenko, Andriy, FDA/CVM; Nemser, Sarah, FDA/CVM; Guag, Jake, FDA/CVM; Rotstein, David, FDA/CVM*

Plain Language Synopsis: FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN), was established to collaborate with veterinary diagnostic laboratories to exchange scientific information, build laboratory capacity for routine and emergency response, and train scientists. The network's 38 laboratories conduct in-depth case investigations which have led to multiple recalls of contaminated products.

Abstract:
In late 2010, CVM's Office of Research initiated a project, the Veterinary Laboratory Investigation and Response Network (Vet-LIRN), to collaborate with veterinary diagnostic laboratories to exchange scientific information, build laboratory capacity for routine and emergency response, and train scientists. The overall goal for CVM is for participating laboratories to be ready, willing, and able to help investigate potential problems with animal feed and animal drugs providing a rapid response to reports of animal injury. The Vet-LIRN network is comprised of 38 laboratories. Vet-LIRN laboratories also participate in 11 network cooperative agreement projects designed to evaluate, harmonize and validate chemical or microbial tests using animal diagnostic samples which are not typical food matrices (e.g. urine, blood, feces, saliva, liver, kidney etc.). In addition, the Vet-LIRN conducts approximately three network-wide chemical or microbial proficiency tests per year to demonstrate that the participating laboratories provide accurate and meaningful testing data to FDA. Vet-LIRN laboratories, in collaboration with USDA's laboratory network, are monitoring antibiotic susceptibility and obtaining genomic sequences of select veterinary pathogens. Vet-LIRN laboratories conduct between 30-50 in-depth case investigations per year. The network is actively conducting tests to investigate the root cause of the jerky pet treat associated illness and has conducted more than 1000 tests on products and over 600 tests on diagnostic samples from affected animals. Vet-LIRN case investigations have led to multiple voluntary recalls of animal foods for contamination with bacteria such as Salmonella and Listeria monocytogenes and chemicals such as pentobarbital. Vet-LIRN has leveraged the resources of state-of-the-art veterinary diagnostic laboratories in a remarkably cost effective way to provide FDA with rapid information regarding potential animal feed related contamination events.

### 40. Trastuzumab, but Not Pertuzumab, Dysregulates HER2 Signaling to Mediate Inhibition of Autophagy and Increase in Reactive Oxygen Species Production in Human Cardiomyocytes

*Authors: Mohan, Nishant, FDA/CDER; Shen, Yi, FDA/CDER; Endo, Yukinori, FDA/CDER; Wu, Wen Jin, FDA/CDER*

Abstract:
Dysregulation of autophagy has been implicated in various cardiovascular diseases. Trastuzumab, a humanized monoclonal antibody, binds to HER2 domain IV and is approved for the treatment of HER2-positive breast cancer. Trastuzumab therapy is associated with considerable cardiotoxicity, the mechanism of which remains unclear. HER2 signaling plays a pivotal role in cardiomyocyte development and survival and is essential for the prevention of cardiomyopathy. However, a direct link has not been confirmed between trastuzumab-induced cardiomyopathy and impaired HER2 signaling. Our data reveal a novel mechanism by which trastuzumab dysregulates HER2 signaling and impairs basal autophagic process in human primary cardiomyocytes. Specifically, trastuzumab treatment leads to the phosphorylation of HER1-Y845 and HER2-Y1248 and the activation of Erk. This in turn results in upregulation of mTOR signaling pathway and subsequently inhibition of autophagy in primary cardiomyocytes and C57BL/6 mice. Trastuzumab-induced downregulation of autophagy is further supported by the fact that trastuzumab treatment reduces protein levels of autophagosome-associated signaling molecules such as Atg 5-12, Atg 7, Atg 14, and Beclin 1. We further demonstrated that trastuzumab-mediated inhibition of autophagy resulted in the increased production of reactive oxygen species (ROS) in cardiomyocytes.

Pertuzumab, another anti-HER2 therapeutic mAb binding to HER2 domain II, fails to modulate HER2 signaling and is unable to inhibit autophagy and to increase ROS production in cardiomyocytes. This study provides novel mechanistic insights into trastuzumab-induced cardiotoxicity, which may assist in formulating novel approaches for clinical management of trastuzumab-induced cardiomyopathy.

### 41. Tap Water-Grown Biofilms: A Real-Time Infection Model for Water Containing Medical Devices

Authors: Jon W Weeks, Aprajita Garg, Elizabeth Gonzalez, Poulomi Nandy, and Victoria Hitchins

Plain Language Synopsis: Biofilms on medical devices containing water pose a significant risk to human health. Recent outbreaks from heater-cooler units contaminated with Mycobacterium chimaera have caused serious health risks to patients after cardiothoracic surgery. Here we have analyzed growth conditions of bacterial biofilms and their contributions to resistance to disinfection procedures.

Abstract:
Heater-Cooler Units (HCUs) are important medical devices for patient thermoregulation during cardiothoracic surgeries. Manufacturer's Instructions for Use (IFUs) recommend the use of filter-sterilized tap water for reprocessing and filling of tanks. However, as common practice, HCUs are filled with non-sterile water, though not recommended. The heating and cooling of this water is used to regulate patient body temperatures across a metal or polymer membrane. Inside the tanks and tubes, biofilms can develop on metal and polymer surfaces. Biofilms are known for their tolerance to cleaning and disinfection. Mycobacterium chimaera, a member of the M. avium complex (MAC), has been isolated from patients and HCUs. Pseudomonas aeruginosa is an additional water-borne opportunistic pathogen that is able to grow biofilms in the tanks of devices. Hydrogen peroxide is added to HCU water in order to inhibit microbial growth. Furthermore, HCUs are disinfected using chlorine bleach or peracetic acid. In order to detect M. chimaera and other Mycobacterium spp., chemicals such as N-acetyl-L-cysteine (NALC)-NaOH and cetylpyridinium chloride are used to decontaminate samples of fast growing organisms such as P. aeruginosa. In the current studies, we have investigated the growth of M. avium, a surrogate for M. chimaera, on stainless steel coupons in Middle Brook 7H9 or tap water to determine the ability of the organism to develop into biofilms. Additionally, for biofilms grown in tap water we are looking at the ability of M. avium to develop biofilms when treated with hydrogen peroxide or grown in the presence of P. aeruginosa. We have investigated the effects of chemical agents used to disinfect HCUs of their bioburden. Finally, we have analyzed the effects of decontamination processes on M. avium and mixed biofilms.

### 42. Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for the rapid identification of fungal pathogens isolated from FDA regulated products

*Authors: Cole, Stephanie, FDA/OC; Randell, Clifton, FDA/WEAC; Nevins, Crystal, FDA/WEAC; and Karbiwnyk, Christine, FDA/WEAC*

Plain Language Synopsis:

Fungi are naturally found throughout the environment and can easily contaminate medical devices, such as contact lenses, catheters, and implants, where they may cause local or systemic infections. Validation of this new method will improve the FDA's capability to identify fungi and protect the public from potential health hazards.

Abstract:
Recent outbreaks of fungal contaminated medicines and medical devices have made it imperative that the Food and Drug Administration (FDA) be prepared to identify these microbes in order to quickly recall contaminated products and thereby protect public health. Conventional phenotypic methods to identify fungi are generally time-consuming and some identification problems have arisen from the fact that closely-related species have identical morphologies. Currently, FDA field

labs lack the expertise to accurately identify fungal contaminates in drug products and medical devices by traditional phenotypic methods. The FDA has recently adopted rDNA sequencing to identify fungal isolates from food, drug products and environmental samples. While the sequencing method has proven to be accurate, the method is laborious; requiring several "hands-on" manipulations and many different chemicals and reagents. Here, we are developing a MALDI-TOF MS based protocol for identifying molds and yeast by utilizing microbe specific protein profiles and comparing profiles to reference protein profile libraries. To date, 73 known mold and yeast strains that are commonly pathogenic in humans have been analyzed. Isolates were grown for five to seven days on Sabouraud dextrose agar and a small amount of fungus was used for an ethanol and formic acid protein extraction. Fungal protein extracts were then analyzed by MALDI-TOF MS. Spectra from the isolates were compared to reference libraries containing spectra compiled from the National Institutes of Health (NIH) and Bruker Daltonics, Inc. Based on the quality of the alignment between the experimental spectra and the reference spectra a score between 3.0 - 0 was assigned (3.0 – 2.0 positive identification of both genus and species; 1.9 - 1.7 positive identification of only the genus; 1.6 - 0 no identification). Of the 73 known isolates evaluated, 72.6% were properly identified at either the genus or the species level. In addition, 30 unknown environmental isolates were collected and classified by MALDI (57% were identified). This MALDI-TOF MS method will continue to be evaluated as a quicker and more cost effective alternative method to identifying fungi in the future for the FDA, thereby protecting the public from potential health hazards.

### 43. Detection and Quantification of Alpha and Beta Radioactivity in Food

*Authors: Lin, Zhichao, ORA/ WEAC; Healey, Stephanie ORA/WEAC; Regan, Patrick ORA/WEAC*

Plain Language Synopsis: WEAC analysts have made significant efforts to develop rapid and sensitive radioanalytical methods that are essential to emergency response and risk management.

Abstract:
Globalization of food production, trade, and distribution presents enormous food safety challenges to public health authorities worldwide when local food contamination can rapidly become a national and global emergency. A large number and variety of foods could potentially become contaminated and need to be examined in the event of a nuclear or radiological incident such as the Fukushima Daiichi nuclear accident. To address this growing food safety concern, considerable efforts were put forth by FDA into developing a broad range of radioanalytical methods for enabling more effective emergency response and risk management. Despite that capabilities exist for high-throughput screening and accurate quantification of gamma-emitting radionuclides in food, rapid detection and quantification of alpha and beta radioactivity are still major challenges in protecting food safety and public health due to methodological complexity and time-consuming procedure involved. Analysis of alpha- or beta-emitting radionuclides such as plutonium (238Pu, 239Pu, 240Pu), americium (241Am), uranium (234U, 235U), strontium (89Sr, 90Sr), and tritium (3H) in food requires extensive matrix/interference removal for achieving sensitive detection and reliable quantification with respect to their FDA levels of concern. In support of FDA regulatory compliance and emergency response programs, various radioanalytical methods were developed by FDA Winchester Engineering and Analytical Center (WEAC) to meet program needs. This presentation summarizes various methodological studies and outcomes on developing mission-critical radioanalytical capabilities that apply novel sample preparation techniques and advanced instrument technologies. The method performance characteristics including robustness, detectability, accuracy, precision, and uncertainty as well as their applications for strengthening FERN analytical capability and sample-surge capacity will also be discussed.

## 44. Rapid Detection of Diarrhetic Lipophilic Biotoxins in Seafood by HPLC-MS/MS

*Authors: Li Yang; Avi Singh; Shelley K. Lankford; James Stuart; Daniel Rice; Wen-Hsin Wu; James Hungerford*

Plain Language Synopsis: There are emerging seafood safety issues recently in US. We developed a rapid HPLC-MS/MS method to detect relevant marine biotoxins in seafood to protect public health

Abstract:
Diarrhetic shellfish poisoning (DSP) and azaspiracid shellfish poisoning (AZP) are emerging seafood safety issues. Humans can develop symptoms including vomiting and diarrhea after consumption of seafood contaminated with DSP and AZP marine biotoxins. Currently there is no official regulatory method endorsed by the US FDA to determine if these biotoxins are present in seafood.

A liquid chromatography coupled with triple quadrupole mass spectrometry (Thermo TSQ Quantum Ultra) method for the separation and detection of DSP and AZP biotoxins is described in this study. Separation was achieved using a C18 Hypersil gold column (50mm × 2mm, 1.9µm particle size) with a single run of 7.2 minutes. The mobile phase was comprised of a gradient acetonitrile/water containing ammonium formate and formic acid. Both positive and negative modes were used for multiple reaction monitoring. The limits of quantification of okadaic acid (OA), dinophysistoxin (DTX)1, DTX2, Azaspiracid (AZA) 1, AZA2 and AZA3 in mussels are 0.40, 0.21, 0.30, 0.31,0.49,0.34µg/kg, respectively. The recoveries of OA, DTX1, DTX2, AZA 1, AZA2 and AZA3 in oysters are 88.6; 121; 133; 92.0; 84.0; and 87.1% respectively. The recoveries of these toxins in clams and mussels show similar results.

Twelve Puget Sound shellfish samples including mussels, clams and oysters were provided by WAPHL with no information regarding presence of toxin. Data obtained from these samples using the proposed method agree well with data from a different LC-MS/MS method used in harvest control by WAPHL (Y = 0.89X + 5.6; R squared = 0.998; P<0.0001). Levels of (total free and esterified) DTX1 in 11 samples are below the regulatory action level (160µg/kg) and range from 0.21 to 112 µg/kg. The level of total DTX1 in one Blue Mussel sample is 1539µg/kg, which is well above the regulatory action level.

The proposed method shows sufficient sensitivity, linearity, and reproducibility, and could provide rapid testing for DSP and AZP biotoxins in shellfish.

## 45. Potential Role of Actigraphy as Measured by a Wearable Device as a Clinical Assessment Tool in Children with Pulmonary Artery Hypertension

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Plain Language Synopsis: Pediatric pulmonary arterial hypertension (PPAH) is a serious disorder with high morbidity and mortality. No drug has been approved to treat PPAH in United States partly due to the lack of age appropriate clinical assessment tool. This study investigates the use of actigraphy as a clinical assessment tool in PPAH.

Abstract:
Background: Pediatric pulmonary arterial hypertension (PPAH) is a severe disorder with high mortality and morbidity, but very limited treatment options. Improving outcomes of PPAH has been constrained by a lack of data from interventional clinical trials in young children due to the absence of consistent, reliable and developmentally-appropriate pediatric endpoints that can be readily applied for disease assessment. It is unknown if continuous measures of exercise and activity in the ambulatory setting through the use of actigraphy in children with PPAH can provide a useful tool.

Objectives: Determine if the measurement properties of actigraphy can serve as a potentially useful clinical assessment tool in PPAH.

Design/ Methods: We are conducting a prospective non-interventional clinical study in 100 PPAH patients (pts) and 100 healthy children (age of 8-14 years). The study was approved by the Institutional Review Boards at the FDA and the University of Colorado. We are reporting data from the first 17 subjects enrolled. Two types of actigraphy devices with different measurement metric outputs, chest-worn Actiheart™ and wrist-worn Fitbit Charge HR™, were used to record heart rates (HR) and physical activities (PA). Actiheart uses a 1-axis accelerometer to obtain total daily vector magnitude counts and mean counts/min. Fitbit Charge HR uses a 3-axis accelerometer to obtain total daily step counts and mean steps/min. Subjects were asked to wear both devices for 14 days after written consent/assent was obtained. Actigraphy data obtained during awake hours each day were analyzed.

Results: All 17 subjects wore both devices for at least 14 days with good compliance. Actigraphy data were successfully collected in each subject:

| | **Actiheart** | | **Fitbit Charge HR** | |
|---|---|---|---|---|
| | PPAH pts | Healthy Children | PPAH pts | Healthy Children |
| # of subjects; %Female | 4; 50 | 13; 44 | 4; 50 | 13; 44 |
| Mean±SD age (years) | 10±3 | 11±2 | 10±3 | 11±2 |
| Total Day PA, x104 counts (Actiheart) or steps (Fitbit) | 8.7±3.5 | 10.5±5.4 | 7700±700 | 9600±3000 |
| Mean±SD Daytime PA, counts or steps/min | 120±49 | 145±75 | 11±1 | 13±4 |
| Mean±SD HR (BPM) | 92±12 | 97±11 | 87±13 | 83±10 |

Conclusions: Data from our early experience of this ongoing study suggest that actigraphy as a measure of physiologic responses to various levels of activity provides a feasible and well-tolerated measure of PA in PPAH pts and healthy children. We speculate that actigraphy has potential utility as a reliable clinical assessment tool for PPAH pts, especially in the setting of future interventional trials. (This study is supported by FDA Chief Scientist Grant and PPHNet U01 grant: 1U01HL121518, https://projectreporter.nih.gov/project_info_description.cfm?projectnumber=1U01HL121518-01)

## 46. Development of a Rapid and Efficient Method for Hepatitis A Virus Concentration from Green Onions

*Authors: Zheng, Yan, FDA/ORA/NRL and Hu, Yuan FDA/ORA/NRL*

Plain Language Synopsis: Inclusion of beef extract and pectinase facilitated the elution of HAV from green onions. Carboxyl beads provided a rapid and efficient HAV concentration method. Solubilizing host-derived membrane improved affinity-based HAV concentration.

Abstract:
Hepatitis A virus (HAV) can cause serious liver disease and even death. HAV outbreaks are associated with the consumption of raw or minimally processed produce, making it a major public health concern. Infections have occurred despite the fact that preventive vaccine has been available. Development of a rapid and sensitive HAV detection method is necessary for an investigation into an HAV outbreak. Detection of HAV is complicated by the lack of a reliable culture method. In addition, due to the low infectious dose of HAV, these methods must be very sensitive. Current methods rely on efficient sample preparation and concentration steps followed by sensitive molecular detection techniques. We have used carboxyl-derivatized magnetic beads to capture virus particles from green onion samples. Carboxyl beads like antibody-coated beads or cationic beads, detect HAV at a level as low as 100 pfu/25 g of green onions. RNA from virus concentrated in this manner can be released by boiling for molecular detection without sacrificing sensitivity. Bypassing the RNA extraction procedure saves time and removes multiple manipulation steps, which makes large scale HAV screening possible. We have also demonstrated that the inclusion of beef extract and pectinase rather than NP40 in the elution buffer improved the HAV liberation from the food matrix over current methods by nearly 10 fold. Our proposed method provides a promising tool to improve food risk assessment

and protect public health.

### 47. Naloxone Prize Competition Catalyzes Innovative Approaches to Reducing Fatal Opioid Overdoses

*Authors: Cruz, Marisa (FDA/OC); Thompson, Camelia (FDA/OC); Lurie, Peter (FDA/OC)*

Plain Language Synopsis: Opioid addiction and abuse is now a public health epidemic in the United States. Naloxone, a prescription medication, can effectively reverse an opioid overdose if administered rapidly. In 2016, FDA launched a prize competition to design a mobile phone app to quickly connect carriers of naloxone with opioid overdose victims.

Abstract:
Since 1999, rates of overdose deaths involving opioids have nearly quadrupled; in 2015, 91 Americans died from an opioid overdose each day. By antagonizing opioid effects in the central nervous system, the prescription medication naloxone functions as an antidote for opioid overdoses. Through the efforts of government agencies and community-based organizations, naloxone is becoming increasingly available in many communities. Both first responders and laypersons, such as family members and other drug users, are now frequently able to carry naloxone. To effectively reverse an opioid overdose, however, naloxone must be administered rapidly. FDA launched the Naloxone App Competition to engage individuals from public health, technology, and business communities in developing innovative approaches to improving the timeliness of naloxone delivery. Teams had six weeks to develop and submit a concept for a mobile phone app connecting carriers of naloxone to persons experiencing an overdose. Participants were also offered the opportunity to engage with other teams in an optional code-a-thon held midway through the competition. Solutions, which were restricted to a five-minute video and a three-page written summary, were judged on four criteria: innovation, usability, functionality, and adaptability. The submissions showcased a variety of ideas from the use of SMS messaging and voice activation to respiratory sensors and other wearable components. Many of the submissions considered interfacing with the 911/Emergency Medical System and social networking sites to establish a support system for the opioid user. Judges from FDA, NIDA and SAMHSA selected the winner and honorable mention; the winning team was awarded $40,000. The winning concept, "OD Help," connects naloxone carriers to at-risk opioid users through a simple smartphone interface. The app can be tailored for use in rural and urban settings, and offers users the option of establishing a trusted support network in order to minimize safety concerns. Should an opioid overdose victim be alone and unable to summon help, the app also optionally pairs with a wearable spirometer, alerting nearby naloxone carriers to an overdose if persistent respiratory depression is detected. All teams were encouraged to further develop and test their concepts through the NIH Small Business Innovation Research program.

### 48. Collaborative Efforts Between Federal and Hawaii State Partners Helped Identify Hepatitis A Adulterated Scallops

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Plain Language Synopsis: Hawaii Department of Health identified a local restaurant chain and, scallops as common food exposures for a hepatitis A outbreak. FDA's SAN-DO collected, FDA's CORE traced back and CFSAN's Gulf

Coast laboratory analyzed the scallop samples, using matrix extensions of standard FDA methodologies. Collaboration between partners resulted in regulatory actions.

Abstract:
On July 8, 2016, the FDA San Francisco District Office (SAN-DO) notified the Coordinated Outbreak Response and Evaluation (CORE) Network of an outbreak of 31 cases of hepatitis A virus (HAV) infections in Hawaii. The Hawaii Department of Health (HDOH) identified a local sushi chain restaurant as a common point of service. The reported common food exposures included scallops, tuna, tobiko, and masago. Following an extensive epidemiologic investigation, scallops were determined to be the suspect food vehicle. On August 16, 2016, SAN-DO collected two frozen scallop adductor-muscle samples from a distributor to the Hawaii chain restaurant. FDA assisted Hawaii with laboratory analysis of scallops from suspect lots imported from a Philippines firm, identified by CORE through traceback investigations. FDA's Center of Food Safety and Applied Nutrition's Gulf Coast Seafood Laboratory utilized matrix extensions of FDA standard methodologies to test for HAV presence in frozen scallops, tuna, masago, and tobiko. The RT-qPCR and gel analysis utilized amplicons from the 5' untranslated region of the HAV genome to detect and further distinguish the HAV strains. The lots of frozen scallop samples tested positive for HAV. Characterization through sequencing of the VP1-2A region of the HAV genome and genetic analysis revealed 100% homology between the recovered scallop and clinical strains. HDOH embargoed the frozen scallop products and closed the associated restaurants. FDA immediately notified the distributor of the implicated scallop and placed the imported scallops from the implicated firm on increased surveillance. On August 18, 2016, the distributor recalled the implicated lots of scallops produced on November 23, 2015 and November 24, 2015. On September 15, 2016, FDA issued an import alert for molluscan shellfish produced by the implicated firm. The collaborative efforts between federal and state partners identified the adulterated product and resulted in regulatory actions, thus effectively stopping the outbreak and protecting public health.

## 49. Murine model of urinary tract infection to study the impact of antibiotic drugs on in vivo emergence of antibiotic resistance

*Authors: Chockalingam, Ashok, FDA/OCP/DARS; Stewart, Sharron, FDA/OCP/DARS; Xu, Lin, FDA/OCP/DARS; Shea, Katherine, FDA/OCP/DARS; Zadrozny, Leah, FDA/OCP/DARS; Matta, Murali, FDA/OCP/DARS; Rouse, Rodney FDA/OCP/DARS*

Plain Language Synopsis: The evolution of bacterial resistance to antibiotics has led to national action plan to combat antibiotic resistance. At FDA, the hollow fiber and mouse model studies are conducted to provide efficacy and safety data for a clinical trial of urinary tract infection using single or combination therapies to deter resistance.

Abstract:
Bacterial resistance to antibiotics has been observed with increasing frequency over the past several decades. The escalating evolution of resistance coupled with a diminished antibiotic pipeline has led to the development of a national action plan for combating antibiotic resistance and addressing urgent and serious drug resistant threats. The FDA's Office of Medical Policy (OMP) and the Division of Applied Regulatory Science (DARS/OCP/OTS) in CDER are collaborating to investigate strategies for suppressing emergence of antibiotic resistance using the in vitro hollow fiber system and in vivo animal models. Urinary tract infection (UTI) is both a common clinical entity and a frequent environment for emergence of resistant bacteria. E. coli is the most common bacteria involved in clinical UTI and has demonstrated the ability to develop resistance to antibiotics commonly used to treat UTI. Therefore, a murine model of UTI was selected to assess the impact of antibiotic exposure on in vivo emergence of antibiotic resistance. This information will be used to determine the ability of novel antibiotic therapeutic strategies to suppress the emergence of antibiotic resistance. Initial experiments to study UTI in the mouse model have been established using the E. coli strain CFT073. The murine

model of acute UTI in our pilot studies showed successful bacterial infection in the urinary bladder and kidneys. Over the course of 10 days post-inoculation, bacterial counts decreased spontaneously but bacteria were not eliminated. The impact on emergent resistance of repeat exposure of E. coli strain CFT073 to ciprofloxacin, fosfomycin, or ampicillin is detailed.

**50. Development of high yield reassortant influenza viruses for evaluation as candidate vaccine viruses**

*Authors: Alvarado-Facundo, Esmeralda, FDA/CBER; Couzens, Laura, FDA/CBER; Jelinek, Christine, FDA/CBER; Asgari, Sepideh FDA/CBER; Eichelberger, Maryna, FDA/CBER*

Plain Language Synopsis: We are developing high-yield virus isolates from recent circulating influenza A viruses for evaluation as vaccine candidates. This will facilitate timely manufacture of influenza vaccines against antigenically-similar viruses causing human morbidity and mortality.

Abstract:

Influenza virus undergoes antigenic drift of its hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins, resulting in the need to update the seasonal vaccine on an annual basis to ensure the vaccine includes viruses that match the circulating strains. There is a short window for the vaccine manufacturing process and the number of doses manufactured is limited by the yield of each virus grown in eggs, the main substrate used for vaccine production. To ensure that candidate vaccine viruses (CVV) which replicate well in eggs are available to manufacturers in a timely manner, we are preparing high yield (HY) influenza isolates that are representative of emerging circulating strains. A classical method is used to select reassortant viruses that contain the HA and NA of the circulating virus and internal gene products from a donor virus that replicates well in eggs. In some cases, viruses that replicate efficiently in eggs were isolated without incorporating donor virus gene segments. The virus isolates are tested for antigenic relatedness to the parent wild type virus and those with highest HA content are selected for further analysis by a WHO Collaborating Center and certified as a CVV. We have selected and characterized six influenza high yield viruses: A/Victoria/361/2011 CBER X-001 (H3N2), A/Saitama/103/2014 CBER X-002 (H3N2), A/Indiana/21/2016 CBER X-003 (H1N1), A/Yokohama/94/2015 CBER X-004 (H1N1), A/Alaska/232/2016 CBER X-005 (H3N2), and A/Idaho/33/2016 CBER-06 (H3N2). RT-qPCR was performed to determine the gene constellation, hemagglutination inhibition assays were used to evaluate antigenicity, and isotope-dilution mass spectrometry was conducted to measure HA concentration. The production of high yield viruses at CBER contributes to ensuring the availability of CVVs for timely production of influenza vaccines that are well-matched to circulating viruses.

**51. An Extraction Free Assay for Quantifying Residual Protein and Microbial Biofilms On Surfaces**

*Authors: Guan, Allan, FDA/CDRH; Wang, Yi, FDA/CDRH; Phillips, Kenneth, FDA/CDRH*

Plain Language Synopsis: Inadequate decontamination during device reprocessing is a leading cause of post-endoscopic infection. Quality control of cleaning processes, therefore, plays an important role in mitigating this risk. This project develops a method to quantify difficult-to-remove residual contaminants on reprocessed endoscopes for use in healthcare facilities.

Abstract:
According to a 2016 U.S. Senate report, from January 2012 through April 2015, 19 outbreaks of patient infections with antibiotic-resistant bacteria were reported in the United States following endoscopic retrograde cholangiopancreatography (ERCP) procedures with closed-channel duodenoscopes. FDA investigation into the association between multidrug-resistant bacterial infections and duodenoscopes revealed that complex device design impeded adequate cleaning. For instance, microscopic crevices formed by the moving joints of the elevator mechanism may not be reached by cleaning brushes. Retention

of bodily fluids and organic debris in these areas could harbor pathogenic microbes and spread infection between patients. As a result, FDA recommended that healthcare facilities implement a comprehensive quality control program for reprocessing duodenoscopes.

An important aspect of quality control in medical device reprocessing is validation of cleaning performance. Current validation methods for detecting surface-adherent contamination require extraction of biological soil (such as protein or biofilm) from the surface of interest. However, physical inaccessibility of contaminated surfaces and poor water solubility of contaminants can underestimate the level of residual bioburden. In this work, we demonstrate how the o-phthalaldehyde (OPA) protein assay can be modified to measure residual protein or biofilm on a surface without the need for extraction. The assay limit of detection (LOD) was 1.6 µg/cm2—four times lower than the current benchmark for protein residuals on reprocessed endoscopes (<6.4 µg/cm2) and eight times more sensitive than the gold standard (protein test strips). Intra- and inter-assay reproducibility were 0.83% and 1.98%, respectively. The detection threshold for Staphylococcus epidermidis biofilm on stainless steel coupons was 117 µg/cm2. A preliminary study is underway at a local hospital to optimize the assay under real-world conditions. By enabling the detection and quantification of soils in complex or hard-to-reach areas, this method has the potential to improve the margin of safety in medical device reprocessing.

References:
Murray, P. Preventable Tragedies: Superbugs and How Ineffective Monitoring of Medical Device Safety Fails Patients. (United States Senate, 2016).

Design of Endoscopic Retrograde Cholangiopancreatography (ERCP) Duodenoscopes May Impede Effective Cleaning: FDA Safety Communication. (2015). Available at: http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm434871.htm.

## 52. Validation of an LC-MS/MS Method for Analysis of Anti-diabetic, Anti-obesity, and Cholesterol-lowering Drugs in Botanical Dietary Supplements Labelled for Blood Sugar Management

*Authors: Jun Ma, Rahul S. Pawar, and Erich Grundel*

Plain Language Synopsis: An LC-MS/MS method was developed and validated to detect and quantitate fourteen anti-diabetic, two anti-obesity, and three cholesterol-lowering drugs in botanical dietary supplements sold for blood sugar management. This method can be used to analyze dietary supplements for possible adulteration with these compounds.

Abstract:
Many botanical dietary supplements which carry label statements related to blood sugar management are available over the internet. Potential adulteration of such dietary supplements with anti-diabetic and other prescription drugs, some of which have been removed from the market due to adverse events, is of concern. In this study, we developed and validated an LC-MS/MS method to detect and quantitate fourteen anti-diabetic, two anti-obesity, and three cholesterol-lowering drugs in botanical dietary supplements sold for blood sugar management. Dietary supplements were extracted with methanol. No significant matrix effects were observed over a wide range of analyte concentrations. Mean recoveries of all 19 analytes from a single product ranged from 88 to 113% at spike concentrations from 500 to 2000 µg/g. Mean recoveries of three analytes (metformin, phenformin, and sibutramine) from composites prepared from mixtures of four or five products ranged from 93 to 115% at a spike concentration of 100 µg/g. The relative standard deviations (RSDs, %) of intra-day analyses ranged from 0.2 to 13 for all recovery studies. Eighty botanical dietary supplements obtained in the U.S. and carrying label statements related to blood sugar management were analyzed using this method and none were found to be adulterated with the above 19 compounds. Two products obtained outside of the U.S. and known to be adulterated were also

analyzed by this method and found to contain the target analytes (phenformin, glibenclamide, and sibutramine). This method can be used to analyze dietary supplements for possible adulteration with these compounds.

### 53. Complete Sequences of Five Multidrug Resistant Salmonella enterica serovar Dublin

*Authors: Li, Cong; Hsu, Chih-Hao; Abbott, Jason; Ayers, Sherry; Tyson, Gregory; McDermott, Patrick; Zhao, Shaohua; FDA/CVM; Hoffmann, Maria; Yao, Kuan; FDA/CFSAN*

Plain Language Synopsis: This work helps FDA understand the ecology of antimicrobial-resistant bacteria and can be used to assess the risks of both new and previously approved antimicrobials for food animals.

Abstract:
Salmonella enterica serovar Dublin is a host-restricted serovar associated with typhoidal disease in cattle. While rare in humans, it causes more severe illness in humans, including invasive infections. The National Antimicrobial Resistant Monitoring System (NARMS) data showed that multidrug resistance has emerged recently in S. enterica serovar Dublin from animal, food, and human sources. To compare the genetic makeup of its virulence and resistance features, and to serve as a reference for future sequencing, we have sequenced and closed five genomes of S. enterica serovar Dublin using the PacBio platform. Three strains were isolated from sick cattle and two from retail ground beef and were selected based on their resistance profiles. All five strains carry one or two plasmids with sizes between 36 kb and 329 kb and each plasmid carries one or two replicons. The resistance genes including tetA, blaTEM-1B, blaCMY-2, sul1, sul2, floR, strA, strB, aadA,aph(3')-Ia were found to be located on the IncA/C2 or chimeric plasmids, the bigger of two plasmids. All five strains also carried plasmid-borne spv operons (spvRABCD), which are important for Salmonella virulence. One isolate from a sick cow carried a single plasmid of 329 kb, showing 99% homology to pSDVr and contained multiple plasmid replicons including IncA/C2 and IncFIC. This plasmid carries two copies of blaCMY-2 and tetA located at different sites, likely reflecting separate recombination events. Together these data reveal the complicated plasmid structures and the recombination events occurring in S. enterica serovar Dublin.

This study helps us to better understand the co-selective pressures of antibiotic resistance and virulence in this important serotype.

FDA Mission Relevance: The objective of this study is to characterize Salmonella enterica serovar Dublin through WGS analysis. S. enterica serovar Dublin is highly virulent serotype that can cause severe salmonellosis in cattle and humans. They have recently developed resistance to multiple antimicrobial drugs, including ceftriaxone. This work helps FDA understand the ecology of antimicrobial-resistant bacteria and can be used to assess the risks of both new and previously approved antimicrobials for food animals.

### 54. Aggregation and innate immune response modulating impurities

*Authors: Polumuri, Swamy kumar FDA/CDER; Haile, Lydia,FDA/CDER; Rao, Roshni, FDA/CDER; and Verthelyi, Daniela, FDA/CDER;*

Plain Language Synopsis: Therapeutic proteins are manufactured and formulated to be remaining as monomers, stress during the complex manufacturing process, can lead to the formation of oligomers, subvisible and visible particles. These aggregated particles induce innate immune response by engaging different receptors on the immune cells and induced various cytokine and chemokines.

Abstract:
Therapeutic proteins can induce immune responses that affect the safety and efficacy even if derived from human sequences. The factors that contribute to the immunogenicity of this therapeutics are poorly understood, but protein aggregation is thought to facilitate it. Previously, our lab showed that trace levels of product or process derived innate immune response modulating impurities (IIRMIs) can also increase product immunogenicity. In this

study we hypothesized that protein aggregates and IIRMIs may synergize to facilitate product immunogenicity and explored it using clinical grade Human Serum Albumin (HSA) and Intravenous Immunoglobulin (IVIG) proteins as model. Protein aggregations formed by mechanical stress (shaking and stirring) were characterized by SDS-PAGE, turbidity, MFI and FlowCAM. Cellular responses to aggregated proteins with trace levels of IIRMs were tested in human PBMC. We show that stirring and shaking resulted in the formation of stable aggregates with size ranges of 2 - 50 µm for stirred HSA; and 2-100 µm for IVIG. Despite the similarity in size, the functional assays showed that aggregates of IVIG result in increased mRNA expression of IL-8, IL-6, IL-1ß and CCL-2 whereas HSA aggregates did not. Further, we analyzed how the aggregated product changed the transcriptome using Nanostring and showed that aggregated IVIG (shaken or stirred) significantly increased the mRNA of CCL2, CCL7, CCL3, CCL24, CSF1, CXCL2, IRAK1, EGR2, CEBPß, PPARg, TNFSF15 and whereas HSA aggregates did not. Similarly MAPKs (pp38, pERK and pJNK) and pAKT were activated when PBMC were stimulated with IVIG aggregates but not HSA aggregates. Lastly, we examined whether the effect was mediated by Toll like receptors.

### 55. Neonatal mouse model to study Zika virus pathogenesis: Host immune response determines ZIKV tropism and outcome of disease.

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Plain Language Synopsis: In mice, as in healthy individuals, innate and adaptive immune responses are essential to control and clear ZIKV infection. Interferon deficient mice are susceptible to the virus, develop bilateral paralysis and die within days of infection. We developed an immunocompetent model of infection for ZIKV using neonatal C57BL/6 mice.

Abstract:

Zika virus (ZIKV) is a mosquito-borne flavivirus that has spread to more than 70 countries and has infected more than 2 million people worldwide. The recent ZIKV outbreak has resulted in CNS complications including microcephaly, Guillain Barre and polyneuropathy. Current ZIKV infection models use interferon (IFN) deficient mice that develop high viral loads systemically and die within a week. Establishing an Immunocompetent animal model that replicates ZIKV neurotropism is critical to understand ZIKV pathology and testing of immunotherapies. We developed an immunocompetent neonatal mouse model in wild type C57BL/6 mice by challenging 1 day old mice with contemporary ZIKV isolate PRVABC59. Infected mice developed unsteady gait, loss of balance, kinetic tremors, severe ataxia and seizures beginning 13 days post infection (dpi) that resolved by 28 dpi. Following challenge the mice developed a systemic infection with high virus titers in spleen, liver and kidney that was cleared by 10 dpi. In the Central Nervous System (CNS) and the eye, the virus was detected starting 6 dpi, peaked at 9-15 dpi, and then decreased over 6 weeks. Unlike IFN deficient mice that have a significant increase in mRNA expression of complement (C3), Cox2, Il1a, Il1b, and Il6, the CNS of infected B6 mice showed increased expression of genes associated with T cell responses including CD3, CD4, CD8, MHC-II, CD80, IFNg, perforin and granzyme B. , Despite the absence of clinical disease in convalescent B6wt mice, there remain increased expression of inflammatory and T cell associated genes that suggest that the virus could have long-term effects on the CNS. Further investigation including behavioral studies is needed to explore possible long term CNS sequelae of ZIKV infection.

### 56. T cell infiltration associated with neurodegeneration and Zika virus control in the CNS of immunocompetent, neonatal mice.

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Plain Language Synopsis: This study uses a novel neonatal animal model to characterize the immune response to Zika virus infection in the brain and determine what role immune cells play in the resulting disease.

Abstract:
The recent spread of Zika virus (ZIKV) and its association with neurological disorders as well as congenital defects has created an urgent need to develop animal models that allow the elucidation of pathogenesis following ZIKV infection and to develop and test therapeutic agents that will prevent or eliminate CNS disease. We have developed a novel model of ZIKV infection in mice, using B6 WT, neonatal mice that are susceptible to contemporary ZIKV (PRVABC59) CNS infection following peripheral, subcutaneous inoculation at P1. Immunocompetent B6 WT mice develop a discrete neurological disease that includes: unsteady gait, kinetic tremors, severe ataxia and seizures by 12-15 dpi that gradually resolve. Gene expression and immunohistochemistry demonstrate inflammation in the CNS of ZIKV infected B6 WT mice starting after 9 dpi and infiltration of immune cells into the parenchyma of the CNS beginning concomitantly. At the peak of disease (15 dpi), immune cell infiltrates in the CNS consisted primarily of T cells, particularly CD8+ T cells. Gene expression analysis indicated an upregulation of genes consistent with this infiltration. Immunohistochemistry of the CNS indicates a concomitant increase in apoptosis and neurodegeneration after 9 dpi in the CNS particularly in the granular and Purkinje cell layers of the cerebellum. The role of T cells in the control of ZIKV infection in the CNS and the periphery will be explored.

## 57. Dependence Behaviors and Nicotine Pharmacokinetics in Electronic Cigarette Users

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Plain Language Synopsis: The objective of the exploratory study to investigate dependence behaviors and nicotine exposure associated with ad libitum use of three e-cigarette products. The study conclusions are as follows:

- Study products contained approximately 40% less nicotine than indicated on the label.
- Despite differences in topography and nicotine exposure, all products suppressed MNWS withdrawal symptoms similarly. Therefore, the relationship between ENDS use and withdrawal suppression may be independent of topography or nicotine exposure, but based on product use.
- Topography measures indicate some behavioral compensation based on products or e-liquid nicotine content.
- OWN product puff topography indicates that these products (primarily 2nd and 3rd generation) may aerosolize e-liquid more efficiently than the study products.
- Participants had greater nicotine exposures when using Own products than low nicotine study products.
- Overall, these results suggest that e-cigarette users may adjust use behaviors according to e-liquid nicotine content or product familiarly.

Abstract:
In an ongoing scientific debate on e-cigarettes and their impact on public health, some argue that e-cigarettes may have a potential for decreasing the death and disease toll by offering a less harmful alternative to combustible cigarette smoking. However, there are also concerns that e-cigarettes may renormalize smoking behaviors and support or maintain nicotine dependence. Thus, it is important to understand how constantly evolving e-cigarette products may affect withdrawal symptoms, nicotine dependence, and nicotine exposure.

Studies indicate that experienced e-cigarette users use their products differently than traditional cigarettes and can achieve nicotine exposures similar to those of cigarette smokers or persons using nicotine replacement therapy.

Furthermore, the nicotine pharmacokinetic curve following e-cigarettes use may be similar to cigarettes, suggestive of these products' addictive potential. Furthermore, e-cigarette users may able to titrate their nicotine exposure by altering e-cigarette use behaviors (i.e., compensation) based on e-liquid nicotine concentration or product.

Due to the variable nicotine exposures and use behaviors associated with e-cigarette use, we conducted an exploratory study to investigate dependence behaviors and nicotine exposure associated with ad libitum use of three e-cigarette products.

### 58. CBER-OBRR Response to Public Health Emergency: Development of Zika Virus RNA Reference Reagents and Lot-Release Panel

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Plain Language Synopsis: Reference reagents for Zika virus nucleic acid are needed to support the development of novel blood screening assays and the evaluation of existing assays. Our laboratory has produced and fully characterized lyophilized material for use as Zika reference reagents and a liquid-frozen lot-release panel for use upon assay licensure.

Abstract:
Since its first reported appearance in the Western Hemisphere in 2015, Zika virus (ZIKV) has spread through the Americas. ZIKV infection has been associated with severe neurological outcomes such as Guillain-Barré syndrome and fetal microcephaly. ZIKV poses a transfusion-transmission risk because viremia appears before symptoms develop, and approximately 80% of infections never produce symptoms. Four cases of transfusion-transmitted ZIKV from three donors have been reported in Brazil, and rates of viremic blood donors during outbreaks were 2.8% in French Polynesia in 2014 and 1.1% in Puerto Rico in 2016. Nucleic acid testing (NAT) is the most sensitive method to screen blood donors for ZIKV infection. Two ZIKV NAT assays are in use for blood screening under IND, but none are currently FDA-approved. Our laboratory has produced prototype ZIKV RNA Reference Reagents to facilitate evaluation of existing NAT assays and development of novel ZIKV assays. The aim of this project was to produce and fully characterize lyophilized material for use as reference reagents and liquid-frozen lot-release panel for use upon assay licensure.

Two strains of ZIKV (PRVABC59, Puerto Rico-2015; and FSS13025, Cambodia-2010) were used to produce cell-culture-grown stocks, heat-inactivated at 56°C for 60 minutes, and diluted in human plasma (BaseMatrix). Heat-inactivation was confirmed by back-titration, and the material was further characterized by 21 proficient laboratories as part of studies to establish the WHO ZIKV International Standard. Study participants tested the reagents for ZIKV-RNA using their NAT assay(s) in serial dilution to determine the end-point, followed by testing half-log dilutions around that end-point to confirm the titer. Estimated units /mL were calculated using Probit analysis. The ZIKV Reference Reagents had an estimated overall mean of 6.04 log10 units/mL for PRVABC59 and 5.59 log10 units/mL for FSS13025, and are currently undergoing accelerated and real-time stability studies for the lyophilized formulation. A lot-release panel ranging from 200 – 0 copies/ml has been produced using the reference reagent materials. The panel is currently being assessed in collaborative studies as done previously. Additionally, we have performed complete genomic sequencing including the 5' and 3' non-coding regions for strains PRVABC59 and FSS13025 and uploaded these to Genbank.

### 59. Automated and High Throughput Reactive Accelerated Aging System to Evaluate Performance of Neural Implants

*Authors: Street, Matthew, FDA/CDRH/OSEL/DBCMS; Takmakov, Pavel, FDA/CDRH/OSEL/DBCMS*

Plain Language Synopsis:
Failure of neural implants in vivo limits their therapeutic potential. Development of new designs to address this issue requires expensive animal testing. Reactive

accelerated aging system that simulates in vivo environment speeds up this testing. The purpose of this project is to design an automated, high throughput testing platform.

Abstract:
Explosive growth of therapeutic applications for neuromodulation is associated with miniaturization of neural implants. Miniaturization is implemented with novel fabrication techniques and novel materials that do not have a proven history of clinical use. Pilot clinical studies with these novel devices demonstrated that failure of the implants in the body and their poor durability presents a major bottleneck for this technology. Evaluation of durability of different neural implant designs in animal models requires a lot of time and resources. To facilitate innovation process and replace chronic animal testing, we developed reactive accelerated aging (RAA) that uses reactive oxygen species to simulate foreign body response and high temperature to accelerate degradation reactions in vitro. Although the first version of the RAA system proved to be useful in mimicking degradation of these devices, it still required daily monitoring and maintenance to ensure functionality as well as had some variability in performance. To address these issues, we developed a new system that implements automation, modularity and multiplexing. First, we designed the RAA system to be scalable and modular to facilitate multiplexing for high-throughput testing. Second, we replaced the thermostats with mineral oil thermal transfer with electrical heating. Third, we implemented automatic control of hydrogen peroxide concentration using an in-line UV spectrophotometer. Fourth, through using a Raspberry Pi we are able to perform in situ optimization of operating parameters and increased control of the system. These improvements will enable us to perform experiments in parallel for a high-throughput approach to investigating optimal test conditions for different materials or device geometries. Lastly, we implemented automatic sampling and analysis of leachable compounds to establish and quantify neural implants failure modes. Through these improvements, we developed a cost-effective tool for rapid evaluation of durability of neural implants in vitro and provide the framework to improve the functional lifespan of these devices.

## 1. Utility of FDA ECID Microarray for Comprehensive Molecular Serotyping of Escherichia coli


*Authors: Kyson Chou, Isha Patel, Jayanthi Gangiredla, Nelly Tran, Donna Williams-Hill,*

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Plain Language Synopsis: Validation of the FDA-ECID microarray provides another efficient and effective tool for the Agency to identify pathogenic E. coli and help facilitate decision-making by CFSAN's Office of Compliance.


Abstract:

Introduction: Shiga toxin-producing Escherichia coli (STEC) serogroups, including novel hybrid pathotypes, are increasingly causing food borne illness. As a result, serotyping of E. coli has become an important element for regulatory decisions. Not all STECs are pathogenic, and since E. coli strains are serologically complex, the identification of the serotype as well as specific genetic loci deemed necessary for severe virulence, must be determined. Laboratory analysis has shown that over half of the E. coli isolated from foods are untypeable or only yield partial serotypes. Therefore, a means to quickly determine the serotype of STECs would be useful in surveillance programs and outbreak responses to expedite product recall, thus reducing the distribution and consumption of contaminated foods, minimizing outbreak impact. The FDA-ECID microarray has the requisite attributes to fill this critical need, not only for FDA/ORA Field Laboratories, but for any food analytical laboratory.

Purpose: Following the guidelines of the FDA Methods Validation Subcommittee, the current study aims to validate the FDA-ECID microarray, as a rapid means for robust genetic serotyping of E. coli isolates.

Methods: Genomic DNA from a panel of reference E. coli strains was assayed in triplicate on the FDA-ECID microarray. Each strain was analyzed to determine both the O and H determinants as well as for specific STEC virulence genes that may be indicative of potential for severe human health risks. The array typing results were then compared with the known profiles of the corresponding strains.

Results: To ensure accurate identification of isolates analyzed, modified standard operating procedure for analysis and quality control were developed and implemented. Analysis using quality controlled results showed that for all 85 isolates analyzed, replicates of each isolate clustered together in the dendrogram and had the same serotype that matched the known profiles of the corresponding strains, thus, demonstrating the reliability and the validity of the FDA-ECID microarray for genetic serotyping.

Significance: Validation of the FDA-ECID microarray provides another efficient and effective tool for the Agency to identify pathogenic E. coli and help facilitate decision-making by CFSAN's Office of Compliance.


## 2. Platelet septin-2 and septin-6 are regulated by miR-223


*Authors: Chattopadhyay, Maitreyi, FDA/CBER; Dahiya, Neetu, FDA/CBER; Kulkarni, Sandhya, FDA/CBER; Chintamani, Atreya, FDA/CBER*


Plain language synopsis: MicroRNAs are small non-coding RNA molecules that regulate gene expression of their target mRNAs. MicroRNA 223, a differentially expressed microRNA during platelet storage, has been hypothesized to modulate biological functions of platelet through its interaction with its targets, septin-2 and septin-6.


Abstract:

Septins are expressed in non-dividing cells such as platelets, which are GTP-binding proteins and are shown localized surrounding alpha-granules in platelets, suggesting that septins may play a role in platelet biology and aggregation. Ex vivo stored Platelet concentrates are lifesaving transfusion products in case of trauma and severe bleeding. During storage, platelets' quality deteriorates since they undergo physiological and morphological changes collectively known as storage lesions (SL). Understanding the SL would help facilitate improvements to the

quality and shelf-life of platelets. Platelets contain functional microRNA-processing machinery. MicroRNAs are short non-coding RNA molecules that modulate post-transcriptional gene expression by binding to the target sites of within their corresponding messenger RNAs (mRNAs) and regulate their translation. We have identified several miRNAs to be differentially expressed during storage and identified that miR-223 expression in platelets to be inversely correlated with platelet aggregation. Further, Septin2 and septin6 mRNAs were identified to have the target sites for miR-223 in their 3' Untranslated regions (UTRs). These septin:miR-223 interactions are being tested using a luciferase reporter gene expression system in mammalian cells. The effect of ectopic expression of miR-223 on platelet aggregation is being confirmed.

**3. One protein, many functions: the diverse roles of transcriptional regulator IRF1 in innate immune signaling and antiviral defense**

*Authors: Erisa Gjinaj, Debasis Panda, and Ronald L. Rabin, Laboratory of Immunobiochemistry, DBPAP, CBER, FDA*

Plain Language Synopsis: Much has been elucidated regarding host responses to viral infection. Although interferons and interferon stimulated genes play a greater role in antiviral defense, there are interferon-independent mechanisms that also play a key role to subvert viral infection. We discovered a multifaceted role of human protein IRF1 in antiviral defense.

Abstract:
Types I and III interferons such as IFNß and IFNλ1, respectively, are often considered redundant because they share canonical JAK1/Tyk2 signaling pathways and similarly induce expression of interferon stimulated genes (ISGs). Recent reports suggest that types I and III IFNs non-redundantly protect against viral infection, but the mechanism is unknown. We recently reported that among a panel of transcription factors expressed in response to these IFNs, BEAS-2B human respiratory epithelial cells (REC) preferentially express IRF1 in response to IFNß. This suggests that IRF1 may mediate non-redundant functions of types I or III IFNs. To further explore the role of IRF1 in viral protection of REC, we created CRISPR/Cas IRF1 KO BEAS-2B cells, and compared their susceptibility to vesicular stomatitis virus (VSV) to the parent cell line. We found that IRF1 KO BEAS-2B cells are highly permissive to VSV, which is reversed by pre-treatment with IFNß or IFNλ1. However, JAK1/Tyk2 inhibitors do not enhance infection of the parent BEAS-2B cells, suggesting that protection is IFN-independent. To further investigate the mechanism of IRF1 protection, we treated IRF1 KO cells with poly I:C, and found decreased activation of IRF3 and STAT1, and decreased expression of IFNß and IFNλ transcripts compared to parent BEAS-2B cells. Overall, our data demonstrate that while IRF1 contributes to optimal signaling and transcript expression of IFNß and IFNλ, it is dispensable for IFN-mediated protection against VSV. Furthermore, our data demonstrate that IRF1 regulates IFN-independent viral protection, including activation of IRF3.

**4. Generation Assembly and Annotation of Whole Genome Sequences of Cyclospora cayetanensis Isolates Directly From Human Stool samples**

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Plain Language Synopsis: The globalization of the food supply has contributed to the spread of Cyclospora cayetanensis worldwide. This project will fill in an important knowledge gap, genome sequence information of C. cayetanensis, which is crucial in order to develop molecular methods for outbreak investigation and development of sensitive molecular detection methods.

Abstract:
The increasing globalization of the food supply has contributed to the spread of Cyclospora cayetanensis worldwide. This is a human specific coccidian parasite associated with food

and waterborne outbreaks in developing and developed nations. Whole Genome Sequencing (WGS) of C. cayetanensis presents challenges due to the absence of culture methods for the organism. Genomic DNA must be extracted from naturally infected individuals' stool samples that also contain intestinal bacterial flora and other contaminants. Furthermore, the only accessible biological stage of the organism is the oocyst, which is very resistant to physical and chemical disruption. Here we present a laboratory workflow for human fecal samples containing C. cayetanensis oocysts. The workflow involves sieving, density gradient centrifugations, surface sterilization treatments with bleach and detergents for oocyst purification, followed by genomic DNA extraction using physical shearing with glass beads. Genomic libraries are constructed using the 'Ovation® Ultralow System' to allow genome sequencing using Next Generation Sequencing (NGS) on Illumina MiSeq platforms. CLC workbench was used for de-novo genome assembly and mapping reads to a draft reference genome. Using this workflow, we were able to obtain assemblies of whole genomes [ranging from 35 to 46 megabases] and organelle genomes of five C. cayetanensis isolates originating from Nepal (3 isolates) and Indonesia (2 isolates). Our workflow seamlessly combines both conventional laboratory techniques and Next Generation Sequencing approaches. A subset of predicted genes from the assembly was confirmed in our sample collection using PCR. This will facilitate molecular methods development for detection and differentiation of C. cayetanensis isolates in clinical, food, and environmental samples.

**5. FDA's Office of Women's Health: Funding Innovative Omics Research to Advance Sex and Gender-Specific Women's Health**

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Plain Language Synopsis: The Office of Women's Health has supported advancing regulatory science through funding multiple projects focused on Omics technology. Moreover, OWH support has made unique contributions in greater understanding sex differences in response to therapeutic products and addressing women's health issues.

Abstract:
Omics technology is aimed at universal detection of related sets of biological molecules from a cell, tissue or an organism. Depending on the target molecules, examples of Omics include the study of genes (genomics), mRNA (transcriptomics), proteins (proteomics), microorganisms (microbiomics), methylated DNA or modified histone proteins (epigenomics), and metabolites (metabolomics). Omics technology generates a large data set, which provides an unprecedented opportunity for greater understanding of normal physiology or disease etiology and progression. At the same time data analysis is more complex and poses unique challenges; it requires database tools for interpretation and validation. Omics technology has increasingly been used in drug discovery and assessment of drug toxicity and efficacy. Over the past 20 years, the Office of Women's Health (OWH) has funded 368 research projects across FDA. Of these, 9% involved Omics-related technology. These 34 funded Omics-related projects have wide application to FDA-regulated products and contributed to the following areas:

(1) assessment of FDA-regulated product safety (drugs, devices, dietary supplements);

(2) detection of drug-induced toxicity (cardiotoxicity, liver and kidney toxicity);

(3) data analysis for novel drug therapeutic targets;

(4)identification of implanted device long-term biocompatibility (biomaterial leachable/extractable-induced toxicity);

(5) study biological mechanism of sex differences at molecular genetic level;

(6) application of personalized medicine in the etiology, diagnosis, and treatment for diseases prevalent in women, such as breast cancer and osteoporosis.

By funding Omics-related research projects, OWH has supported larger, Agency efforts

with other centers/offices to accomplish the agency's mission to protect public health through advancing emerging technology. Moreover, OWH support has made unique contributions in unleashing the potential to understand sex differences at molecular genetic levels and addressing women's health issues.

Key Words: Omics, sex differences, drug toxicity, medical product safety, Office of women's health; personalized medicine

### 6. An Epigenome-Wide Association Study (EWAS) of Peripheral Blood Mononuclear Cells from African American and European American Women with and without Lupus

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Plain language synopsis: Systemic lupus erythematous is an autoimmune disease that is complex and known to have a strong genetic basis. However, recent date indicate the role of epigenetic mechanisms. An epigenome-wide association study conducted on lupus and non-lupus patients revealed a highly significant interferon signature possible biomarkers for early diagnosis of lupus.

Abstract:
Systemic lupus erythematous (SLE) is an autoimmune disease that is complex and occurs in different ethnic groups at different incidence rates. DNA methylation plays an important role in the pathogenesis of lupus. Here, we performed an epigenome-wide DNA methylation study in lupus and healthy control (non-lupus) subjects to identify epigenetic patterns in lupus based on ethnicity and SLE disease activity index (SLEDAI). A total of 57 lupus patients (ethnicity: 39 AA and 18 EA) and 33 healthy controls (ethnicity: 17 AA and 16 EA) were included in the study. Differential DNA methylation between lupus patients and controls was quantified for approximately 485,000 sites across the genome. These sites demonstrated differentially hypomethylated sites in genes that are regulated by type-I interferon. We identified 41 differentially methylated sites between lupus and controls subjects (associated with 30 genes), 85% of which were hypomethylated. Significant hypomethylation of differentially methylated sites was associated with interferon related genes, MX1, IFI44L, PARP9, DT3XL, IFIT1, IFI44, RSAD2, PLSCR1, and IRF7. The hypomethylation of several interferon related genes were also found to be associated with ethnicity and lupus disease activity. We observed significant hypomethylation of sites associated with interferon related genes in African American lupus patients, and lupus patients with SLEDAI score greater than 6 (SLEDAI>6). The IFN-signaling pathway has emerged as an important focus for drug development. The data identified epigenetic patterns in lupus and epigenetics susceptibility of CpGs based on SLEDAI score and ethnicity. Gene expression studies confirmed these findings. These findings support the importance of type 1 interferon pathway in lupus pathogenesis, and highlights the variations in DNA methylation pattern of lupus based their SLEDAI score and ethnicity. The identification of these interferon-inducible genes are potential biomarkers for diagnosis of this disease.

### 7. Development of a renewable blood group genotyping reference panel for Rh variants

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Plain language Synopsis: Blood group genotyping is critical for transfusion safety and reference reagents are needed to evaluate the quality of assay performance. We aim to produce a reference panel for Rh-variants by characterizing the Rh gene in existing cell lines produced during the development of the first CBER blood group genotyping panel.

Abstract:
The Rh blood group system is highly polymorphic with over 250 RHD and 100 RHCE alleles described. Rh blood group genotyping is becoming routine in many reference

laboratories worldwide and, consequently, DNA reference reagents to ensure the quality of the tests are needed. Our aim is to perform Rh characterization of 53 B-lymphoblastoid cell lines from an existing CBER - RBC genotyping panel in order to select samples with Rh variants to be included in a renewable blood group genotyping reference panel for the Rh system. To characterize the RHD gene of the samples, we performed Multiplex PCR for exons 3, 4, 5, 7 and 9 and based on the results samples were subjected to PCR-RFLP reactions to identify RhD variants already described in the literature. The RHD zygosity was determined by PCR-RFLP and the presence of possible novel RHD variant alleles was verified by Sanger sequencing. Among the 53 panel members, 42 (79%) were RhD seropositive, and the relevant RHD alleles have been characterized. Eleven panel members (21%) that were RhD seronegative are undergoing further investigation to determine the molecular basis for this phenotype. Of the 42 RHD positive, 7 (17%%) had at least one RHD variant allele and 35 (83%) had standard RHD allele. Among these 7 RHD-variant samples, the findings were as follows: (a) 2 samples had RHD*DAU0 allele in the homozygous state; (b) 2 samples had one deleted RHD allele (RHD*01N.01) along with either RHD*DAU5 or RHD*DHMi; (c) 2 samples had one standard RHD allele (RHD*01) each, one along with RHD*DAU0 and the other with RHD*DIII.a; (d) 1 sample had heterozygous RHD variant (compound) comprised of RHD*DIVa.2 along with RHD*DAU5. The data generated by Rh investigation will define the allelic composition of existing CBER panel cell lines and assist with the selection of samples to be included as members of a dedicated renewable reference panel for Rh genotyping.

**8. Resistome of multidrug resistant Klebsiella pneumoniae**

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Plain Language Synopsis: Antibiotic resistance is a borders-transcending issue, requiring international cooperation among institutions and a multidisciplinary approach for its control. Particularly concerning has been the acquisition by Enterobacteriacee of carpapenemases, i.e. enzymes able to inactivate most beta-lactam antibiotics. We used whole-genome sequencing to further characterize carbapenem-resistant Klebsiella pneumoniae isolates.

Abstract:
Particularly concerning has been the recent acquisition by Enterobacteriacee of carpapenemases, i.e. enzymes able to inactivate most beta-lactams, including last resort antibiotics such as carbapenems. Whole-genome sequencing (WGS) can play a significant role in rapid and accurate differentiation of the existing and emerging carbapenemases, which will be essential for surveillance and controlling their spread. We characterized the occurrence of carbapenemase genes and extended-spectrum beta-lactamase (ESBL) genes in 10 multidrug-resistant (MDR) Klebsiella pneumoniae isolates from Pakistan.

The resistance profiles for the isolates were determined using Vitek. WGS data from sequencing on Illumina platforms was used for multilocus sequence typing (MLST), as well as antimicrobial resistance gene and plasmid replicon sequence analyses.

Resistance was observed for 15 of the 25 tested antibiotics in all strains except one. Seven isolates were resistant to colistin, and all were susceptible to tigecycline. The highest number of resistance genes was observed for aminoglycosides (n=12) and beta-lactams (n=11), with at least two genes of each class present in every isolate. The blaNDM-1 and blaOXA-48 genes were detected in 7 and 5 samples, respectively. In 2 isolates, both genes were present. Several ESBL genes were identified: blaCTX-M-15, blaTEM-1B, blaSHV-11 and blaSHV-28. No plasmid-mediated colistin resistance genes were detected, but

disruptions in chromosomal loci (i.e. mgrB and pmrB) were observed. Six sequence types (STs) were detected: ST11 (n=3 isolates), ST14 (n=3), ST15 (n=1), ST101 (n=2), and ST307 (n=1). The IncL/M(pOXA-48) replicon, indicating the presence of a ~60kb plasmid carrying no other resistance genes, was found in the OXA-48 positive samples.

The numerous potential transmission routes at the human-animal-environment interface underline the importance of an integrated approach for effective control and prevention. Global movement of people, animals and food is amplifying the geographic distribution of MDR isolates making antibiotic resistance a borders-transcending issue, requiring international cooperation among institutions and multidisciplinary approach for its control. WGS-applying molecular epidemiology studies will provide a better understanding of the worldwide dissemination of MDR isolates and a surveillance tool useful in detecting possible new emerging threats.

**9. Agnostic Classification of Single Cell Transcriptomes**

*Authors: Fochtman, Brian, FDA CBER; Simonyan, Vahan, FDA CBER*

Plain Language Synopsis: We present a novel classification method for single cell transcriptome analysis.

Abstract:
Analysis of single cell transcriptome data presents a difficult problem given the small sample sizes relative to the high dimensionality of transcripts measured. In this work, we present an agnostic classification method, based on grand canonical monte carlo simulations (GCMC), which seeks to identify clinically relevant subpopulations of genes and cells based on expression. GCMC is commonly used in physical and atomistic simulations to identify low energy states from high dimensional data. Here, instead of ordering particles in space, we use GCMC to order cells and gene expression based on similarity in expression patterns. This poster describes the theory and implementation behind this method and demonstrates an example classification. We believe that this method is well suited to handling large data sets and can be used to elucidate other "omics" scale questions.

**10. Development and application of next generation sequencing toward detection of norovirus at low copy number and/or within multiple viral species from celery**

*Authors: Yang, Zhihui, FDA/CFSAN/OARSA; Mammel, Mark, FDA/CFSAN/OARSA; Papafragkou, Efstathia, FDA/CFSAN/OARSA; Elkins, Chris, FDA/CFSAN/OARSA; Kulka, Michael, FDA/CFSAN/OARSA*

Plain Language Synopsis: Currently limited methods are available for detection of viruses directly from foods due to the low virus load in contaminated food items. The objective of this project was to develop a genomic approach for detection of amplification-independent foodborne viruses at low copies or/and in a multiple species from food.

Abstract:
Next generation sequencing (NGS) holds promise as a single application for both detection and sequence identification of foodborne viruses; however, technical challenges remain due to anticipated low quantities of virus in contaminated food. In this study, with a focus on data analysis with various bioinformatics tools, we developed and established a NGS approach for the detection of amplification-independent norovirus at low copy or in multiple strains from celery. Celery samples were inoculated either with single norovirus strain (from norovirus positive stool suspension), or with multiple strains (GII.4 and GII.6) or different species (hepatitis A virus and norovirus). Fifty-gram portions of virus-inoculated celery samples were subjected to extraction (viz. elution and ultracentrifugation following one hour incubation) to obtain pelleted virus. Viral RNAs were isolated from these celery preparations using three RNA kits and quantified by real time RT-PCR. cDNA libraries were generated using the TruSeq stranded mRNA prep kit followed by sequencing on MiSeq. First, the overall assessment of viral genome coverage of each sample varying in copy numbers (1.1x103 to

1.7x107) and genomic content (single or multiple strains in various ratios) was conducted by reference-based alignment. Second, the samples were then treated as unknowns, and data was analyzed using (i) sequence-based alignment with local database (ii) an "in-house" k-mer tool, and (iii) metagenomics softwares cosmosID and Kraken. Norovirus was successfully identified at low copy or within multiple strains using both our in-house k-mer tool and local database alignment, but not with cosmosID and Kraken. In summary, a sequencing protocol was developed and optimized for the viral RNA isolation, library generation and sequencing of norovirus from celery. The local database alignment and in-house k-mer tool outperformed cosmosID and Kraken for the detection of norovirus at low copy or within multiple strains. The results of this investigation reveal the potential for further development/integration of these tools toward the identification and detection of foodborne viruses.

**11. Evaluation of Enriched Microflora of Raw Milk Cheese Spiked with E. coli O157:H7 and E. coli O103 using Next-Generation Sequencing Technology**

*Authors: Pfefer, Tina Lusk, FDA/CFSAN; Kase, Julie A., FDA/CFSAN; Ramachandran, Padmini, FDA/CFSAN; White, James, FDA/CFSAN; Reed, Elizabeth, FDA/CFSAN, Mammel, Mark, FDA/CFSAN; Ottesen, Andrea, FDA/CFSAN*

Plain Language Synopsis: Between 1998 and 2014, there were 804 illnesses, 172 hospitalizations, and 3 deaths linked to unpasteurized cheese. Enrichment broths are an essential component of detection methods used to isolate bacterial pathogens from foods, including cheese, implicated in outbreaks. Here we use powerful metagenomics technologies to assess and improve enrichment broths.

Abstract:
Between 1998 and 2014, there were 38 outbreaks in the U.S. directly linked to unpasteurized cheese resulting in 804 illnesses, 172 hospitalizations, and 3 deaths. Enrichment broths are an essential component of detection methods used to isolate bacterial pathogens from foods implicated in outbreaks. A better understanding of enrichment dynamics will inform efforts to improve detection of diarrheagenic Escherichia coli in this difficult matrix.

Here we describe microflora diversity during enrichment of raw milk cheese spiked with diarrheagenic E. coli and enriched with multiple broths. Multiple sample prep and data analysis approaches were evaluated to assess which could provide resolution down to the serotype level. This level of resolution is necessary to compare performance of the broths in amplification of diarrheagenic E. coli.

Raw milk cheese was spiked with either E. coli O157:H7 or O103 and enriched overnight using R&F Escherichia coli O157:H7 Enrichment Broth or mBPWp broth used in the FDA BAM. High-throughput 16S rRNA amplicon sequencing and shotgun sequencing resulted in a survey of bacterial species for each treatment. 16S rRNA sequencing data was analyzed using Resphera Insight, samples shotgun sequenced on a NextSeq 500 were analyzed using Cosmos ID and in-house tools.

Shotgun sequencing on a NextSeq 500 followed by data analysis using Cosmos ID was by far the most informative approach, giving resolution down to strain level for E. coli O157:H7, but not E. coli O103, and showed that R&F outperformed the FDA BAM mBPWp broth. Data analysis of NextSeq 500 data using in-house software tools gave resolution down to E. coli phylogroup level. Enriched test portions that underwent 16S rRNA amplicon sequencing using MiSeq and data analyzed using Resphera Insight showed that, after enrichment, L. lactis and total possible E. coli (E. coli and Escherichia spp.) dominated all samples at 24-68% and 16-68%, respectively.

These data will be used to improve diarrheagenic E. coli detection technologies for the FDA Bacteriological Analytical Manual as well as serve as an important step towards culture independent diagnostic characterization of diarrheagenic E. coli directly from challenging food matrices such as dairy.

## 12. Impact of MDR1 Genotype In Collies On The Pharmacokinetics of Fexofenadine and Its R- And S-Enantiomers

*Authors: Myers, Michael J., FDA/CVM; Martinez, Marilyn, FDA/CVM; Li, Fei, FDA/CVM; Howard, Karyn, FDA/CVM; Yancy, Haile F., FDA/CVM; Troutman, Lisa: FDA/CVM; Sharkey, Michele; FDA/CVM*

Plain Language Synopsis: This study examined the impact of a naturally occurring mutation in the major drug efflux mechanism using fexofenadine, a racemic mixture of two mirror images of this drug. The results demonstrated genotype and phenotype affect the efflux of one form of fexofenadine, which may impact toxicity in other racemic drugs

Abstract:
P-glycoprotein (P-gp) is encoded by the Multidrug Resistance Protein 1(MDR1) gene. In some dogs, a mutation of the MDR1 gene results in the generation of a non-functional P-gp membrane protein. For the drug fexofenadine (Fex), P-gp is responsible for its active efflux from the basolateral to apical surface of the intestinal membrane, thereby limiting its oral absorption. Since P-gp has been reported to exhibit stereospecificity for some drugs, the question was whether the two enantiomers of Fex are equally influenced in dogs possessing one (heterozygotes) or two (homozygous recessive) genes with this mutation. To answer that question, stored serum sample from thirty three Collies (14 male and 19 female) were used to determine the impact of MDR1 genotype and phenotype (ivermectin sensitivity, which is a clinical marker for the presence of the genetic mutation) on the pharmacokinetics of Fex and its R- and S-enantiomers. Genotypes and phenotypes were known prior to study enrollment. Plasma pharmacokinetics were determined for total Fex as well as the R- and S- enantiomers (single dose and steady state). Wild-type (WT) Collies exhibited lower blood levels of Fex and the two separate enantiomers as compared to heterozygous IVM non sensitive (HNS), heterozygous mutant IVM sensitive (HS) and homozygous mutant (Mut) Collies. This was consistent with a lower fraction absorbed in the dogs. Across all dogs, blood levels of R exceeded that of S, with the ratio of the two enantiomers differing as a function of genotype. This suggested a stereospecific effect on Fex. The mean ratio of S/R AUC values were (in rank order lowest to highest) WT, HNS, HS, Mut. Additional analysis was consistent with this effect being a function of absorption and not clearance. However, there appeared to be a trend toward a greater likelihood for active efflux and reabsorption during the terminal portion of the profile of WT dogs. These results underscore the importance of considering the individual enantiomers (rather than total drug) and genotype when assessing population variability in drug dose-exposure-response relationships.

## 13. GenomeTrakr: characterization of Listeria monocytogenes isolates obtained from Piedmont, Italy over a 12-year period.

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Plain Language Synopsis: Given the frequent multi- state or national nature of outbreaks, it is important to have molecular typing data for a large number of diverse well-characterized food and environmental isolates. We performed whole genome sequencing on a large collection of Listeria monocytogenes isolates collected in Northern Italy between 2003 and 2014.

Abstract:
Given the frequent multi-state or multi-national nature of outbreaks, it is important to have molecular typing data for a large number of diverse well-characterized environmental isolates. Whole genome sequencing (WGS) has high resolution, can provide multiple data in a single step and assist in locating the source of an outbreak. FDA-CFSAN collaborated with the University of Turin and the Regional Food Safety and Animal Health Institute (IZS Torino) in Italy to perform WGS on L. monocytogenes isolates from Italy, as part of the GenomeTrakr project.

A total of 362 L. monocytogenes isolates collected between 2003 and 2014 from different

sources (i.e. food, facility, animal, feed, etc.) were sequenced on Illumina platforms. Virulence types (VTs) were assigned based on extracted consensus sequences for 6 virulence genes. Thirty-three VTs were assigned for 324 isolates, of which 27% corresponded to VTs previously observed in Epidemic Clones. VT11 was found in different environments and across the years (44% of all isolates). WGS data for 343 L. monocytogenes isolates was also analyzed at the NCBI Pathogen Isolates Browser tool to determine clustering and closest relatives. Most isolates (n=311, 90.6%) were grouped in 43 clusters, whereas 32 isolates did not belong to any cluster. The Italian VT11 isolates were divided into 8 clusters of which PDS000003111.19 grouped 130 isolates (84%). Overall, PDS000003111.19 contains 306 isolates, mostly from environmental/food or generic sources from the US and Europe. In previous studies VT11 was found as the main type in different processing environments and further research will be needed to determine why this VT appears to be a predominant and persistent environmental clone.

Overall, more than 90% of the L. monocytogenes isolates from Italy currently available at the NCBI Pathogen Isolates Browser belong to this project, therefore providing a large increase in geographical reference for Italy.

### 14. Guidance for evaluating next generation sequencing data using the new 2-channel chemistry

*Authors: Wu, Wells, FDA/CBER; Phue, Je Nie, FDA/CBER; Lee, Chun-Ting, FDA/CBER; Shen, Rong-Fong, FDA/CBER*

Plain Language Synopsis: CBER's FBR core facility provides next generation sequencing (NGS) using Illumina sequencers based on sequencing by synthesis (SBS) technology, which has recently been changed from 4-channel to 2-channel method. We thoroughly compared data generated from the different chemistry to provide much needed guidelines for NGS research and data submission.

Abstract:
Illumina's next generation sequencing (NGS) is the most widely adopted NGS sequencing technology worldwide. Its sequencing by synthesis (SBS) technology is the foundation of all Illumina sequencers. Briefly, during each sequencing cycle, labeled dNTPs are successively added with each added dNTP serving as a reversible terminator for polymerization. After each dNTP incorporation, the flow cell is imaged to identify the incorporated base, then the 3' blocking group is chemically cleaved to allow incorporation of the next nucleotide to achieve cycle-by-cycle sequencing. Base calls are made based on distinct signals from fluorescent colors (A, C, T, and G) of successive images. In the past several years, CBER's FBR has assisted FDA researchers with applications listed below based on the 4-channel chemistry using HiSeq 2500 and MiSeq:

- DNA sequencing
  - o Whole genome sequencing
  - o Targeted sequencing of fixed panels or amplicons
  - o Epigenetic sequencing (methylation- and ChIP-sequencing)
  - o Metagenomic sequencing (16S rRNA gene sequencing)
- RNA sequencing
  - o Messenger RNA sequencing
  - o Non-coding RNA sequencing
  - o Ribosome-protected mRNA fragment profiling

However, NGS is rapidly evolving. Recent Illumina sequencing system (e.g., NextSeq 500) and the newest NovaSeq system change the way image data are acquired from the 4-channel (using 4 fluorescent dyes for each of the 4 bases) to 2-channel (a mix of 2 dyes) system. Briefly, in the 2-channel SBS, clusters seen in red and green images are flagged as C and T bases, respectively, those observed in both red and green images (appearing as yellow) are interpreted as A bases, and the unlabeled clusters are flagged as G bases. This latest method speeds up image acquisition and simplifies imaging system. However we found it often introduces an error in calling

G bases, which has not yet been reported or discussed in the literature. We systematically studied this abnormality in both DNA- and RNA-sequencing applications mentioned above and provided options to mitigate the issues. The aim of this study is to provide the much needed information for NGS researchers as well as to establish useful guidance for evaluating data submitted, using this new technology.

### 15. Optimization and implementation of an internal next-generation sequencing analysis pipeline to analyze antiviral drug resistance data

*Authors: Lee, Stella S., FDA/OC; Simonyan, Vahan, FDA/CBER; Karagiannis, Konstantinos, FDA/CBER; Voskanian-Kordi, Alin, FDA/CBER; Donaldson, Eric F., FDA/CDER*

Plain Language Synopsis: Reviewing Next Generation sequencing (NGS) data submitted with NDA/BLA applications is a time-consuming and complex process that is critical for regulatory decision-making. For this project, we optimized an internal NGS analysis pipeline and then tested it. This optimized pipeline will increase the efficiency of the antiviral drug approval process.

Abstract:
Viral resistance has important implications for the durability of antiviral drugs and the potential impact that resistance pathways may have on future treatment options for infected patients. The Division of Antiviral Products (CDER\OND\OAP\DAVP) performs independent analyses of resistance data associated with antiviral drugs to ensure that the emergence of resistance is carefully characterized and explained in the label of newly approved antiviral drug products. Over the past few years, antiviral drug sponsors have been rapidly adopting the use of next-generation sequencing (NGS) technology, and have been submitting NGS data to support resistance analysis studies for antiviral drugs. Most of the sponsors have been using their own bioinformatics analysis pipelines that can provide a unique interpretation of the data. To better review these data, DAVP developed and evaluated independent bioinformatics analysis pipelines to analyze NGS resistance datasets submitted by sponsors.

To deal with the increasing number of NGS data submissions and the time-sensitive nature of the regulatory review of such complex data, we wanted to optimize our internal analysis pipeline. Two platforms were used: 1) HIVE (High-performance Integrated Virtual Environment), which is a platform developed by CBER's High Performance Computing Core staff, and 2) CLC Genomics workbench, a proprietary software package. Through collaboration with other centers, including CBER's HIVE team, we implemented several new computational tools in HIVE and optimized the parameters used for the read mapping and variant calling algorithms.

Using the optimized pipeline, we successfully assessed a mock NGS dataset recently submitted as part of a rolling BLA. This dataset was unique because the amino acid changes associated with resistance that conferred a reduced susceptibility to the drug in vivo occurred in a variable region of the viral envelope glycoprotein and represented changing glycosylation sites. Our independent analysis results were largely in agreement with the sponsor's, but identified a few discrepancies that need to be further addressed by the sponsor. In conclusion, the optimized internal NGS analysis pipeline will allow for efficient regulatory assessments of NGS resistance data, which is essential to facilitate the antiviral drug approval process.

### 16. Development of a proteomic approach for the identification and characterization of post-translational modifications in transfusion products using high resolution accurate mass (HRAM) mass spectrometry

*Authors: Strader, Michael, DBCD/OBRR/CBER/FDA; Sirsendu, Jana, DBCD/OBRR/CBER/FDA; Meng, Fantao, DBCD/OBRR/CBER/FDA; Hicks, Wayne, DBCD/OBRR/CBER/FDA; Kassa, Tigist, DBCD/OBRR/CBER/FDA; , Tarandovskiy, Ivan, DBCD/OBRR/CBER/FDA; De Paoli, Silvia, DBCD/OBRR/CBER/FDA; Simak, Jan, DBCD/OBRR/CBER/FDA; Miller, Jeffrey, NIDDK/NIH; Mendelsohn, Laurel, NHLBI/NIH; Nichols*

*Jim, NHLBI/NIH; Thein, Swee Lay, NHLBI/NIH; Belcher, John, Division of Hematology, Oncology, and Transplantation, University of Minnesota Medical School; Vercellotti, Gregory, of Hematology, Oncology, and Transplantation, University of Minnesota Medical School; Alayash, Abdu, DBCD/OBRR/CBER/FDA*

Plain Language Synopsis: Our lab employs a quantitative proteomic method for hemoglobin (Hb) toxicity studies aimed at designing Hb-based oxygen carriers (HBOCs) and to gain insight into sickle cell Hb as a pathophysiology model.

Abstract:
Our lab focuses on hemoglobin (Hb) toxicity studies aimed at providing insight into safety evaluation and development of blood products. The design of a quantitative proteomic method has therefore been invaluable for several of our studies including those focused on the design of Hb-based oxygen carriers (HBOCs) or to gain insight into sickle cell Hb as a pathophysiology model. Specifically, we employed a proteomic approach using high resolution accurate mass (HRAM) mass spectrometry (MS) to 1) characterize Hb oxidative post translational modifications (PTMs) and to 2) study the red blood cell (RBC) derived microparticle (MP) proteome from sickle cell disease (SCD) blood. In the first study, HRAM data provided mechanistic insight by quantifying oxidation in amino acids near the reactive heme or at specific "oxidative hotspots". To better understand oxidative toxicity we evaluated mutant Hbs originally discovered from patients with hemoglobinopathies and utilized this information toward designing against (or for) these reactions in acellular oxygen therapeutics. The results from this work led to successfully engineering one protein that is oxidatively more stable and is currently being evaluated as a candidate HBOC. In the second study, our goal was to focus on the mechanistic impact of HbS oxidative toxicity and polymerization; these HbS specific outcomes lead to events that cause vaso-occlusive crisis. We employed a coordinated HRAM-MS workflow to characterize proteomic changes of RBC-derived microparticles (MPs) of SCD patients for comparison with ethnically matched control subjects or transgenic sickle mice with control wild type. These studies collectively indicated that human and mice SCD MPs (compared to control MPs) exhibited markers associated with oxidative stress which included increased Hb oxidation, elevated antioxidant enzyme levels and phosphorylation of the band 3 protein. HRAM data also revealed, for the first time, subunit specific ubiquitination at ßLys96 and ßLys145. This ubiquitin proteasome system modification is added to defective proteins for degradation targeting. The presence of ubiquitination supports our previous studies showing HbS ß subunits to be oxidatively unstable and subceptible to turnover and concomitant heme release. These studies collectively will inform our regulatory oversight of some high profile products that impact blood safety.

## 17. HIVE Platform for Next-Generation Sequencing Analysis

*Authors: Voskanian, Alin,FDA/CBER; Dingerdissen,Hayley, FDA/CBER;Golikov,Anton FDA/CBER; Karagiannis, Konstantinos,FDA/CBER;VinhNguyen Lam, Phuc, FDA/CBER; Santana-Quintero, Luis,FDA/CBER ;Mazumder, Raja, GWU;Simonyan,Vahan FDA/CBER*

Plain Language Synopsis: The High-performance Integrated Virtual Environment (HIVE) is a cloud-based environment optimized for the storage and analysis of extra-large data, primarily Next Generation Sequencing (NGS) data. This environment will provide secure web access for authorized users to deposit,retrieve, annotate and compute on High-Throughput Sequencing (HTS) data, and to analyze the outcomes using web-interface visual environments appropriately built in collaboration with research scientists and regulatory personnel.

Abstract:
The High-performance Integrated Virtual Environment (HIVE) is a cloud-based environment optimized for the storage and analysis of extra-large data, primarily Next Generation Sequencing (NGS) data. This environment will provide secure web access for authorized users to deposit,

retrieve, annotate and to compute on High-Throughput Sequencing (HTS) data and analyze the outcomes using web-interface visual environments appropriately built in collaboration with research scientists and regulatory personnel.

HIVE is a multicomponent cloud infrastructure where the distributed storage library and the distributed computational powerhouse are linked seamlessly. Unlike many massively parallel computing environments, HIVE uses a cloud control server which virtualizes services, not processes. It is both very robust and flexible due to the introduced abstraction layer between computational requests and OS processes. The novel paradigm of moving computations to the data instead of moving data to computation nodes implemented in HIVE has proven to be significantly less taxing for hardware and network infrastructure.

The honeycomb data model developed for HIVE differs from traditional relational databases by coalescing the metadata into object oriented model, but unlike other object oriented databases implements unified API interfaces to search, view and manipulate different data types. This model simplifies the addition of new data types and minimizes the necessity for restructuring of the database, streamlining the developments of new integrated information systems. The honeycomb model implements highly secure hierarchical access control and permission system, allowing determining data access privileges in a finely granular manner without flooding the security subsystem with multiplicity of rules.

The HIVE infrastructure allows r&d engineers and scientists to perform HTS analysis in an efficient, secure manner without sacrificing either. HIVE is actively supported in a public domain and pilot project collaborations are welcomed.

### 18. Proteomics-based evaluation of nano silver cytotoxicity to human cells

*Authors: Narayanasamy, Suresh, FDA/CDRH; Wickramasekara, Samanthi, FDA/CDRH; Sussman, Eric, FDA/CDRH*

Plain Language Synopsis: Silver, owing to its antimicrobial properties, has been widely explored for medical device applications. To address toxicological concerns arising from use of silver nanoparticles (AgNPs), here we quantitate changes in protein expression levels in cells upon silver nanoparticle treatment to gain detailed information about cellular responses to AgNPs.

Abstract:
Silver in both ionic and nano form is known for its antimicrobial properties and has been widely used in various industrial and medical applications. Mainly, silver nanoparticles (AgNPs) have emerged as an important class of nanomaterials for a wide range of medical devices, but there has been concern over possible Ag-induced toxicity. Various studies demonstrate that common causes of Ag-induced toxicity include oxidative stress, DNA damage and apoptosis. Though individual toxicity assays are specific and robust they lack in providing a broad toxicological perspective. In order to achieve global proteome expression level changes upon treating cells with ionic and nano silver, we established SILAC metabolic labeling workflow in our lab.

Silver nano particles (10nm) with PVP coating were used for toxicity studies. Nanoparticle size was determined by both TEM and DLS methods. Human embryonic kidney cells (HEK 293) were cultured in SILAC DMEM media. SILAC light isotopes labeled cells were treated with varying concentrations of silver nitrate (AgNO3), AgNPs and gold nanoparticles (AuNPs). Heavy labeled cells were used as controls. A treatment concentration for nano silver was determined by dose response. Light cells were lysed and mixed with heavy lysate 1:1. Proteins were reduced, alkylated, digested with LysC/Trypsin, and cleaned with C18 spin columns. Peptides were subjected to high pH reverse-phase fractionation. Samples were injected to LC-MS/MS analysis on an Agilent 6530 QTOF LC/MS system with a chip cube nanospray interface. The MS/MS data were subjected to Swissprot database search with Trans Proteomics Pipeline (TPP) software for identification and SILAC quantitation. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA).

Incubation of HEK 293 cells with AgNO3 and AgNPs followed a dose dependent inhibition of cell proliferation. First, 24-hour LC50 values for AgNO3 and AgNP on HEK 293 cells were determined to be 5µg/mL and 50µg/mL respectively. Pathway analysis by IPA software resulted in the following top canonical pathways like EIF2 signaling, HIPPO signaling, NRF2 mediated oxidative stress response and protein kinase A signaling. The above pathway analysis has revealed the protein expression among the samples is common to cellular processes involved in cellular growth and proliferation, cell death and survival.

**19. Ribosome Profiling: Investigating the role of the genetic sequence in recombinant therapeutics.**

*Authors: Gaya K. Hettiarachchi[1], John Athey[1], Gokhan Yavas2, Vijaya L. Simhadri[1], Upendra K. Katneni[1], Aikaterini Alexaki[1], Brian C Lin1, Nobuko H. Katagiri1, Ryan C. Hunt[1], Sujata Jha3, Kazuyo Takeda1, Wenming Xiao2, Darón I. Freedberg1, Anton A. Komar3, and Chava Kimchi-Sarfaty1*

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Plain language synopsis: Ribosome profiling is a revolutionary technology that provides an unprecedented nucleotide-level magnification of protein translation. Here it is used to explore the translational consequences of synonymous mutations as an underlying mechanism that may modulate critical quality attributes of recombinant therapeutics.

Abstract:
Background: Ribosome profiling is a cutting-edge technology that highlights global and gene-specific translation patterns at nucleotide-level resolution. This method does so by isolating non-uniform populations of actively translated mRNA originating from differential ribosome occupancy over specific codons.

Ribosome profiling has been used to investigate a myriad of factors governing protein biogenesis including translation kinetics. Evidence suggests that ribosome processivity along an mRNA transcript may modulate protein co-translational folding and define protein attributes. The appearance of folding intermediates emerging from the ribosome depends on non-uniform elongation rates along the mRNA which is thought to be modulated by factors like codon usage.

Here, ribosome profiling is used to investigate the translational consequences of adopting codon optimization to bolster the production efficiency of recombinant biologics. This method employs hundreds of synonymous mutations despite their demonstrated modulation of protein expression, structure and function in disease. The extent to which these mutations impact the safety and efficacy of recombinant biologics remains unclear. A mechanistic understanding how these changes come into effect may reveal the role of the genetic code in protein biogenesis.

Methodology: Coagulation Factor IX (FIX) was used as a model for this work. A series of codon optimized (CO) constructs were synthesized and compared to wild type (WT) FIX.

Constructs were first characterized using a variety of specialized assays to assess and confirm variances in protein expression, structure and function.

The ribosome profiling method was optimized and a novel bioinformatics workflow was developed to elucidate both gene-specific translation and global codon usage patterns.

**20. Quantitative Proteomics, Post-Translational Modification Characterizations, and Biomarker Discovery**

*Authors: Phue, Je Nie, FDA/CBER; Wu, Wells, FDA/CBER; Shen, Rong-Fong, FDA/CBER*

Plain Language Synopsis: Proteomic analyses using mass spectrometry (MS) are new services provided by CBER's FBR (Core Facility). We offer a variety of MS-based cutting-edge technologies to assist FDA laboratories in regulatory and scientific research.

Abstract:
The technologies provided by FBR core facility consist of the following four general categories:

- Protein/peptide ID (identification of recombinant and unknown proteins/peptides in complex mixtures using bottom-up and top-down mass spectrometry).
- Post-translation modification (characterization of phosphorylation, glycosylation, sumoylation, and carbonylation in proteins).
- Biomarker discovery (fractionation of complex samples using liquid chromatography/ gel electrophoresis, removal of abundant proteins in plasma, followed by mass spectrometric methods).
- Quantitative proteomics (quantification of proteins among different conditions using label-free and label-dependent mass spectrometry).

Representative results from each of the above four categories, published in peer-reviewed journals as our collaborative efforts or in-house method development, are showcased to highlight these powerful technologies available to FDA researchers.

**21. Title: GenomeTrakr database: WGS network for foodborne pathogen traceback.**

*Authors: Timme Ruth FDA/ORS, Sanchez Maria FDA/ORS, Allard Marc FDA/ORS, Stevens Eric FDA/ORS, Hoffman Maria FDA/ORS, Kastanis George FDA/ORS, Lindley Sabina FDA/ORS, Muruvanda Tim FDA/ORS, Strain Errol FDA/OAO, Payne Justin FDA/OAO, Pightling Arthur FDA/OAO, Rand Hugh FDA/OAO, Pettengill James FDA/OAO, Luo Yan FDA/OAO, Gonzalez-Escalona Narjol FDA/ORS, Melka David FDA/ORS, and Brown Eric FDA/ORS.*

Plain Language Synopsis: Update on FDA GenomeTrakr and NCBI Pathogen Detection web sites

Abstract:
In 2012 a pilot project was set up using whole genome sequence data to track foodborne outbreaks. In this network, public health agencies collect and publically share WGS data in real time. This high-resolution, rapidly growing database is actively being used in outbreak investigations at state, national and international level.

**22. Whole genome sequencing of live attenuated Leishmania donovani parasites reveals novel biomarkers of attenuation and enables product characterization**

*Authors: Gannavaram, Sreenivas, FDA/CBER; Torcivia, John, FDA/CBER; Kaul, Amit, FDA/CBER; Ismail, Nevien, FDA/CBER; Simonyan, Vahan, FDA/CBER; Nakhasi, Hira, FDA/CBER.*

Plain Language Synopsis: No licensed vaccines or donor screening tests are available against blood borne protozoan parasitic agents. Live attenuated parasite vaccines have the potential to reduce disease burden and enhance the blood transfusion safety. We identified biomarkers of attenuation using whole genome sequencing methods that enable more robust product characterization.

Abstract:
No licensed human vaccines are currently available against leishmaniasis. Several anti-leishmanial vaccines are currently undergoing testing, including genetically modified live-attenuated parasite vaccines. Studies with live attenuated Leishmania vaccines such as centrin deleted Leishmania donovani parasites (LdCen-/-) showed protective immunity in animal models. Such studies typically examined the biomarkers of protective immunity, however the biomarkers of attenuation in the parasite preparations have not received adequate attention. As several candidate vaccines enter clinical trials, a more complete product characterization to enable maintenance of product quality will help meet regulatory requirements. Towards this goal, we have determined the complete genome sequence of LdCen-/- and its parent strain Ld1S-2D (LdWT) and characterized the LdCen-/- vaccine strain using bioinformatics tools. Results showed that the LdCen-/- parasites, in addition to loss of the centrin gene, have additional deletions ranging from 300bp to 2200bp in non-contiguous loci on several chromosomes, most commonly in untranslated regions. We have experimentally verified a subset of these adventitious deletions that had no impact on the attenuation of the LdCen-/- parasites. Our results identified hitherto unknown features of attenuation of virulence that could be used as markers of

product quality in production lots highlight the importance of product characterization in parasitic vaccines.

### 23. Vibrio parahaemolyticus sequence type 631, an emerging foodborne pathogen in North America

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Plain Language Synopsis: Vibrio parahaemolyticus ST631 USA

Abstract:
Vibrio parahaemolyticus is the leading seafood-transmitted bacterial pathogen worldwide. A pandemic complex of strains, most identified as sequence type (ST) 3, has dominated infections world-wide. The most prevalent clinical strain in the US, ST36, recently spread from the Pacific into the Atlantic where it has caused outbreaks since 2012. Here we report a new lineage of V. parahaemolyticus, ST631 which is rapidly emerging as the predominant pathogenic clade endemic to the Atlantic coast of North America. Genome comparisons were used to understand the potential relationships and diversity of ST631 and we found that ST631 shares no recent ancestry with and differs substantially from ST36 and ST3 (> 3600 out of 3909 common loci contained variation). A custom core genome multilocus sequence typing scheme, using as reference genome MAVP-Q, on draft genomes of 29 clinical and one environmental isolate representing the entire geographic distribution and time span of infections; identified only 124 single nucleotide polymorphisms (SNPs) in the population. This analysis confirmed that clinical ST631isolates were highly clonal with limited diversification. The fact that an increasing number of cases have been traced to sources in the northwestern Atlantic over the last decade suggests ST631 poses a continuing and mounting public health threat, and calls for surveillance of this lineage to reduce illnesses

**24. Improving Communications with Minority Groups while Closing the Health Disparity Gap"**

*Authors: Jovonni Spinner, FDA OMH; Katherine Bravo, FDA OMH; Cariny Nuñez, FDA OMH; Shakia Baskerville, FDA OMH; Gloria Sánchez-Contreras, FDA OEA*

Plain Language synopsis: The Food and Drug Administration's (FDA) Office of Minority Health (OMH) and the Office of External Affairs (OEA) work across FDA to improve outreach and education communicating critical health information to minority groups and people with limited English Proficiency (LEP).

Abstract:
Learning Objectives:

1. Be able to identify strategies used to communicate findings to priority populations, partners, and stakeholders.
2. Be able to identify levels of literacy of intended audience and to understand the importance of translating materials to improve communication with people with limited English proficiency.
3. Be able to identify methods used to deliver messages using media and communication strategies to minority audiences.

Introduction: The Food and Drug Administration's (FDA) Office of Minority Health (OMH) in collaboration with the Office of External Affairs (OEA) work across FDA to improve outreach and education to underserved populations and communicate critical health information to minority groups and people with limited English proficiency (LEP). In the past two years OMH and OEA has implemented practical strategies to improve communication for minority populations; including translating materials for LEP groups; using innovative digital tools to communicate health information to minorities; and building strategic partnerships to achieve common health literacy goals.

Methods: In 2014, OMH launched a multi-faceted Outreach and Communication Program (OCP) to improve communications for underserved populations and address stakeholders' in their places of needs and where information is more relevant to them. We used digital platforms and social media to outreach to minority populations in various languages and strengthened our communication network to amplify information about FDA-regulated products. Examples include social media, webinars, website, electronic newsletters, and campaigns. Content is disseminated via Twitter; through consumer updates, blogs, and quarterly newsletters; creating infographics and electronic brochures; email blasts; and keeping a current website. We launched our first multilingual campaign; and content is translated into Spanish and Asian languages, as needed.

Evaluation Methods and Results: We use various metrics tools to measure our impact in the community like Google Analytics, Google AdWords; email metrics and stakeholder engagements. Since its inception, we have increased our Twitter followers to more than 6,500; we have over members 26,000 on our list-serv, and over 10,000 YouTube playlist views. Qualitative information suggests that our stakeholders value and rely on receiving our information to inform their practices.

Conclusions: OMH and OEA together have demonstrated value in building the FDA brand by leveraging the benefits of using various digital platforms by meeting consumers at their places of need and efficiently delivering culturally and linguistically appropriate information to diverse users. Effective communication is critical to everyone; it can also make the difference in a life or death situation. Most importantly is key to further the agency's mission of "Protecting the Public's Health."

**25. Patient-Reported Outcomes: Observations of Regulatory Approvals by the Center for Biologics Evaluation and Research**

*Authors: Ezzeldin, Hussein, FDA/CBER; Moncur, Megan, FDA/CBER; Luo, Yuqun Abigail, FDA/CBER; Irony, Telba, FDA/CBER*

Plain Language Synopsis: Advancing Science of Patient Input (SPI) is critical to incorporating patient's voice in drug development. To identify

challenges and opportunities for regulatory science on patient-report outcomes, we summarize the reporting of PROs in past regulatory approvals by the Center for Biologics Evaluation and Research.

Abstract:
Objective: As a first step to identify challenges and opportunities in the contribution of Patient-Reported Outcomes (PROs) to the clinical development of investigational products, we examine and summarize our observations of the past regulatory approvals by the Center for Biologics Evaluation and Research (CBER).

Methods: We reviewed all approvals of original Biologics License Applications (BLAs) by CBER, listed at the FDA Biological Approvals website [1], for the years of 1998 through 2016. For each BLA approval where at least a PRO was mentioned, we reviewed the publicly posted approval information, which may include package insert, summary basis for regulatory action (summary basis for approval), clinical review memo, and statistical review memo. We catalogued the PRO instruments used, whether the PRO was a primary or a secondary endpoint, and the description of the PRO results.

Results: During 1998-2006, there were 296 BLA approvals. We exclude BLAs on reagents, assays, blood components, etc., and focus on the 80 (27%) approvals with clinical studies. Of these 80 approvals, 21 (26%) reported PROs as primary or secondary endpoints: 2014 with the highest percentage at 75%, followed by 2016 and 2008, at 60% and 50%, respectively. Sixty-two percent (13/21) of the PRO-reported approvals were indicated for blood disorders: Hemophilia A (7), Hereditary Angioedema (3), Hemophilia B (2), and Factor X deficiency (1). Another 14% (3/21) of approvals were for Grass/pollen induced allergies. The remaining 24% (5/21) of indications are: primary immunodeficiency replacement therapies, cartilage defects of the knee, snake envenomation, nasolabial fold wrinkles, and mucogingival.

Conclusion: About a quarter of past BLA approvals by CBER reported PRO results, including both single-item and multi-domain instruments. There appears to be an increase in the number of approvals mentioning PROs, from 12.5% in the 9 years of 1998 through 2006, to 32% in the 10 years of 2007 through 2016, after the FDA draft PRO guidance was issued in 2006.

[1] http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/BiologicalApprovalsbyYear/default.htm

### 26. Using patient and consumer perspectives to develop strategic communications about medical devices: Commonly used techniques in the CDRH approach

*Authors: Ovelmen, Heather, FDA/CDRH/OCE/DHC; Witters, Alicia, FDA/CDRH/OCE/DHC; Coffey, Aisha, FDA/CDRH/OCE/DHC;*

Plain Language Synopsis: An analysis of how the CDRH Division of Health Communication gathers patient input from social media and other resources to help develop products that communicate risk and benefit information about medical devices to the public.

Abstract:
Patients play an increasingly important role in managing their health care. As patients and consumers become more involved with health care decisions, the need for clear, easy-to-understand communication grows. The Center for Devices and Radiological Health's (CDRH) Division of Health Communication (DHC) leads the Center's collaborative communication process which helps assure the safety and effectiveness of medical devices by developing and facilitating public communication, and educating medical device industry. The purpose of this poster is to demonstrate how we gather patient and consumer input from sources such as traditional and social media, consumer and press inquiries, data analytics measuring consumer engagement with CDRH websites, message testing, and stakeholder webinars and webinar survey results; and how we use those results to inform the messaging, language, tone, and reading levels of communications developed for the public. Using a combination of research tools and techniques, DHC has successfully developed clear communications that address the most common questions and

use consumer-recognized terminology and which leverage opportunities for outreach to targeted audiences. DHC will discuss our common approaches and the findings of our techniques in developing public-facing communications for medical device topics, including hearing aids, artificial pancreas, and facial fillers and injectables.

### 27. Drug Trials Snapshots: Representing Diversity among Clinical Trials Participants

*Authors: Goetzel, Noah, FDA/CDER; Wang, Junyang, FDA/CDER; Lolic, Milena, FDA/CDER; Whyte, John, FDA/CDER*

Plain Language Synopsis: In order to increase transparency regarding the demographics of clinical trial participants, the FDA Center for Drug Evaluation and Research initiated Drug Trials Snapshots in 2015. The Snapshots stratify trial participants by sex, race, and age, then share this data with the public in a centralized consumer-friendly way.

Abstract:
Objective: This poster shares descriptive statistics highlighting the demographic information of clinical trial participants in all New Molecular Entities (NMEs) or original biologic (BLA) products approved by the U.S. Food and Drug Administration (FDA). As part of the 2012 Food and Drug Administration Safety and Innovation Act (FDASIA 907), the U.S. Congress required FDA to report on the diversity of participants in clinical trials and the extent to which safety and effectiveness data is based on demographic factors such as sex, age, and race. In response, the FDA Center for Drug Evaluation and Research (CDER) created a new transparency initiative called the Drug Trials Snapshots.

Methods: Since January 2015, CDER has been publishing a Drug Trials Snapshot for each novel drug approved within a month of the official approval date. The following poster summarizes the first two years of the Drug Trials Snapshot program, broken down by calendar years 2015 and 2016. The results provide a detailed summary of how members of each sex, race, and age are represented per clinical trial for every novel drug the FDA approved. Additionally, the poster includes the overall percentage of participants from each highlighted demographic subset that participated in the clinical trials.

Results: In calendar year 2015, CDER approved 45 novel drugs, either as either as NMEs under New Drug Applications (NDAs) or as new therapeutic biologics under Biologics License Applications (BLAs). Overall, 105,826 patients participated in these trials. The results share demographic information on the participants in each specific drug's clinical trial. Among the participants for all 2015 drug trials, 40 percent were female, 5 percent were African American, 12 percent were Asian, 79 percent were White, 4 percent were part of "Other" racial groups, and 37 percent were ages 65 and older.

In 2016, CDER approved 22 novel drugs, either as NMEs or BLAs. Overall, 31,468 patients participated in these trials. The results share demographic information on the participants in each specific drug's clinical trial. Among all participants in 2016, 48 percent were female, 7 percent were African American, 11 percent were Asian, 76 percent were White, 7 percent were part of "Other" racial groups, and 21 percent were ages 65 and older.

These findings underscore FDA's commitment to enhancing transparency and better understanding of patient representation in clinical trials.

### 28. Patient Reported Outcome (PRO) Questionnaires for Total Knee Arthroplasty (TKA): A Systematic Review of Psychometric Properties and Methodological Quality using the Consensus-based standards for the selection of health Status Measurement Instruments (COSMIN)

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*MD, PhD, Department of Cardiothoracic Surgery, Weill Cornell Medical College; Nilsa Loyo-Berrios, PhD, MS, DEPI/OSB; Benjamin Eloff, MS, PhD, DEPI/OSB; Manuel Bayona, MD; MS, Ph, DEPI, OSB; Danica Marinac-Dabic, MD, PhD, DEPI/OSB; Faisal Mirza, MD, FRCSC, OrthoSynthesis Inc.*

Plain Language Synopsis: TKA PROs are prevalent in the literature, but studies often have poor methodological quality. When choosing an instrument to monitor TKA PROs, the instrument quality is important. The COSMIN method was used to evaluate these studies. Out of 18 instruments, OAKHQOL had the best scores for TKA outcomes.

Abstract:
An aging and increasingly obese US population suggests TKA will be performed more frequently as time passes, making evaluation more critical for minimizing economic burden and quality of life reduction. Having quality evaluation instruments will inform clinical decision making and begin to streamline TKA outcome data for improved comparability that can be used for regulatory, scientific and clinical reasons. There is no systematic review using recent guidelines for evaluations of PROs in patients at risk for TKA. The aims for this study are to identify currently available patient reported outcome instruments used in patients who will undergo TKA and to critically appraise, compare and summarize the psychometric properties of the identified PRO instruments. A systematic literature review for PRO evaluation using the COSMIN method was conducted. PubMed, Embase, Web of Science, Cochran Library, and CINAHL were used to identify any article that evaluated PROs related to TKA. From a total of 1978 articles, 59 met the COSMIN inclusion criteria. Few studies looked at measurement error, content validity, structural validity or criterion validity, highlighting a gap in the literature. Most responsiveness evaluations received poor methodological ratings due to poor statistical methods, thus illustrating a need for standards in the TKA research community. Womac and OKS were the most evaluated instruments. Out of 18 evaluated instruments, OAKHQOL had the most "excellent" scores, suggesting that it could be a viable PRO for assessing TKA patient reported outcomes.

**29. Systematic Review on Psychometric Properties and Methodological Qualities on Patient Reported Outcome (PRO) Questionnaires for Patients that Undergo Total Hip Arthroplasty (THA) using the COnsensus-based standards for the selection of health Status Measurement INstruments (COSMIN)**

*Authors: Gagnier, Joel, Department of Epidemiology, School of Public Health, University of Michigan; Huang, Hsiaomin, Department of Epidemiology, School of Public Health, University of Michigan; Mullins, Megan, Department of Epidemiology, School of Public Health, University of Michigan; Stone, Heather, OMPT/CDER/OMP; Ghambaryan, Anna, NIAAA/NIH; Sedrakyan, Art, Department of Cardiothoracic Surgery, Weill Cornell Medical College; Loyo-Berrios, Nilsa, DEPI/OSB; Eloff, Benjamin, DEPI/OSB; Bayona, Manuel, DEPI, OSB; Marinac-Dabic, Danica, DEPI/OSB; Mirza, Faisal Mirza, MD, FRCSC, OrthoSynthesis Inc.*

Plain Language Synopsis: Many PROs questionnaires/instruments have been developed for THA, and many studies evaluated these instruments' qualities. Literature reviews suggested that most of these studies have poor methodology. The COSMIN method was used to further evaluate these studies. Out of 18 instruments, WOMAC had the strongest positive qualities followed by HOOS, OAKHQOL, OHS, and PASI.

Abstract:
There is no up-to-date systematic review using recent guidelines for evaluations of PROs in patients at risk for THA or after THA. Thus, the objectives of this study were: (a) To identify currently available PRO questionnaires used in THA patients , (b) To appraise the methodological quality of the studies that evaluate the identified instruments, (c) To assess the psychometric evidence of these instruments, and (d) To provide a summary of the overall evidence for and against each included questionnaire. We conducted a systematic literature review for PRO evaluation using the COSMIN method. We

searched PubMed, Embase, Web of Science, Cochran Library, and CINAHL for any article that evaluated PROs related to THA. From a total of 4,002 articles, 49 met the COSMIN inclusion criteria. The WOMAC, the OHS, and the HHS were the most commonly assessed PRO instruments for use in THA patients. Out of 18 evaluated instruments, WOMAC had the strongest positive internal consistency, structural validity, hypothesis testing, and responsiveness followed by the HOOS, OAKHQOL, OHS, and PASI. Studies assessing psychometric properties are encouraged to follow the COSMIN and the FDA psychometric evidence guidelines. Development of alternative overall final psychometric rating schemes should be encouraged and tested. Identification of core outcome for patients undergoing THA is recommended to be established as are guidelines to evaluate them. Validated core outcomes will be useful for regulatory, scientific and clinical work.

### 30. Healthy Citizen @ FDA: working to enhance public health through citizen-centric communication

*Authors: Phipps, Josh, Conceptant; Mikhalchuk, Andrey, Conceptant; Johanson, Elaine, FDA/OC; Bright, Roselie, FDA/OC; Bandler, Ruth, FDA/OC; Griffin, Amber, FDA/OC; Hall, Letria, FDA/OC; Houle, Lisa, Conceptant.*

Plain Language Synopsis: Healthy Citizen @ FDA will be a holistic, citizen-centric, mobile platform for FDA to collaborate and communicate with citizens to improve public health outcomes. It will empower patients to securely contribute their direct health experiences to FDA, receive timely, personally-relevant FDA alerts, and sign up to participate in clinical research.

Abstract:
Challenges/Facts: Recent law encourages FDA to more fully engage patients. There has not been a way to send targeted, consistent and timely recall information and safety alerts to the general public.

The FDA has faced challenges with obtaining electronic, comprehensive, coded adverse event reports and consumer complaints and being able to reach back to the submitters. Citizens possess a wealth of personal and other relevant data that they have been unable to share with the FDA for safety surveillance and safety studies.

There has been a lack of connection between researchers needing study participants for clinical studies and appropriate citizens willing to participate.

Proposed Solution: Healthy Citizen @ FDA will allow for the consistent, targeted and timely dissemination to the general public of recall information and safety alerts for all FDA regulated products.

Healthy Citizen @ FDA will also allow citizens to securely submit adverse event reports, product problem reports, and consumer complaints, from a mobile application, as they happen, with comprehensive and correctly coded electronic submissions. FDA surveillance staff would have the ability to contact the submitter to obtain more information or get any updates. Citizens will be able to review/manage/share their personal and clinical (and in future release genomic) data, track their daily lifestyle activities, and record usage of FDA regulated products.

This solution will allow citizens to choose to share their personal and clinical data to create a large scale de-identified data pool for analysis and research, as never before. Researchers will have the ability to identify cohorts based upon these rich datasets and send invitations for relevant clinical studies to citizens that fit the research profile. If a citizen opts into the study, the researcher would have the ability to collect study specific data and to reach back to the enrollee, all through the Healthy Citizen platform.

Goals: We believe that the Healthy Citizen @ FDA platform will enable population health outcome gains through new partnerships between citizens, FDA staff and researchers to help citizens lead healthier, longer, and more productive lives.

### 31. Comparison between Single-Incision Mini-Slings (SIMS) and Transobturator Tension-free Vaginal Tape (TOT) in the Treatment of Female Stress Urinary Incontinence: Systematic Literature Review and Meta-analysis of Complications

*Authors: Lu, Xiaoxiao, FDA/OSB/DEPI; Du, Dongyi (Tony), FDA/OSB/DEPI; Loyo-Berrios, Nilsa, FDA/OSB/DEPI*

Plain Language Synopsis: There are few studies comparing the long-term complications of Single-Incision Mini-Slings (SIMS) with other procedures for SUI treatment. Evidence from published literatures was synthesized to compare the long-term safety between SIMS and transobturator tension-free vaginal tape (TOT).

Abstract: Background: After reviewing the complaints in the MAUDE database, FDA issued a communication on "Considerations about Surgical Mesh for SUI". It is critical to evaluate the long-term safety of Single-Incision Mini-Slings (SIMS), particularly to understand how SIMS compares to transobturator tension-free vaginal tape (TOT) with respect to safety, when used for treating SUI.

Methods: A literature search was performed for randomized clinical trials (RCTs) comparing SIMS with TOT.

Results: Seven hundred and sixty five (765) studies that compared SIMS and TOT were identified, of which 21 were included in the study, based on study design and clearly defined outcomes. Our meta-analysis showed that compared with TOT, SIMS had significantly lower rates of groin or thigh pain (relative risk [RR]: 0.32; 95% confidence interval [CI]: 0.26 – 0.41), long-term chronic pain (RR: 0.14; 95% CI: 0.04 – 0.54), and urinary retention (RR: 0.60; 95% CI: 0.39 – 0.94); meanwhile, SIMS showed higher complication rates of vaginal exposure of mesh (RR: 2.16; 95% CI: 1.11 – 4.21) and reoperation (RR: 2.03; 95% CI: 1.22 – 3.38). The complication rates between the two approaches were similar for bladder or urethral perforation, vaginal wall perforation, de novo urgency, and infection.

Conclusion: This meta-analysis shows that SIMS is associated with lower risks of pain related outcomes but exhibits higher risks of vaginal exposure of mesh and additional surgical intervention. However, these results should be interpreted with caution due to the heterogeneity of the trials included.

### 32. A systematic review of the state of validation of patient reported outcomes (PROs) for urinary incontinence (UI)

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Plain Language Synopsis: UI adversely impacts Quality of Life (QoL) and PROs are important for UI QoL evaluation. Quality PRO measures should be reliable, valid, responsive and interpretable. PRO evaluation is essential for regulatory and clinical purposes. To date, a systematic review of PRO psychometric properties for patient with UI has not been performed.

Abstract:
In order to assess and evaluate the reliability, validity and responsiveness of PROs for UI, we conducted a systematic literature review for PRO evaluation using the COnsensus-based Standards for the selection of health status Measurement INstruments (COSMIN) method. We searched PubMed, Embase, Web of Science, Cochran Library, and CINAHL for any article that evaluated PROs related to UI. From a total of 6,349 articles, 105 met the COSMIN inclusion criteria assessing the properties of 32 instruments. The most commonly used instruments were the Incontinence Impact Questionnaire (IIQ), The Incontinence Quality of Life Questionnaire (I-QOL), The Urogenital Distress Inventory Questionnaire (UDI) and the King's Health Questionnaire (KHQ). The

instrument with the overall best quality was the Urgency Symptom Profile (USP) with positive evidence on 4 properties followed by the I-QOL with positive evidence on 3 properties. Eight other measures had positive evidence on 1 property but all other 22 measures had either unknown evidence or no psychometric evidence. Therefore, the vast majority of patient reported outcome measures designed or tested in patients with UI have no evidence for their properties. Much more research is required to test these instruments. Only two measures showed any promise of having acceptable properties. Regulatory decisions on devices in this area must be careful to note what PRO measures were used and if there is psychometric evidence for them or not. The results of the study and this standardized methodology could be readily applied to registries and the National Evaluation System for Health Technology, informing regulatory decision making and strengthening PRO-based research at CDRH. This project demonstrates the capability of the COSMIN-based methodology in a high profile area, allowing its applications for other medical device areas.

### 33. Fellows Day: A Targeted Approach to Physician Outreach and Education in the Oncologic Drug Space

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Plain Language Synopsis: We performed a needs-based assessment and gap analysis to look at improving education of oncologists in drug development and regulatory science. To address the gap, the Food and Drug Administration–American Society of Clinical Oncology Semi-Annual Hematology and Oncology Fellows Day Workshop was tailored to meet specific educational needs.

Abstract:
Using Janetti's representation of incorporating needs assessment and gap analysis into education design, we assessed the current educational and outreach programs for early-career oncologists as it relates to regulatory science and oncology drug development. The assessment looked at subject matter expertise and creation of a three-tiered system of knowledge. Within this three-tiered system, the Food and Drug Administration's regulatory medical officers have unique experience in first hand application, knowledge, and perspective of oncologic drug development.

First, a baseline was created to analyze the education gap in drug development regulations and processes promulgated by the Food and Drug Administration. Then ways to close this gap were assessed. To address the drug development knowledge gap facing clinical oncology fellows, the Food and Drug Administration-American Society of Clinical Oncology (FDA-ASCO) Semi-Annual Hematology and Oncology Fellows Day Workshop was developed. This one day workshop incorporates drug development topics that are relevant for oncology fellows, who will be writing and conducting clinical trials during their career.

Recognizing a lack of regulatory science training for oncology fellows, the Office of Hematology and Oncology Products (OHOP) created a specific oncology drug development workshop that uses didactic and interactive learning modules based on current regulatory science topics. The specified learning modules are: oncology drug regulations, the investigational new drug process, expanded access programs, disease-specific considerations, expedited development programs, clinical trial design, common errors in oncology drug development, and biomarker and companion diagnostic development. The information provided at this workshop facilitates an oncology fellows' knowledge and understanding of the regulations associated with clinical trials and could be applied throughout their career.

In addition to the learning modules, several specialty specific breakout sessions were created to foster small group discussion and engagement. In the February 2017 Fellows Day, the six breakout sessions were leukemia/

lymphoma, breast cancer, gastrointestinal oncology, immunotherapy, genitourinary oncology, and thoracic oncology. During these breakout sessions, over two dozen subject matter experts within OHOP led small group discussions to focus on regulatory questions related to these specific disease areas.

This workshop model also accounted for evaluation of the activity through post-workshop surveys of all the participants. This feedback mechanism allows OHOP to continually improve the workshop.

Currently, the success of the FDA-ASCO Fellows Day has been shown by the positive feedback from the fellows attending the workshop and by the expanding number of fellowship programs and states represented by the workshop participants; in its fourth iteration, the February 2017 Fellows Day hosted hematology and oncology fellows from 19 states plus the District of Columbia.

### 34. Progress in Including Women in Clinical Trials for FDA-Approved Products

*Authors: Elahi, Merina, FDA/OC/OWH; Kallgren, Deborah, FDA/OC/OWH; Fadiran, Emmanuel, FDA/OC/OWH; Scott, Pamela, FDA/OC/OWH*

Plain Language Synopsis: FDA has continued to monitor the progress in the participation of women in clinical trials. Participation has improved in many areas, although not to the same extent across all the therapeutic areas.

Abstract:
Background: FDA has implemented both regulations and guidance documents to improve the participation of women and other subgroups in clinical trials. FDA has continued to monitor the progress in participation of women and ethnic/racial minorities throughout the past several years. In addition, it is part of the mission of the FDA Office of Women's Health to advocate for the participation of women in clinical trials and for sex, gender and subpopulation analyses. In collaboration with other FDA centers, OWH has supported and conducted demographic studies of product applications (NMEs, NDAs, BLAs) for the past 20 years.

Methods: Participation data was compiled from previous demographic studies which reported on the demographics of clinical trial participants by sex across phases of clinical trials (phase 1, 2, 3) and which were used for the approval of drug and biologic products approved by FDA from 1983-2015. Additional demographic data including age group, race and ethnicity were also compiled. Studies were compared to determine variations in methodology for determining inclusion or the level of participation.

Results: Participation of women has remained close to 50% in late phase drug and biologic trials for several years, but varies across indication. Participation of women in early phase drug trials has increased from 22% to 31%. The availability of participation data over the years has varied by product type and indication. Additionally, the methodology used in studies which report on participation has varied.

Conclusion: The participation of women in clinical trials has improved over the past 30 years and women are generally included in clinical trials. However, low enrollment has persisted in some disease areas and for ethnic/racial minorities. New FDA initiatives have greatly improved the public availability of clinical trials participation data and continue to seek improvement in disease and health areas that have persistent low enrollment of women.

### 35. Using Global Surveillance Sampling and Testing to Inform U.S. Patients of Generic Drug Quality

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Plain Language Synopsis: Patients are concerned about sub-standard quality for many imported generic drugs that are dispensed in the U.S. each year. The Drug Quality Sampling and Testing program at the FDA provides patients with quality testing assessment for many of these drugs. This information can enable patients to better protect their health.

Abstract:
The Office of Surveillance's (OS) Drug Quality Sampling and Testing (DQST) program seeks to protect public health by minimizing patient exposure to non-conforming and/or sub-standard quality of drug products. Through the DQST program, OS can: 1) assess the quality of post-market drug products used by U.S. patients or consumers regardless of their source and 2) inform patients about drug quality in the U.S. market by disseminating sampling and testing results. To address patient concerns of sub-standard quality for imported generic drugs, 31 drugs were selected for DQST. These drugs were selected by multiple approaches: risk-based model, historical quality data review, post-marketing reports, and for-cause and triggered inspections. The sampled generic drugs represent a wide range of therapeutic indications with multiple strengths and were all produced by foreign pharmaceutical manufacturers. All 31 imported generic drugs were subjected to multiple quality tests, including: assay, content uniformity, dissolution, and identity. The standard methods used for these quality tests were the current methods from the U.S. Pharmacopeia (USP). When there were no current standard compendial methods or USP monographs for a test product, FDA used the methods from the approved NDA/ANDA for that drug product. Our testing results showed that all 31 imported generic drugs selected for sampling and testing met their quality requirements. In conclusion, via global surveillance sampling and testing the DQST program can help protect U.S. patients and inform them about the status of U.S. drugs.

### 36. Consumer Perceptions about Restaurants -- Health Inspections Information and Consumer Advisory Statements

*Authors: Lando, Amy, FDA/CFSAN; Williams, Laurie, FDA/CFSAN*

Plain Language Synopsis: The objective of this project is to understand consumers' understanding and use of restaurant health inspection information and advisory statements about the risks of eating raw and undercooked meat, poultry, fish, and eggs. FDA can use this information as it considers ways to update and improve the Food Code.

Abstract:
The FDA Food Code is a model code that represents FDA's best advice for a uniform system of regulation to ensure that food at retail is safe and properly protected. Within the Food Code, FDA provides recommendations on how information about restaurant inspections and certain risks from eating raw or undercooked meat, poultry, fish, and eggs should be communicated to consumers. We explored these topics in a series of eight consumer focus groups conducted in September and October 2016 in four U.S. cities.

Health Inspection Information: Consumers rely on many factors when selecting where to eat including, prior experience, food cravings, customer traffic, convenience, readings from social media websites, as well as health department inspection information. We found familiarity with health inspection reports and scores depended upon local guidelines for displaying this type of information. Those living in cities where health inspection information is required to be publically posted were more familiar with the health inspection information. There was a range in how consumers used the health inspection information, but most thought the information was valuable.

Consumer Advisory Statement: We found that participants were generally aware that some foods such as sushi, raw shellfish, and undercooked hamburgers were more risky choices, and most indicated that they had seen warnings about risky foods on restaurant menus. Participants were asked to review the following consumer advisory statement, "Consuming raw or undercooked meats, poultry, seafood, shellfish, or eggs may increase your risk of foodborne illness." We found that while the wording and meaning of the statement was clear to participants, there were two areas that cause some confusion. The term "foodborne illness" was not familiar to all participants. Some suggested using more familiar terms such as "stomach flu" and "food poisoning." Also, seeing "poultry" was surprising. When participants saw

"poultry" they thought of "chicken" which is not a food that traditionally would ever be served intentionally undercooked. This caused some to think the advice was about unintentional mistakes that the restaurant could have made and could result in illness.

**37. Assessment of Patient Tolerance for Risk Associated with High Intensity Focused Ultrasound (HIFU) for the Ablation of Prostate Tissue in men with Localized Prostate Cancer**

*Authors: Olufemi Babalola, MHS; Joyce Lee, MHS; Charles Viviano MD, PhD; John Baxley; Glenn Bell, PhD;*

Plain Language Synopsis: HIFU devices were approved to ablate (destroy) prostate tissue. The devices are being used to treat prostate cancer, but their effectiveness at treating cancer is unknown for US patients. Our project seeks to gauge patients' willingness to accept the risks of HIFU in this setting.

Abstract:
Rationale: The Food and Drug Administration (FDA) recently cleared to market two HIFU tools for prostate tissue ablation after rejecting prior premarket applications indicated to treat prostate cancer, as they failed to demonstrate appropriate clinical efficacy in a United States (US) population. Although patient relevant outcome data such as disease free- and metastasis free- survival are unavailable in the U.S., 12 month post-treatment prostate biopsy data and the adverse event profile of HIFU are known. Patients must currently make an informed decision regarding HIFU treatment in the absence of relevant clinical outcome data but in light of known potential adverse events. Patient preference information (PPI) regarding tolerance of risks associated with HIFU ablation of tissue in patients with localized prostate cancer could provide important information to patients and clinicians who may choose to use the device, current and prospective device sponsors, and the FDA.

Methods: This is a prospective study investigating patient tolerance for risk associated with HIFU prostate tissue ablation in men diagnosed with localized prostate cancer. We will conduct focus groups with patients to understand what is clinically important to prostate cancer patients. This data will then inform the design of a threshold technique survey, by which we will elicit the level of adverse event risk patients are willing to tolerate for increased ablation effectiveness of a device. The primary outcome is defined as maximum acceptable risk (MAR). We will compare MAR results according to population variables including ethnicity, age, and disease severity.

Results: Results are pending. We are awaiting University of Maryland Medical Center IRB approval to begin recruiting patients for the focus groups.

Conclusions: This data will be useful to the FDA for evaluating the risk-benefit profile of future prostate tissue ablation devices in the absence of patient relevant cancer effectiveness data.

The results will also allow patient input to inform the FDA and prospective device sponsors to include patient-based risk and benefit acceptance in devices and device protocols. The incorporation of PPI into the device decision making process is also one of the strategic goals of the CDRH at the FDA.

**38. An examination of the role of advertising and promotional labeling in adult immunization disparities**

*Authors: Elekwachi, Oluchi, FDA/CBER*

Plain Language Synopsis: For this research, the barriers to adult vaccination in adults of racial/ethnic minority background were reviewed, specifically examining health messaging through vaccine advertising and promotional material, with specific focus on Herpes Zoster Vaccine in those aged 65 and older. The messaging was evaluated for health literacy and cultural competence.

Abstract:
Vaccination rates for ethnic/racial minorities (i.e, Asian, Latino, Black) fall well below Healthy People 2020 targets for adult vaccination. Specifically, the Healthy People 2020 targets for herpes zoster are 30% in those aged 60 and over. While the vaccine was approved

by the FDA for use in adults at least 50 years old, the Advisory Committee on Immunization Practices (ACIP) recommended that the vaccine be used in adults age 60 and over. These ACIP recommendations are based on factors such as age, health condition, risk, occupation, travel, and other factors. The adult immunization schedule was approved by the Advisory Committee on Immunization Practices (ACIP) in October of 2015. Improvement in adult vaccination rates is paramount to reducing the unfavorable health consequences, hospitalizations, morbidity, and mortality of vaccine-preventable diseases. Among the vaccines recommended for elderly adults, the herpes zoster vaccine, has one of the lowest adult immunization rates at 24% overall, however the rate is only 11% in Blacks as opposed to 27% in Whites. The cause of low rates of vaccination among minorities is often thought to be an issue of access, however it can be multifactorial. One of these factors, specifically the focus of this research, relates to the cultural competence and health literacy levels of the advertising and promotional messaging around these vaccines, as well as the extent to which they have or are able to impact the disparities in vaccination rates and awareness among minority populations.

### 39. A Bayesian Approach for Benefit-Risk Assessment in Medical Devices

*Authors: Chul Ahn and Ram Tiwari*

Plain Language Synopsis: Three measures are proposed to simultaneously evaluate benefit and risk in ophthalmic devices. The measures will be expressed as the ratios involving benefit and risk, and the confidence intervals of these ratios will be calculated using Bayesian method.

Abstract:
One of the themes observed at the Institute of Medicine workshop (Caruso and Claiborne, 2014) was the importance of developing methods that can help convey to patients and providers quantified information about benefit and risk in a concise and meaningful way. In this poster, we present measures that can simultaneously evaluate benefit and risk in medical devices. As an example, we consider ophthalmic devices with a main objective of demonstrating improvement in uncorrected (without glasses or contact lenses) near or intermediate visual acuity (UCNVA), uncorrected distance visual acuity (UCDVA) may be used to assess risk, since subjects may be giving up distance vision for some gain in near or intermediate vision. In this case, a joint analysis of the benefit and risk of the device may be the assessment of how much distance vision the subjects give up for how much near or intermediate vision they gain. We define three measures of benefit-risk assessment for a four category random variable. The visual acuities from subjects can fall into four mutually exclusive quadrants, Quadrant 1 (Q1) with improvement in UCNVA and without loss in UCDVA, Quadrant 2 (Q2) without improvement in UCNVA and without loss in UCDVA, Quadrant 3 (Q3) without improvement in UCNVA and with loss in UCDVA, and Quadrant 4 (Q4) with improvement in UCNVA but with loss in UCDVA. We assume that the number of individuals in each of four quadrants follow a multinomial distribution with size N and probabilities p = (p1, ..., p4), and calculate three measures of benefit-risk assessment based on the ratio of their probabilities, Q1/Q3 (the ratio of the best quadrant over the worst quadrant), (Q1+Q4)/(Q3+Q4) (the ratio of benefit over the risk), and (Q1+Q4)/(Q2+Q3) (the ratio of benefit over no-benefit). We will calculate the confidence interval of these ratios with Bayesian method by assuming Dirichlet distribution as prior for multinomial probabilities (Zhao et al, 2014). The results will be compared with the intervals calculated using the Delta method (Oehlert, 1992).

### 40. A quantitative method for assessing the association between an objective endpoint and patient reported outcome measures

*Authors: Ahn, Chul, FDA/CDRH; Zhang Bo, FDA/CDRH; Silverman, Phyllis FDA/CDRH; Zhang, Zhiwei, UCR; Fang, Xin, FDA/CDRH*

Plain Language Synopsis:

1. Introduction: why we need the new association statistic

2. Data structure to be used in this poster, the theoretical construct of the new statistic
3. Motivation for the New Statistic:
4. Characteristics of the New Statistic
5. Application to a clinical trial data
6. Discussion and Conclusion

Abstract:
One approach to assess the quality of a patient reported outcome measure (PROM) is to investigate the association between the PROM and an objective clinical endpoint measuring the status of a disease/condition. In this paper, we propose a method to assess such association with any type of sample with or without correlation. The method involves estimating the probability of concordance between an objective endpoint and the PROM. The probability of concordance reflects the true status of a subject's disease/condition with respect to the subject-specific latent objective threshold. A consistent estimator for the probability of concordance is derived. The operating characteristics of the consistent estimator are illustrated using simulation. Then the estimator for this probability is applied to a real medical device clinical trial to assess the quality of the PROM used in the trial. Finally, pros and cons of utilizing this probability of concordance reflecting the true disease status in drug and medical device clinical trial and future research are discussed.

**41. Demographics and Risk Perceptions of Unpasteurized Milk and Unpasteurized Cheese Consumers in the United States**

*Authors: Bazaco, Michael, FDA/CFSAN; Lando, Amy, FDA/CFSAN; Parker, Cary Chen, FDA/CFSAN; Czabaranek, Christina, FDA/ORA; Wolpert, Beverly, FDA/CFSAN*

Plain Language Synopsis: We wanted to better understand the types of people in the United Sates who drink unpasteurized milk or eat cheese made with unpasteurized milk. With a better understanding, public health agencies can develop more effective ways to communicate the health risks to these consumers.

Abstract:
Consumption of unpasteurized (raw) milk as well as cheese made from unpasteurized milk (raw milk cheese) is a significant public health concern because these products do not undergo the pasteurization process that is essential for killing foodborne pathogens such as Campylobacter spp., Brucella spp., and Listeria monocytogenes. In 1987, the US Food and Drug Administration banned the interstate distribution of raw fluid milk packed in final packaging for direct sale to consumers. However, a small percentage of the U.S. population consumes raw milk either regularly or occasionally and outbreaks of illness have been linked to consumption of raw milk. Other outbreaks have been linked to raw milk cheese. To better understand raw milk and raw milk cheese consumers' demographics and risk perceptions, we analyzed data from the 2016 Food Safety Survey conducted by the U.S. Food and Drug Administration (FDA) in collaboration with the U.S. Department of Agriculture (USDA). We looked at the differences in demographics as well as risk perceptions among raw milk and raw milk cheese consumers. People who had reported consuming raw milk in the past 12 months were significantly more likely to be younger, male, non-Hispanic white and live in rural settings. Those who had consumed raw milk cheese in the past 12 months were more likely to be younger, white non-Hispanic, white-Hispanic or nonwhite Hispanic, and to have higher levels of education. Both consumers of raw milk and raw milk cheese were significantly less likely to perceive known food safety risks (such as inadequate hand washing and cross-contamination) as risky. Although there was some overlap between these two consumer groups, they do make up two distinct populations. Overall this study helps to identify demographic features and risk perceptions of raw milk and raw milk cheese consumers. The two groups of consumers have distinct features that may require different public health messaging. The findings of this study will be useful in crafting educational interventions to convey the risk of consuming both unpasteurized milk and cheese made from unpasteurized milk in the future.

## 42. Association Between The Real Cost Media Campaign and Smoking Initiation Among Youth — United States, 2014–2016

*Authors: Farrelly, Matthew, RTI, International; Duke, Jennifer, RTI, International; Nonnemaker, James, RTI, International; MacMonegle, Anna, RTI International; Alexander, Tesfa, FDA/CTP; Zhao, Xiaoquan, FDA/CTP; Delahanty, Janine, FDA/CTP; Rao, Pamela, FDA/CTP; Allen, Jane, RTI, International*

Plain Language Synopsis: This study describes findings from FDA's first tobacco-focused public education campaign, The Real Cost, aimed at reducing tobacco use among 12-17 year olds in the United States.

Abstract:
In the United States, approximately 900,000 youth smoke their first cigarette each year (1). Health communication interventions are evidence-based strategies for preventing the initiation of tobacco use, promoting and facilitating cessation, and changing beliefs and attitudes about tobacco use (2, 3). This presentation describes the association between the Food and Drug Administration's (FDA's) first national tobacco public education campaign, The Real Cost, and rates of smoking initiation among youth in the United States from 2014 to 2016. A nationally representative cohort study of youth (N = 5,185) was conducted during November 2013–March 2016. Results from a discrete-time survival model indicate that, among youth who reported never having smoked a cigarette in the baseline survey, the odds of reporting smoking initiation at follow-up were lower among youth with frequent exposure to campaign advertisements than among those with little or no exposure (adjusted odds ratio [aOR] = 0.70, 95% confidence interval [CI] = 0.55–0.91). Based on the results of the model, The Real Cost is associated with an estimated 348,398 U.S. youth aged 11–18 years who did not initiate smoking during February 2014–March 2016. Sustained youth-focused tobacco education campaigns, such as The Real Cost, can help speed progress toward preventing tobacco use among youth in the United States.

## 43. Using Personal Electronic Devices in the Kitchen

*Authors: Lando, Amy, FDA/CFSAN; Bazaco, MIchael, FDA/CFSAN; Chen, Yi, FDA/CFSAN*

Plain Language Synopsis: The objective of this project is to understand how consumers use smartphone and other personal electronic devices in the kitchen while preparing food. FDA can use this information to better target their food safety education and outreach.

Abstract:
Since their introduction in the mid 2000's smartphones, tablets, and other personal electronic devices have become ubiquitous in American's daily lives. These devices are mobile and multifunctional and used by people throughout the day including while preparing food. For example, they may be used to look at recipes and therefore be touched multiple times during preparation. Previous research has shown that cell phones can harbor bacteria including opportunistic human pathogens (such as Staphylococcus and Klebsiella spp.). Using data from the 2016 Food Safety Survey (FSS) and subsequent focus groups we investigated the frequency of consumers using their personal electronic devices in the kitchen while preparing food, what type of devices they use, and their hand washing behaviors after handling their device. The 2016 FSS is the 7th wave of a repeated cross-sectional survey conducted by the Food and Drug Administration (FDA) in collaboration with the U.S. Department of Agriculture (USDA). The goal of the survey is to evaluate U.S. adult consumers' attitudes, behaviors, and knowledge about food safety. The FSS surveyed 4,169 adults using a dual frame (landline and cell phone interviews) random-digit-dial (RDD) sampling process. The personal electronics module was the first of three food safety topics discussed in each of eight consumer focus groups which were conducted in four U.S. cites in the fall of 2016. Results from the 2016 FSS found that of those who use personal electronic devices while cooking; only about a third reported washing their hands after touching their device before they continue cooking. This is significantly lower than the self-reported hand washing

behavior after touching risky products such as raw eggs, meat, chicken, or fish. Results from the focus groups highlight the varied usage of these devices while cooking and the related strategies consumer are using to incorporate personal electric devices into their cooking routines. FDA can use this information to better target food safety education and outreach.

### 44. The CDRH - SMRT MRI Safety Posters

*Authors: Terry O. Woods & Jana G. Delfino*

Plain Language Synopsis: CDRH engineers collaborated with MR (Magnetic Resonance) technologists from the Society for MR Radiographers & Technologists (SMRT) Safety Committee, to create posters addressing MRI safety. The posters: MRI Burn Prevention: Tips for Keeping Patients Safe, Tips for Scanning Patients with Implants, and Understanding MRI Safety Labeling are available at http://www.fda.gov/Radiation-EmittingProducts/RadiationEmittingProductsandProcedures/MedicalImaging/MRI/ucm482788.htm.

Abstract:
Magnetic resonance imaging (MRI) is a widely used diagnostic modality with an excess of 30 million scans being performed every year in the U.S. Although MRI is a safe and effective diagnostic tool for many patients, thermal injuries are the most commonly reported adverse event for MRI. Additionally, the MR environment presents unique safety hazards for patients with implants, external devices and accessory medical devices. Conducting an MRI scan on a patient with an MR Unsafe medical device or failing to follow the MR Conditional labeling for a medical device can lead to serious adverse health consequences, including death.

MR technologists must follow accepted medical practices and take certain precautionary steps before performing an MRI exam. For patients with a medical device, these include determining whether a device is MR Safe, MR Conditional, or MR Unsafe, selecting appropriate scan parameters based on the MRI safety labeling and required diagnostic imaging, monitoring the patient during the scan and assessing the patient and their device to ensure its functionality after the scan.

CDRH's Office of Science & Engineering Laboratories and Office of In Vitro Diagnostics and Radiological Health collaborated with the Safety Committee of the SMRT (Society for MR Radiographers & Technologists, a Section of the International Society for Magnetic Resonance in Medicine (ISMRM)) to create three posters intended to provide tips for preventing burns, to raise awareness of the standardized MRI safety terminology and to raise awareness of safety procedures to be followed before, during, and after conducting an MRI scan on patients with implants. The posters are freely available to the clinical diagnostic community and formatted for posting near MRI sites.

The posters: MRI Burn Prevention: Tips for Keeping Patients Safe, Tips for Scanning Patients with Implants, and Understanding MRI Safety Labeling are available at http://www.fda.gov/Radiation-EmittingProducts/RadiationEmittingProductsandProcedures/MedicalImaging/MRI/ucm482788.htm.

### 45. The Adoption of Generic Immunosuppressant Medications Among Kidney and Liver Transplant Recipients Using the Colorado All Payer Claims Database

*Authors: Liu, Jane, Arbor Research Collaborative for Health; Smith, Abby,Arbor Research Collaborative for Health; Park, Jeong,U of Michigan Ann Arbor; Oguntimein, Murewa, FDA/OGD; Dutcher, Sarah, FDA/OGD; Bello, Ghalib,Arbor Research Collaborative for Health; Helmuth, Margaret, Arbor Research Collaborative for Health; Turenne, MarcArbor Research Collaborative for Health;Balkrishnan Rajesh, U of Virginia; Fava, Melissa, Arbor Research Collaborative for Health; Sharma, Pratima,U of Michigan Ann Arbor; Beil, Claire,Arbor Research Collaborative for Health; Leichman Alan, Arbor Research Collaborative for Health; Zee, Jarcy,Arbor Research Collaborative for Health;*

Plain Language Synopsis: Using the Colorado All Payer Claims Database to identify generic and brand immunosuppressant use from January 2009 through September 2014.

Abstract:
Substitution of generic for brand-name immunosuppressants has increased in solid organ transplantation following the expiration of brand patents. Our aim was to describe the patterns of brand vs. generic immunosuppressant use among kidney and liver transplant recipients.

Methods: The Scientific Registry of Transplant Recipients database was used to identify kidney and liver recipients transplanted between 2009 and 2013 and linked to the Colorado All Payer Claims Database to identify generic and brand immunosuppressant use from January 2009 through September 2014. Percentages of patients using brand and generic tacrolimus (TAC), mycophenolate mofetil (MMF), and mycophenolate sodium (MPS) were plotted by month to illustrate trends over time.

Results: Among 582 kidney transplant recipients, 544 (93%), 234 (40%) and 346 (59%) received TAC, MMF, and MPS, respectively; 193 of 203 (95%) liver transplant recipients were on TAC. In the kidney cohort, generic TAC use increased to 74% within one year of the first generic approval by the FDA (August 2009) and to 85% by the end of the second year. TAC trends were similar in the liver cohort. Among kidney recipients, usage of generic formulations increased to 88% within a year for MMF and to 65% within 9 months for MPS after the transition to generics began.

Conclusion: The use of generic immunosuppressants in transplantation increased rapidly after the introduction of the first few generics and has greatly exceeded brand usage. Generic TAC uptake was more gradual than that of MMF and MPS. This difference in practice may have been a consequence of providers' hesitancy to switch to generic TAC due to a perceived narrower therapeutic window resulting in greater apprehension regarding the efficacy of the generic formulation.

### 46. ZIKV RNA is Present and Persists for Weeks in Stored RBC Units from Infected Blood Donors

*Authors: Chancey, Caren, FDA/CBER; Ok, Suzan, FDA/CBER; Sippert, Emilia, FDA/CBER; Gusmao, Rafaelle, FDA/CBER; Sonoda, Jadine, FDA/CBER and Washington University; Rios, Maria, FDA/CBER*

Plain Language Synopsis: While blood screening and diagnostic assays typically test plasma or serum, Zika virus RNA may also be associated with red blood cells. We have shown that ZIKV-positive RBCs remain positive for ZIKV RNA beyond the 42-day unit expiration date, and assessment of infectivity in tissue culture is ongoing.

Abstract:
Zika virus (ZIKV) is a Flavivirus transmitted to humans mainly by Aedes aegypti mosquitoes. Human infection with ZIKV was rarely reported prior to the 2007 outbreak on Yap Island in Micronesia. Since 2013, large ZIKV outbreaks have been reported in the Pacific region and the Americas, with the first mosquito-borne transmissions occurring in the U.S. in 2016. Transfusion-transmission of ZIKV has been reported in Brazil, and remains an ongoing risk to the U.S. blood supply because viremia emerges before symptoms, and approximately 80% of infected individuals never develop symptoms. While blood screening and diagnostic assays are typically performed using plasma or serum as the testing substrate, we suspected that ZIKV RNA may also be associated with RBCs, as observed for the related flaviviruses WNV and DENV. A study has shown that patient whole-blood samples were ZIKV-RNA-positive out to 58 days while the corresponding sera were positive for only 3 days. The goal of this study was to evaluate persistence of ZIKV RNA and infectivity in RBC units from infected donors. RBC units from blood donors who tested ZIKV-positive on NAT assays for blood screening in use under IND were obtained (Blood Systems Research Institute, San Francisco, CA and Creative Testing Solutions, Tempe, AZ) and stored in the original bag at 4°C. Aliquots were withdrawn from the units upon receipt and weekly thereafter for RNA quantitation and infectivity in tissue culture. Viral RNA, extracted from aliquots using Trizol reagent, was quantified using ZIKV-specific Taqman qRT-PCR. Tissue culture was performed by inoculating Vero cells or monocyte-derived macrophages (MDM) with RBCs for one hour, refeeding with media and incubating at 37°C, 5% CO2 for 7 days. ZIKV-positive RBC units remained RNA-positive for at least 35 days past the date of draw, and all units that were tested beyond the 42-day expiration date of the RBC units remained positive. RNA loads remained relatively constant in tested units. Determination of infectivity to Vero or MDM is ongoing. The source of persistent ZIKV RNA in stored RBC units should be investigated to determine whether ZIKV may be replicating under storage conditions.

### 47. Improving Risk Assessment of Color Additives in Medical Device Polymers

*Authors: Chandrasekar, Vaishnavi, FDA/CDRH; Janes, Dustin, FDA/CDRH; Forrey, Christopher, FDA/CDRH; Saylor, David, FDA/CDRH; Bajaj, Akhil, FDA/CFSAN; Duncan, Timothy, FDA/CFSAN; Zheng, Jiwen, FDA/CDRH; Riaz Ahmed, Kausar, FDA/CDRH; Hood, Alan, FDA/CDRH; Casey, Brendan, FDA/CDRH*

Plain Language Synopsis: Color additives are used in several medical devices, and if leached at toxicologically significant amounts can lead to adverse health effects. The goal of this work is to develop an improved risk assessment approach for color additives using conservative exposure models to predict leaching of color additives from polymer devices.

Abstract:
Color additives are used in a wide range of medical devices for a variety of purposes including labeling, coding for instructions, market appeal and advertising graphics. The potential health risk of color additives may be assessed by comparing the amount of color additive released over time to acceptable threshold levels based on available toxicity data. Potential health risk is typically assessed initially by assuming 100% of the color additive leaches during the first 24 hours, which can result in severe over-estimation of exposure.

Thus, there is a need for a better understanding of color additive release from medical devices.

We propose a conservative exposure model that requires only the diffusion coefficient of the additive in the polymer matrix, D, to be specified. This model is applied here using a model polymer (poly(ether-block-amide), PEBAX 2533) compounded with two sample color additives (quinizarin blue (QB) and manganese phthalocyanine (MnPC)). Experimental transport parameters of diffusion coefficient (D) and matrix solubility (C_s) were obtained from sorption experiments performed in an aqueous dispersion of QB or MnPC into neat PEBAX. Based on these experimentally determined transport parameters, the model was validated by comparing predictions to the leaching profiles of QB and MnPC from a PEBAX matrix into physiologically representative solvent. Available toxicity data for the color additives was evaluated to determine acceptable toxicological threshold values (i.e., provisional Tolerable Intake (pTI) or Threshold of Toxicological Concern (TTC) values). By considering the release of the color additive from PEBAX matrices over time and comparing with an acceptance threshold, we demonstrate that a significant enhancement in device size that would give rise to a toxicological concern can be achieved. These findings suggest that worst-case color additive exposure estimates based on diffusion modeling will improve initial screening-level risk assessments of color additives used in medical devices, and this approach can potentially be extended to other potentially toxic compounds found in device polymers.

## 48. Evaluation of Three Real-Time PCR Methods for Detection of Salmonella in Allspice, Cinnamon, and Oregano

*Authors: Xiaohong Deng, FDA/CFSAN; Aparna Tatavarthy, FDA/CFSAN; Laila Ali, FDA/CFSAN; Lijun Hu, FDA/CFSAN; Thomas S. Hammack, FDA/CFSAN; Guodong Zhang, FDA/CFSAN*

Plain Language Synopsis: Detection of Salmonella in select spices by PCR continues to be a challenge due to inhibitory substances found in those spices. This study compared the relative effectiveness of three real-time PCR platforms for the detection of Salmonella in different spice species and evaluated the efficiency of different DNA extraction methods.

Abstract:
Introduction: Detection of Salmonella in select spices by PCR continues to be a challenge due to inhibitory substances found in those spices.

Purpose: The purpose of this study was to compare the relative effectiveness of three real-time PCR platforms for the detection of Salmonella in allspice, cinnamon, and oregano and to evaluate the efficiency of different DNA extraction methods.

Methods: Eighteen separate trials were conducted using two different cultivars of each spice (allspice, cinnamon, and oregano), each inoculated individually with three Salmonella serotypes, Montevideo, Typhimurium, and Weltevreden. Inoculation levels ranged from 1.7 to 3.5 log CFU/25g. Overnight pre-enrichment cultures were used to extract DNA for PCR, using two different methods, boil lysis and the standard method for each PCR platforms. The 3 real-time PCR platforms evaluated in the study were ABI-MicroSEQ®, FDA-PRLSW, and GeneDisc®.

Results: The detection rate of Salmonella by PCR methods from culture positive samples for all trials was 99.8% for MicroSEQ® (n=509) and 97.6% for both PRLSW (n=510) and GeneDisc® (n=509). The PCR-negative samples that were culture-positive were mostly from cinnamon. Mean CT values for cinnamon (26.86) were significantly higher than those for allspice (22.26) and oregano (20.15) (P<0.0001). This result indicated possible PCR inhibitors in cinnamons. The difference of mean CT values between boil lysis and standard extraction was not significant for allspice (P=0.573) and oregano (P=0.064), but significant for cinnamon (P<0.0001) with the standard DNA extraction having lower CT values.

Significance: The detection of Salmonella was equivalent for all three PCR platforms and is comparable with culture assay results. When considering the choice of DNA extraction methods, different spices' inhibitory substance

should be taken into account. Although boil method is simple, when facing inhibitory problems, the standard DNA extraction protocol suggested by PCR kit manufacturers could perform better.

### 49. Development of a minimally invasive laparoscopic technique for sampling the abdominal lymph node in standing steers

*Authors: Oscar Alberto Chiesa, DVM, MS, PhD, FDA/OF/CVM/OR; Andrea Kouneski, RLAT, FDA/OF/CVM/OR ; Sara Sklenka, MPH, FDA/OF/CVM/OSC ; David Rotstein, DVM, DACVP, FDA/OF/CVM/OSC ; David E. Anderson, DVM, MS, DACVS. University of Tennessee.*

Plain Language Synopsis: A minimally invasive technique was developed to provide a research tool to predict contamination with Salmonella in live animals. Prevalence and characterization of Salmonella in bovine lymph nodes destined for use in ground beef has been reported. Avoiding carcass contamination with Salmonella will ultimately protect human health.

Abstract:
A minimally invasive technique was developed to obtain abdominal lymph node biopsies in standing steers. Five Holstein steers were purchased from USDA-ARS, Beltsville, Maryland. Each animal was kept in a separate box stall in a building designed with 1 indoor pen per animal. Straw was used for bedding. The animals were fed once daily, and the mixed standard feed was supplemented with hay ad libitum. Water was available ad libitum. The steers were provided at least 4 weeks of acclimation and a period of halter training, to become familiar with the new surroundings and the study personnel.

Feed was withheld from each animal 24 h before the procedure, but water was provided ad libitum. Each animal was haltered and led into a head gate, whereupon an intramuscular injection of acepromazine (0.025 mg/kg) was administered. Once tranquilized the animal was lead to the surgical room and confined in a stanchion. A catheter was placed in the left jugular vein for administration of xylazine (0.015-0.025 mg/kg IV). When the steer was adequately sedated, the right and the left paralumbar fossae were clipped, shaved, aseptically prepared, and draped with a laparotomy sheet. After a paravertebral anesthesia (lidocaine 2%), a 2-cm-long incision was made through the skin for placement of the laparoscopic trocar cannula assembly in the peritoneal space. One port of the laparoscope was introduced through the cannula and the abdomen insufflated with CO2 to create a pneumoperitoneum. The laparoscope was directed to the caudodorsal quadrant of the abdomen to identify the retroperitoneal abdominal lymph nodes. A blue dye was injected with a 27gx0.5" winged butterfly needle into the rectal mucosa and the surface of the lymph nodes were identified as blue areas after the colorant was injected. A Blakesley biopsy forceps (43 cm long) was introduced through the cannula and used to obtain all lymph node biopsy specimens. The biopsies were fixed in formalin and sent for histopathologyc confirmation of lymph node in the sample. After the procedure, the steers were humanely sacrificed and a necropsy performed. The results indicate that the technique is feasible and encourage future work in this area.

### 50. Application of a Novel FT-NIR Spectroscopy and a Partial Least Square Procedure to Predict the Authenticity of Extra Virgin Olive Oil

*Authors: Mossoba, Magdi, FDA/CFSAN, Karunathilaka, Sanjeewa, FDA/CFSAN*

Plain Language Synopsis: There is an urgent need for authoritative testing standards and rigorous analytical methods to determine olive oil quality and purity. To detect economically motivated adulteration of olive oil and address food safety vulnerabilities, we applied a novel FT-NIR and PLS1 methodology to rapidly screen for authenticity retail products labeled EVOO.

Abstract:
Economically motivated adulteration (EMA) of extra virgin olive oils (EVOO) has been a worldwide problem and a concern for government regulators for a long time. The United States (US) Food and Drug Administration (FDA) is mandated to protect

the US public against intentional adulteration of foods and has jurisdiction over deceptive label declarations. To detect EMA of olive oil and address food safety vulnerabilities, we used a novel and rapid screening methodology to authenticate EVOO. For the first time, a recently developed FT-NIR spectroscopic methodology in conjunction with partial least squares (PLS1) analysis was applied to commercial products labeled EVOO to rapidly predict whether they are authentic, potentially mixed with refined olive oil (RO) or other vegetable oil(s), or are of lower quality. Of the 88 commercial products labeled EVOO that were assessed according to published specified ranges, 33 (37.5%) satisfied the three published FT-NIR requirements identified for authentic EVOO products which included the purity test. This test was based on limits established for the contents of three potential adulterants, oils high in linoleic acid (OH-LA), oils high in oleic acid (OH-OA), palm olein (PO), and/or refined olive oil (RO). The remaining 55 samples (62.5%) did not meet one or more of the criteria established for authentic EVOO. The breakdown of the 55 products was EVOO potentially mixed with OH-LA (25.5%), OH-OA (10.9%), PO (5.4%), RO (25.5%), or a combination of any of these four (32.7%). If assessments had been based strictly on whether the fatty acid composition was within the established ranges set by the International Olive Council (IOC), less than 10% would have been identified as non-EVOO. These findings are significant not only because they were consistent with previously published data based on the results of two sensory panels that were accredited by IOC, but more importantly, because each measurement/analysis was accomplished in less than 5 minutes.

### 51. Generation and characterization of interferon-lambda 1-resistant H1N1 influenza A viruses

*Authors: Ilyushina, Natalia, FDA/CDER; Sheikh, Faruk, FDA/CDER; Bovin, Nicolai, RAS; Donnelly, Raymond, FDA/CDER*

Plain Language Synopsis: In light of the critical antiviral action of interferons (IFNs) and their potential use as anti-influenza agents, it is important to understand whether resistance to these host proteins can develop. We identified a single mutation in the neuraminidase protein that markedly affects influenza viral sensitivity to IFN-lambda 1.

Abstract:
Influenza A viruses pose a constant potential threat to human health. In view of the innate antiviral activity of interferons (IFNs) and their potential use as anti-influenza agents, it is important to know whether viral resistance to these antiviral proteins can arise. To examine the likelihood and mechanism of emergence of IFN-λ1-resistant H1N1 variants, we serially passaged the A/California/04/09 (H1N1) strain in a human lung epithelial cell line (Calu-3) in the presence of increasing concentrations of recombinant IFN-λ1 protein. To monitor changes associated with adaptation of this virus to growth in Calu-3 cells, we also passaged the wild-type virus in the absence of IFN-λ1. Under IFN-λ1 selective pressure, the parental virus developed two neuraminidase (NA) mutations, S79L and K331N, which significantly reduced NA enzyme activity (1.4-fold) and sensitivity to IFN-λ1 (>20-fold), respectively. These changes were not associated with a reduction in viral replication levels. Mutants carrying either K331N alone or S79L and K331N together induced much lower expression of the IFN genes (IFNB1, IFNL1 and IFNL2/3) and weaker activation of the IFN-responsive transcription factor, STAT1. A separate mutation (V14I) in the polymerase acidic (PA) protein, which occurred without selective pressure, was associated with increased transcription activity of the viral polymerase complex (1.8-fold). Our findings demonstrate that the IFN-λ1-induced K331N mutation in the NA protein markedly inhibits induction of IFN gene expression by human airway epithelial cells. This adaptive mutation provides a novel mechanism by which influenza viruses can develop increased resistance to the antiviral activity of type III interferons.

### 52. TACI-deficient macrophages alleviate metabolic disease symptoms in obese mice

*Authors: Liu, Lunhua, FDA/OVRR; Allman, Windy, FDA/OVRR; Coleman Adam, FDA/OVRR; Siddiqui Shafiuddin, FDA/OVRR; Mustafa, Akkoyunlu, FDA/OVRR*

Plain Language Synopsis: TACI defeciency mice were protected against high fat diet-induced obesity and type 2 diabetes. These mice exhibit less weight gain, decreased body fat, enhanced glucose tolerance and insulin sensitivity, and less inflammation in adipose tissue than that of WT controls.Thus TACI could be the therapeutic target to improve symptoms associated with metabolic disease.

Abstract:
TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) is one of receptors for the TNF superfamily cytokines BAFF (B cell activating factor) and APRIL (A Proliferation Inducing Ligand). In this study, we investigated the role of TACI in high fat diet induced obesity and metabolic disease. Compared to WT mice, TACI KO mice exhibited less weight gain, decreased body fat, enhanced glucose tolerance and insulin sensitivity. Improved metabolic changes in TACI KO mice were accompanied by significantly less accumulation of CD8+ T cells and F4/80+CD11c+ inflammatory macrophages in visceral fat pad. In addition, TACI KO mouse adipose tissue contained elevated numbers of ILC2, eosinophils and M2 macrophages. Underscoring the protective phenotype of M2-skewed macrophages, the transfer of TACI KO macrophages into obese WT mouse significantly improved insulin sensitivity and glucose tolerance in WT mouse. Interestingly, adoptively transferred TACI KO macrophages into WT mice also increased numbers of the host M2 macrophages and eosinophils in the adipose tissue. We identified IL-10 secreted by TACI KO macrophages was majorly responsible for the improved insulin sensitivity because not only TACI KO macrophage culture media contained high levels of IL-10 but also an IL-10 blocking antibody abolished the enhancement of insulin signaling provided by TACI KO macrophage culture media. These findings indicate that macrophage TACI mediated inflammatory signals promote obesity induced metabolic changes. Consequently, TACI blocking reagents may offer therapeutic benefits to obese patients suffering from metabolic complications.

## 53. TACI deficiency delays the onset of systemic lupus erythematosus symptoms and improves the survival of MRL/Lpr mouse

*Authors: Liu, Lunhua, FDA/OVRR; Windy Allman, FDA/OVRR; Adam Coleman, FDA/OVRR; Takeda, Kazuto, FDA/OVRR; Mustafa Akkoyunlu, FDA/OVRR*

Plain Language Synopsis: Loss of TACI fully leading to a delayed onset of lupus nephritis and extended lifescan of lupus mice

Abstract:
Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and immune-complex deposition in organs such as skin and kidney. Ablation of BAFF with anti-BAFF antibodies alleviates SLE symptoms but the receptor responsible for the generation of pathogenic antibodies has been controversial. Since TACI is critical in the development of antibody secreting plasma cells, we evaluated the contribution of TACI in the formation of autoantibodies and the development of SLE in TACI deficient "lupus mouse", generated by backcrossing the lupus prone Mrl/Lpr mouse with TACI-/- mouse. We found that TACI deficiency (Lpr-TACI-/-) increased the survival of lupus mouse as compared to the littermates, LPR-TACI +/+ and LPR-TACI +/- mice. The survival of Lpr-TACI-/- mice correlated with a delay in the development of anti-dsDNA and anti-SAM/RNP antibody titers. The prolonged survival of LPR-TACI -/- mice is likely a result of protection from kidney damage since proteinuria levels and IgG accumulation in the renal glomeruli were significantly less in LPR-TACI -/- mice as compared to the age-matched control mice with disease. Coinciding with the preserved kidney function, four month old LPR-TACI -/- mice kidney macrophages manifested M2-skewed phenotype, while macrophages harvested from kidneys of the age-matched control littermates were M1-skewed. Further supporting the protective role of M2-skewed macrophages, adoptively transferred LPR-TACI-/- macrophages improved the proteinuria and glomerulonephritis in control animals with kidney pathology. Collectively, LPR-TACI -/- mice experiments suggest that blocking TACI function may be a novel approach for

the treatment of SLE disease. (This work was support by FDA Office of Women's Health)

### 54. CDER Engagement with Public Private Partnerships Drives Innovation

*Authors: Maxfield, Kimberly FDA/CDER/OTS/ OCP; Parekh, Ameeta FDA/CDER/ OTS*

Plain Language Synopsis: CDER has collaborated with public-private partnerships for over a decade to streamline drug development and have produced deliverables that are impacting all stages of drug development from preclinical and clinical to post market safety and product quality.

Abstract:
In 2004, the Food and Drug Administration (FDA) published a prospective vision called the Critical Path Initiative (CPI) that aimed to accelerate the stagnating product development pipeline. Following this initial launch, FDA convened internal and external stakeholders to help identify specific research priorities that could facilitate the realization of the CPI vision, which resulted in the 2006 Critical Path Opportunities List (CPOL). Since then, the FDA's Center for Drug Evaluation and Research (CDER) has directed considerable efforts to achieve the drug development goals outlined by the CPI and CPOL through mechanisms such as funding scientific research and engaging in external partnerships. While funded research addresses specific questions that arise during the scientific assessment of FDA regulated products, collaborations, such as Public Private Partnerships (PPPs), can answer broader regulatory questions that require a coalition of resources, expertise, and partnering. Since 2006, CDER/ PPP collaborations have launched multiple initiatives and projects that have impacted FDA regulatory processes and drug development. Given these successes, CDER recently established a formal process for CDER staff to engage in PPPs to avoid the potential conflicts of interest or appearance of undue influence. Through PPP collaborations, CDER is striving to leverage existing knowledge, develop platforms for data sharing, and overcome the challenges of intellectual property that may limit such efforts. Together, CDER and external stakeholders are learning to address both specific and broad overarching issues to yield meaningful results to benefit public health.

## 1. Characterization of Interactive Sites of the Blood Coagulation Factor VIII and the Low-Density Lipoprotein Receptor using Macromolecular Docking Prediction Algorithms


*Authors: Fochtman, Brian, FDA/CBER; Shestopal, Svetlana, FDA/CBER; Simonyan, Vahan, FDA/CBER; Sarafanov, Andrey, FDA/CBER*


Plain Language Synopsis: FVIII products are used to treat its deficiency (Hemophilia A). Understanding mechanisms of FVIII clearance would facilitate generation and regulation of longer-acting therapeutic FVIII. Using the FDA CBER HIVE platform, we built up a model of FVIII interaction with its clearance receptor LDLR and identified critical elements of this interaction.


Abstract:
Background: The deficiency in blood coagulation factor VIII (FVIII) results in excessive bleeding known as Hemophilia A. The disease treatment requires frequent infusions of FVIII due to relatively short circulatory half-life of FVIII (12 h). Thus, the manufacturers of recombinant FVIII products modify the FVIII molecule to prolong its circulatory half-life. Understanding mechanisms of plasma clearance of FVIII will facilitate generation and regulation of such longer-acting FVIII products. The major receptors responsible for the clearance of FVIII are endocytic liver receptors the low-density lipoprotein receptor (LDLR) and the LDLR-related protein 1. The receptors' sites for binding their ligands are formed by complement-type repeats (CRs), which are grouped in clusters. Upon the binding, a particular CR domain interacts with a surface-exposed lysine of the ligand. Previously, we demonstrated that four adjacent CRs (2-5) of LDLR form an extended site for binding FVIII, while the interactive site on FVIII involves an extended area on its light chain (Kurasawa et al, 2013, J. Biol. Chem. 288:22033-41). In present work, we used computer modelling to predict details of this interaction.

Aims: To determine surface-exposed lysines of FVIII potentially involved in its binding to LDLR and assess orientation of both molecules during the interaction.

Methods: Based on available atomic coordinates from the crystal structures of FVIII and LDLR and using the FDA CBER high-performance integrated virtual environment (HIVE) capabilities (DOCK2 program), each of the CRs 2-5 of LDLR was docked individually to FVIII. Promising poses, most similar to the canonical CR domain interaction, were filtered based on the distance of a FVIII lysine to the CR-coordinated Ca2+ ion and the ability of the lysine to interact with a conserved aromatic residue of the CR. Finally, by modeling the linker regions between the CR domains orientations, the individual docking results were combined.

Results: A number of surface-exposed lysines of FVIII were determined as candidates for the interaction and aligned with particular CR domains of LDLR.

Conclusion: A model of the FVIII-LDLR interaction was built up. In particular, a number of lysines on the FVIII LCh were identified as possibly contributing to the binding.


## 2. Quantitative Virtual Reality Test Platform for Sensate Prosthetic Hands


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Plain Language Synopsis: New advanced prostheses are controlled with muscle signals and give a sense of touch through nerve stimulation. Virtual reality software is often used during development of these prostheses, and may be useful for early performance assessment. We describe tests that were created in a virtual environment for assessing these prostheses.


Abstract:
Advanced prostheses are costly, and sensorized

versions are still under development. In order to allow research teams to prototype and test advanced prosthetic control and feedback systems without the finalized robotic limb, a virtual version can be created. MuJoCo (Multi-Joint dynamics with Contact) HAPTIX (Hand Proprioception and Touch Interfaces) is a virtual reality (VR) simulator, in which a virtual prosthesis can be modeled, programmed, and calibrated in the same way that the physical prosthesis would be. MuJoCo HAPTIX programmatically reports tactile and proprioceptive feedback detected by modeled sensors.

Here we report on a series of four tests that can be used to assess user control of the prosthesis and perception of sensory feedback from the prosthesis. The first three tasks examine movement quality and control, quality of sensory feedback, and the combined use of movement control and sensory feedback. The fourth task is a virtual implementation of the Activities Measure for Upper Limb Amputees (AM-ULA), a functional outcome assessment.

Individuals with an amputation completed the four tests, using implanted EMG electrodes to control the virtual prosthesis and receiving stimulation from peripheral nerve implants to provide sensory feedback. Benchmark performance was also measured in able-bodied individuals using a data glove and OptiTrack motion capture hardware to control the virtual prosthesis. Further testing can help validate the use of VR software for early stage device performance assessment.

### 3. Modeling the Impact of Norovirus Transmission in Retail Food Establishments - Results and Lessons Learned from the Risk Assessment

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Plain Language Synopsis: The objective of this risk assessment was to evaluate the dynamics of norovirus transmission from infected food employees during food preparation, and the efficacy of control measures as a function of the degree of food employee compliance with these control measures.

Abstract:
Norovirus is the leading cause of foodborne illnesses globally and within the United States. Most of these illnesses can be traced back to food contaminated from feces or vomit from symptomatic and asymptomatic infected food employees in the retail food establishment (or restaurant) setting. Food in these settings is most frequently contaminated via contact with soiled hands of infected food employees. This poster presents the results of a quantitative risk assessment developed to evaluate the dynamics of norovirus transmission from infected food employees during food preparation, and the efficacy of control measures as a function of the degree of food employee compliance with these control measures. The control measures evaluated include exclusion of ill food employees from retail food establishment, handwashing, facility sanitation frequency, touchless faucets in restrooms, and "no bare hand contact" with ready-to-eat foods. Many of these control measures are contained within the FDA Food Code. The FDA Food Code is a model code that represents FDA's best advice for a uniform system of regulation to ensure that food at retail is safe and properly protected.

### 4. FDA Benchmark Flow Models To Support Computational Fluid Dynamics Techniques In The Evaluation of Medical Devices

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*Affiliation: 1Food & Drug Administration, Silver Spring, MD, 2Rochester Institute of Technology, Rochester, NY, 3RWTH Aachen University, Aachen, Germany, 4Pennsylvania State University, University Park, PA*

Plain Language Synopsis: This Critical Path Initiative study evaluates the credibility of computational modeling used for simulating blood flow in medical devices. Round-robin

studies were performed on two benchmark flow geometries by modelers from 20 independent groups. This poster presents the variabilities in the simulation results when compared with experimental flow data.

Abstract: Computational fluid dynamics (CFD) is increasingly being used to develop and evaluate blood-contacting medical devices such as ventricular assist pumps and heart valves. CFD models are typically used to evaluate if the medical devices can cause flow-induced red blood cell damage (hemolysis) or platelet activation (and thrombosis), both of which are major safety concerns for blood-contacting medical devices. However, the lack of reliable standardized methods for validating CFD simulations and blood damage predictions limits its applicability in the safety evaluation of new products.

As part of an FDA initiative, two benchmark models representative of typical medical device flow geometries (Study #1: nozzle, Study #2: blood pump) were developed and tested over a range of flow conditions in multiple labs to provide experimental fluid dynamics and hemolysis data to support CFD validation. In addition, for each model study, computational simulations were performed by over 20 independent groups from around the world to assess current CFD techniques and limitations.

Marked discrepancies between the CFD results and the measured velocities in the nozzle study motivated the development of "best practice" CFD guidelines and an FDA Guidance Document on what factors to consider when reporting computational studies of devices. In Study #2, CFD simulations of the centrifugal blood pump model were conducted at six flow rate and pump speed conditions (from 2.5–7 L/min and 2500-3500 rpm). Approximately 60% of the computational predictions of pressure head fell within the one standard deviation bounds of the experimentally measured pressures when using porcine blood (during hemolysis testing) and a blood-analog sodium iodide-glycerol solution (in particle image velocimetry experiments).

This poster provides an overview of the CFD modeling challenges and a summary of the comparative results to date for the blood pump model. The goal of this project is to assess and improve CFD techniques for modeling flow and blood damage in blood-contacting devices by providing two publicly available benchmark flow models and data sets that can be used to collaboratively develop guidelines on proper validation and use of CFD simulations in the assessment of medical devices.

## 5. Bioequivalence Test for Sparse Design

*Authors: Li[1], Huaixiang (Helen); Sun[1], Guoying; Schuirmann[1], Donald; Makhlouf[1], Fairouz ; Grosser[1] , Stella; Martinez[2], Marilyn.*

*[1]FDA/CDER/OTS/OB/DB8,*
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Plain Language Synopsis: We extend the sparse sample design for pharmacokinetic bioequivalence studies, where a single measurement is made per subject, to the case where multiple samples but not a complete time profile is available.

Abstract:
In evaluating bioequivalence (BE), a sparse design may be necessary for the pharmacokinetic (PK) bioequivalence study. In such studies, the concentration sample for a given subject is collected at only a single time point rather than at all design time points (as is the case in traditional PK studies). When employing a sparse sample study design, a composite concentration vs time profile is generated based upon a single concentration averaged over subjects at each time point. Accordingly, only a single value for AUC (Area under the Curve) and Cmax at time t can be estimated from that composite profile. Hence, the traditional method for estimating confidence limits about AUC and Cmax values cannot be utilized and alternative approaches are needed.

To this end, the estimation of the standard deviation for BE analysis has been accomplished via the use of a bootstrapping technique to calculate the 90% confidence interval of AUC and Cmax ratio (Test/Reference). Alternatively, we describe a parametric (Non-bootstrap) method that could provide stable and reproducible ratio and 90%

CI for AUC BE test.

We also extend the sparse design to more complex situations, in particular the case where the multiple samples (more than one) per each subject but not a complete profile are available.

**6. Target-Adverse Event profiles for enhanced pharmacovigilance: a pilot study with six new molecular entities**

*Authors: Schotland, Peter, FDA/CDER; Racz, Rebecca, FDA/CDER; Jackson, David, Molecular Health, GmbH; Burkhart, Keith, FDA/CDER*

Plain Language Synopsis: A method for predicting drug adverse events to assist with post-market safety surveillance

Abstract:
Background: Clinical trials often fail to detect rare adverse events (AE). Predictive methods may assist in post-market pharmacovigilance. Target adverse-event (TAE) analysis aggregates adverse event reports from drugs that share molecular targets with a drug of interest (DOI). A multi-label classifier was constructed to assess the ability of TAE to predict potential AEs.

Methods: Pilot study drugs: Six DOIs with at least four years post-market experience were chosen to represent a variety of therapeutic areas: certolizumab, desvenlafaxine, etravirine, liraglutide, pazopanib, and rivoroxaban. Generation of TAE profiles: TAE profiles were generated by aggregating AEs from FAERS reports and product labels using data available prior to marketing approval. TAE profiles from FAERS reports were created using the MH-EFFECTTM software from Molecular Health, Inc. TAE profiles from labels were created using manual curation of FDA product labels. For FAERS generated TAEs, the EFFECTTM software computed a disproportionality score (PRR) and aggregate case count (N) for each AE in the profile. For label generated TAEs, we computed the proportion of labels containing an AE for each AE in the profile (labelscore).

Classification method: We constructed a multi-label classifier such that TAEs (features) were used to predict the product label (output). Predictions were limited to a list of 43 Designated Medical Events (DME). A genetic algorithm was used to choose N, PRR, and labelscore to maximize predictive performance assessed by precision, recall, specificity, and F. Label changes: Original product labels were compared to current labels and 22 label changes were identified. Label changes were compared to classifier predictions made at maximum F. Qualitative error analysis: False positives were investigated as potential real AEs by manual review of contemporary FAERS reports and product labels.

Results: Classifier performance: Predictions were made using TAEs from FAERS data alone and FAERS+label data. For FAERS, F = 0.63; for FAERS+label, F = 0.75. ROC analysis: AUC » 0.68 for both methods. Label changes: 18/22 product label changes (82%) were predicted correctly. Error analysis: Manual review of 62 false positives indicates 36 (58%) merit further review as potential AEs.

Conclusions: TAE analysis shows promise as a predictive method for enhanced pharmacovigilance.

**7. Investigation on the impact of osmotically active excipients on the in vivo performance of orally administered drugs by physiologically based pharmacokinetic (PBPK) modeling to assist in biopharmaceutics assessment.**

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Plain Language Synopsis: A mathematical model was developed to study how certain excipients may impact drug absorption from gut. The preliminary research efforts indicate the potential utility of in silico modeling to study excipient effect on drug absorption, and guide clinically relevant regulatory decision-making.

Abstract:
Excipients can affect bioavailability of the drug product through different mechanisms, one

of which is modification of gastrointestinal transit time (e.g., sugar alcohols including mannitol and sorbitol). In accordance to the Code of Federal Regulations (21CFR320.22) and relevant guidances, the excipient effect is one of the considerations for granting biowaivers [waiver of conducting in vivo bioavailability (BA)/bioequivalence (BE) study]. The preliminary investigation was initiated to study the potential utility of physiologically based pharmacokinetic (PBPK) or mechanistic absorption model for regulatory review of drug product bridging information on quantitative changes in excipients, clinically relevant regulatory decision-making, and life-cycle management of the drug product. Two drug products (A-Solution for sorbitol effect and B-Tablet for mannitol effect) were selected for preliminary investigation belonging to biopharmaceutics classification system (BCS) 1 (high solubility and high permeability) and 3 (high solubility, and low permeability), respectively. The mechanistic modeling was conducted using GastroplusTM (Version-9.0 software). A model was developed with inputs of relevant physicochemical, formulation, and pharmacokinetic parameters of selected drug. The model was appropriately validated and further applied for formulations containing varying amounts of excipient. For drug product A, a significant high percent prediction error (%PE) for the drug exposure parameters [peak plasma concentration (Cmax-19.2%) and area under the curve (AUC-22.14%)] were obtained when the validated model was applied for the oral solution with 3210 mg of sorbitol compared to the formulation with 262.5mg of sorbitol (Cmax-0.1%; AUC-11.1%). The results indicated the potential impact of sorbitol on the oral absorption of BCS class 1 drug and further investigation will be continued for elucidating and confirming the mechanism (e.g., transit time) of the above effect. Efforts will also be made to study the effect of sorbitol for BCS class 3 drugs. For drug product B, a significant difference in %PE for Cmax and AUC between two formulations was not observed potentially due to the small difference, in mannitol amount between the two formulations (130.9mg - %PE: Cmax-6.1%; AUC-10.6% vs 165.4mg - %PE: Cmax-11.3%; AUC-1.12%) and further investigation using formulations with wider difference in mannitol amount will be sought to understand its impact on the drug product performance.

## 8. Modeling and predictions of mutagenic potential of primary and secondary amines

*Authors: Richard Beger[1], Svetoslav Slavov[1], Iva Slavova[1], Chris Barber[2], Jonathan Vessey[2], Mukesh Patel[2]*

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Plain Language Synopsis: 3D-SDAR modeling technique was applied to model mutagenicity of primary and secondary amine mutagenicity. The models were used to decode the structure-activity relationships and identify structural alerts associated with mutagenicity.

Abstract:
Impurities are present in all drug formulations regardless of purification strategies employed. Some of these impurities have the potential to induce permanent transmissible changes in DNA. The Ames assay using Salmonella typhimurium is an early screen for mutagenicity, but requires both time and a large amount of the drug (~1g). Hence, under ICH M7 accurate computational prediction of the mutagenic potential of drug impurities is an alternative to a full GLP Ames assay.

Among all impurities, the aromatic amines are a concern to the FDA and sponsors due to their powerful carcinogenic and mutagenic potential. Datasets of 579 primary and 437 secondary amines provided by Lhasa Limited were used to model the mutagenic potential of aromatic amines. A 3D-SDAR methodology employing a bagging-like PLS approach to process binned molecular fingerprints constructed from the chemical shifts of 13C and 15N atoms augmented with their corresponding atom-to-atom distances was used. To improve the models' discrimination power, the original categorical class assignments of 0 and 1 (encoding for negative and positive consensus Ames assay outcomes) were converted to continuous values.

After curation the number of primary amines was reduced to 554 chemicals, whereas the secondary amines were reduced to 419. Both sets were split into training (3/4 of the total number of compounds) and external test (1/4 of the total) subsets. A tessellation using 4 ppm x 4 ppm x 0.5 Å bins for the C-C region of the SDAR space and 4 ppm x 10 ppm x 0.5 Å bins for the C-N region was found to result in a model whose performance characteristics for the primary amines internal test set were as follows: accuracy of 0.79, sensitivity of 0.82 and specificity of 0.75. The accuracy, sensitivity and specificity for the secondary amines test set were 0.84, 0.79 and 0.90, respectively. The primary and secondary amine mutagenicity models were evaluated by predictions of true external sets. This model enables in silico predictions that could be almost instantaneous, thus saving time and expensive lab testing.

**9. Local Specific Absorption Rate (SAR) in computational models of blood vessel compared to ASTM phantom**

*Authors: Fujimoto, Kyoko, FDA/CDRH; Angelone, Leonardo, FDA/CDRH; Lucano, Elena, Sapienza University of Rome; Serano, Peter, FDA/CDRH; Rajan, Sunder, FDA/CDRH; Iacono, Maria, FDA/CDRH*

Plain Language Synopsis: Heating of tissue during Magnetic Resonance Imaging (MRI) depends on the local energy absorption. A numerical human anatomical model was used to evaluate the energy absorbed by the body during 1.5T MRI. Our simulation results showed higher energy absorption in some anatomical regions compared to that of a gel-based phantom.

Abstract:
Introduction: A stent implanted in a patient can cause heating of surrounding tissues during Magnetic Resonance Imaging (MRI). Radio-frequency (RF) heating due to stents is typically measured as indicated in the ASTM-F2182 standard. The heating effect, however, depends on the local energy exposure near the device, measured as specific absorption rate (SAR), which may be high in specific anatomical regions of the human body. The local SAR in arteries or veins has not been evaluated yet due to the coarse spatial resolution (>2mm) currently used in computational modeling. We studied this quantity using both the ASTM phantom and a 1mm isotropic human model with four different imaging landmarks. Results showed that in-vivo exposure in specific anatomical locations is higher than that in the ASTM phantom.

Methods: Simulations were performed with the Sim4Life platform (Zurich Med Tech, Switzerland) based on the finite-difference time-domain method. The AustinMan model with 1×1×1mm spatial resolution and 64 anatomical structures was utilized. Five simulations were performed at 64MHz with a birdcage body coil: one simulation with the ASTM phantom and four simulations with the AustinMan at brain, heart, hip, and knee imaging landmarks. SAR maps were then averaged over 0.1g and 1g of the surrounding tissues. All the simulations were normalized to obtain an averaged whole-body SAR of 2W/kg.

Results: The maximum 1g SAR value in the ASTM phantom was 8.6W/kg. The femoral vessels at the hip bone landmark showed 3-fold higher local 0.1g SAR (23W/kg) compared to the ASTM. Similarly, the popliteal vessels at the knee landmark resulted in 5-fold higher local 0.1g SAR (40W/kg) compared to the ASTM phantom.

Conclusion: At specific imaging landmarks and anatomical locations, the value of SAR calculated using human body model can be higher than the values calculated with the ASTM phantom. Results suggested that a re-calibration of the ASTM phantom measurements may be needed before extrapolating the results to the clinical scenario.

**10. Developing a mechanistic absorption model to predict the pharmacokinetics of immediate-release weak base drugs with a long Tmax by incorporating lysosomal trapping**

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*CDER; Zhang, Lei, OCP/CDER*

Plain Language Synopsis: Mechanistic absorption models were developed using commercially available software (Gastroplus) to predict the long Tmax and overall PK profiles of two immediate-release drugs: drug X and drug Y by incorporating lysosomal trapping in enterocytes. The model was further verified using PK data for different doses, particle sizes, or administration conditions (fed/fasting).

Abstract:
Objective: The objective of this study is 1) to develop and verify mechanistic absorption models to predict the pharmacokinetics (PK) of two immediate-release weak base drug (WBD) products (Drug X and Drug Y) with a long time to reach maximum plasma concentration (Tmax) (e.g., Tmax>6 h.), and 2) to understand what are the key input parameters for these models.

Method: For each drug product, the absorption model was initially constructed using measured drug physicochemical parameters in the drug product (e.g., particle size, solubility, permeability) while other drug parameters (e.g., precipitation time, FuEn (unbound fraction in enterocytes)) and physiological parameters (e.g., stomach pH and gastric transit time) were kept as default or as predicted values using the Gastroplus (version 9.0) built-in functions. Literature has shown that lysosome could extensively sequester lipophilic WBDs. Following sensitivity analyses, FuEn was optimized to reflect potential lysosomal trapping of the WBDs. The optimized model was then used to predict the drug PK for different doses, different drug particle sizes in the drug product or administration conditions with regard to food to evaluate the model performance. Predictions were considered acceptable if the predicted maximum concentration (Cmax), Tmax and area under the concentration-time curve (AUCinf) were within ±1.5 folds of the observed data.

Results: Absorption models without the lysosomal trapping component optimization could not adequately capture the Cmax and Tmax following single oral dosing of Drug X and Drug Y, although with a good prediction of AUCinf (Figure 1). Sensitivity analyses revealed that varying stomach transit time, small intestinal transit time, precipitation time or permeability did not significantly impact the prediction of the absorption phase (e.g., Cmax) and Tmax. However, the long Tmax (8 hours and 6 hours) of Drug X and Drug Y were well captured after considering lysosomal trapping in the model, by optimizing the FuEn to 1% and 4% from 100% (default software parameter) for Drug X and Drug Y, respectively (Figure 1). Furthermore, the optimized model achieved a good prediction of the drug PK for different doses, particle sizes of drug product or administration conditions with regard to food with the predicted Cmax, Tmax and AUCinf within ±1.5 folds of the observed data.

Conclusion: Our data suggested that incorporating lysosome trapping via optimizing parameter FuEn into the mechanistic absorption model was critical for a better PK profile prediction for two immediate-release WBDs that exhibit a long Tmax.

## 11. Optimizing Data Density for Multivariate Calibration Models

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Plain Language Synopsis: This work aims to minimize the cost of data generation without sacrificing model performance.

Abstract:
Multivariate data analysis has been shown to be an integral part of the biopharmaceutical industry for the real-time monitoring of cell culture processes. These techniques require a sufficient number of data points to establish an accurate and robust model. However, the optimum amount of data to collect for model training and validation is not well defined, and using an incorrect amount may result in poor model performance. Under sampling may result in insufficient data, which will

yield inaccurate model predictions; while over sampling may result in increased data management requirements, which can impose unnecessary regulatory and financial burdens. A CHO DG44 cell culture was grown in a lab scale bioreactor system that was equipped with a BioPAT Trace and Spectro, which allowed for extracellular glucose and lactate concentrations, as well as near infrared and visible light spectra, to be measured every two minutes. Additional datasets were generated by uniformly sampling the measured data at increasingly smaller intervals (every 16 minutes, 8 minutes, etc). While keeping the percentage of the data used for model training constant, multivariate calibration models were then built separately for each dataset to predict the extracellular component concentrations from the multimodal spectra. The relative performance of these models was then assessed by comparing the validation set model predictions with their corresponding measured values, and it is shown that as data density increases, model prediction error decreases and model reproducibility increases.

Disclaimers: This article reflects the views of the authors and should not be construed to represent official FDA's views or policies.

## 12. Evaluating performance of chemical fingerprinting methods and machine learning Algorithms for in silico prediction of Ames mutagenicity

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Plain Language Synopsis: This research focuses on building QSAR (Quantitative Structure-Activity Relationship) models for predicting Ames mutagenicity of food related chemicals. In particular, we build QSAR models using six different chemical fingerprinting methods and six machine learning algorithms available in the public domain and compare their predictive performance.

Abstract:
The Office of Food Additive Safety (OFAS) at U.S. FDA's Center for Food Safety and Applied Nutrition (CFSAN) is responsible for ensuring the safety of all food additives used in the United States. A database called Chemical Evaluation and Risk Estimation System (CERES) was created for reviewers at OFAS to serve as a knowledgebase of chemical, toxicological, and regulatory information on food additives, food contact substances, and GRAS ingredients. Over time, the CERES database was enhanced with data on other chemicals such as colors, cosmetic ingredients, and drugs along with cheminformatics tools such as similarity searching and toxicity prediction. One of our current research efforts at OFAS focuses on building in-house mutagenicity and carcinogenicity predictive models with high prediction accuracy for food related chemicals. In this research, we present an evaluation of different chemical fingerprinting methods and machine learning algorithms available in the public domain and compare their performance for in silico prediction of Ames mutagenicity using the Hansen benchmark dataset. We evaluate six fingerprinting methods, namely MACCS keys, RDKit, Circular, ToxPrint, PubChem, and Atom pairs fingerprints, and six machine learning algorithms, namely k-Nearest Neighbors (kNN), Decision Trees (DT), Random Forest (RF), Artificial Neural Networks (ANN), Support Vector Machines (SVM), and Naïve Bayes (NB). Using 5-fold cross-validation scheme, we calculate sensitivity, specificity, and concordance for all combinations of fingerprinting methods and machine learning algorithms and identify models that give high performance metrics. The predictive performance of these mutagenicity models is then evaluated against empirical data within CERES. In addition, the aforementioned tools are used to evaluate the chemical space of Hansen dataset and compare it to that of the food related chemicals within CERES.

## 13. Seamless Lesion Insertion in Digital Mammography: Methodology and Reader Study

*Authors: Pezeshk, Aria, FDA/CDRH/OSEL; Petrick, Nicholas, FDA/CDRH/OSEL; Sahiner, Berkman, FDA/CDRH/OSEL*

Plain Language Synopsis: Collection of large repositories of clinical images containing verified cancer locations is costly and time consuming. This poses a serious setback for training or validating computer algorithms. We have developed a method to supplement an existing mammography dataset by seamlessly inserting existing lesions into new locations or other mammograms.

Abstract:
Collection of large repositories of clinical images containing verified cancer locations is costly and time consuming due to difficulties associated with both the accumulation of data and establishment of the ground truth. This problem poses a significant challenge to the development of machine learning algorithms that require large amounts of data to properly train and avoid overfitting. In this project we expand the methods in our previous publications by making several modifications that significantly increase the speed of our insertion algorithms, thereby allowing them to be used for inserting lesions that are much larger in size. These algorithms have been incorporated into an image composition tool that we have made publicly available. This tool allows users to modify or supplement existing datasets by seamlessly inserting a real breast mass or micro-calcification cluster extracted from a source digital mammogram into a different location on another mammogram. We demonstrate examples of the performance of this tool on clinical cases taken from the University of South Florida Digital Database for Screening Mammography (DDSM). Finally, we report the results of a reader study evaluating the realism of inserted lesions compared to clinical lesions. Analysis of the radiologist scores in the study using receiver operating characteristic (ROC) methodology indicates that inserted lesions cannot be reliably distinguished from clinical lesions.

## 14. Consideration of Inhalational Studies for a Quantitative Oral Risk Assessment

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Plain Language Synopsis: The relevance of data from inhalation studies for a quantitative oral risk assessment should be investigated on a case-by-case basis. Data from inhalation studies can be supplemented or extrapolated for risk assessment after oral exposure depending on the toxicokinetic and toxicodynamic profile of a compound across exposure routes.

Abstract:
The occurrence of distal site tumors in rodent inhalation bioassays or in humans upon occupational inhalational exposure can be informative when assessing the potential carcinogenic risk of oral exposure to a test substance. Data from inhalation studies where treatment-related systemic tumors are observed are often used in quantitative risk assessment of oral exposure when data from oral toxicity studies are either unavailable or unsuitable for use. However, the toxicokinetic and toxicodynamic profiles of a compound may be dependent on the exposure route. Therefore, the relevance and significance of results from inhalation studies to quantitative oral risk assessment needs to be explored. The objective of this project was to develop a strategy for quantitative oral risk assessment using data from inhalation studies, using two classes of compounds: solvents (styrene) and metals (cobalt) as examples, by evaluating the toxicodynamic and toxicokinetic profiles of oral versus inhalation exposure routes across species and performing an internal exposure-based assessment. We determined that data from inhalational studies can be supplemented for a risk assessment of oral styrene exposure due to the observed similarities in carcinogenic end points and no significant differences in PK profiles across exposure routes. In contrast, inter-route extrapolation of inhalational data is required to perform a risk assessment of oral cobalt exposure due to a lack of oral

carcinogenicity data and significant differences in PK profiles across exposure routes. In conclusion, the use of inhalational studies for a quantitative risk assessment for oral exposures depends on several factors, including pharmacokinetics, and should be performed on a case-by-case basis.

**15. Investigation into the Detection of Reduced Leaflet Mobility in Surgical and Transcatheter Bioprosthetic Aortic Heart Valves**

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Plain Language Synopsis: Findings published in the New England Journal of Medicine reported that bioprosthetic heart valves may have reduced leaflet mobility (RLM) caused by thrombotic deposits situated on the valve leaflets. Our work investigates potential under-diagnosis of valvular dysfunction in the presence of RLM due to limitations of standard ultrasound imaging methods.

Abstract:
Study: Bioprosthetic heart valves, including those implanted surgically (surgical aortic valve replacement (SAVR)), and via a catheter (transcatheter aortic valve replacement (TAVR)), are life sustaining medical devices for tens of thousands of patients who require aortic valve replacement each year. Recent findings published in the New England Journal of Medicine, reported that up to 40% of bioprosthetic heart valves may have reduced leaflet mobility (RLM) caused by thrombotic deposits situated on the valve leaflets [1]. The study noted that some standard ultrasound imaging techniques that are typically used to assess valve hemodynamic function failed to detect irregularities in leaflet mobility. The short and long term effects of RLM on heart valve and cardiac function are not well understood. Our current work investigates the potential under-diagnosis of valvular dysfunction in the presence of RLM due to limitations of the standard ultrasound imaging methods.

Methods: To better understand and assess the potential patient risk, this work investigates whether the current ultrasound imaging methods are able to accurately detect RLM. The project includes (i) an in vitro evaluation of the ultrasound-based imaging methods clinically used for hemodynamic assessments of heart valve function. In particular, the method's theoretical assumptions as applied to RLM detection is assessed; and (ii) computational fluid structure interaction (FSI) modeling of blood flow through the valve, with and without RLM, to understand the effect of RLM on the heart and blood flow properties.

Preliminary Results: Our in vitro model system of the left ventricle mimicked the range of clinically relevant fluid pressure and flow conditions to evaluate ultrasound imaging methods. FSI simulations of nominal valve function have been performed and simulations are currently underway to investigate valvular dysfunction. Computational FSI modeling will complement the bench measurements of flow through the valve with additional flow details, which can help understand the source of flow disturbance that may contribute to misdiagnosis of valve function. An outline of the project and preliminary results from our investigations will be presented.

1. Makkar RR et.al. Possible Subclinical Leaflet Thrombosis in Bioprosthetic Aortic Valves. N Engl J Med 2015;373:2015-24.

**16. Applying PBPK modeling to drug product quality risk assessment (Risk Gauge Tool): Case study -- the impact of microsphere particle size on the in vivo performance of an extended release capsule formulation**

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Plain Language Synopsis: A validated PBPK model of a BCS Class III drug was applied for quality risk assessment of an extended-release (ER) formulation (microsphere-in-capsule). The impact of microsphere particle size on in vivo performance was assessed and the "safe-space" of the size range was defined by the model.

Abstract:
Background: Physiologically based pharmacokinetic (PBPK) modeling could help add clinical relevance to drug product quality risk assessment. This study explored to apply an in-house validated PBPK model of Drug X, a BCS Class III drug, to its extended-release (ER) formulation (microsphere-in-capsule), for the purpose of assessing and mitigating the risk of microsphere particle size on the target in vivo performance.

Method: The z-factor dissolution model tool in GastroPlus™ was used to fit the dissolution profile of the biobatch for Drug X ER capsule, and the in vivo performance was evaluated using the developed PBPK model with two supporting clinical studies. Additionally, the in vivo performance of formulation A and B, with the same microsphere composition but differed in microsphere particle size, was also investigated by this model. Moreover, virtual bioequivalence (BE) was performed to define the lower and upper bound of z-factor using the biobatch as reference. The relationship between microsphere size and z-factor was delineated via regression analysis, which was extrapolated to calculate microsphere particle size corresponding to the defined BE range of z-factor. After the "safe-space" was defined, the updated risk assessment on the impact of critical quality attributes (CQA) such as dissolution was performed.

Result: The PBPK model successfully predicted the in vivo performance of the ER capsules of Drug X with the prediction error within ±20%. For formulation A and B, the ascending colon absorption scaling factor was optimized due to the formulation composition differences from the biobatch. The virtual BE analysis indicated that the microsphere size range could be defined as 275-431um to maintain bioequivalence to the biobatch. Thus, the risk of CQAs (dissolution) was reduced when the microsphere particle size is within the defined range as indicated by the model.

Conclusion: The current study implemented PBPK modeling & simulation as a risk assessment tool (namely RiskGauge Tool) to provide a quantitative basis for evaluating the likelihood of occurrence and detectability of the risk of failure in target in vivo performance. The result indicates that the risk could be mitigated through setting clinically relevant specification of microsphere particle size of Drug X ER capsules.

### 17. Development of a mechanistic PBPK absorption model to predict the in vivo performance of a BCS III drug for risk assessment

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Plain Language Synopsis: A PBPK model was developed for a BCS III drug and it was validated across twelve clinical studies with prediction error within 20%. Preliminary results indicate that it works well with both immediate-release and extended-release formulations. This model could serve as an important tool for future risk assessment.

Abstract:
Background: Physiologically based pharmacokinetic (PBPK) models have increasingly been employed in drug development and regulatory review. This study aims to develop a universal PBPK model for Drug X (BCS Class III) for the purpose of: a) predicting the in vivo performance of different drug formulations b) in future, to perform risk assessment on the effect of changes of critical quality attributes on drug exposure.

Method: The PBPK model was constructed based on collected physicochemical properties

of Drug X; while other parameters were kept as default or as predicted values using built-in functions of PBPK software, Gastroplus™. Parameters for drug distribution and clearance were derived by simultaneous fitting PK data from one intravenous (IV) and one oral (PO) studies using PK Plus. The model was further optimized by incorporating ADME (absorption, distribution, metabolism and elimination) properties (e.g. Km and Vmax value of CYP enzymes, renal clearance, et al). The final model was internally and externally verified with data from literature research paper, new drug applications (NDAs) and abbreviated new drug applications (ANDAs). Furthermore, the model was applied to extended-release formulation of Drug X by incorporating formulation information using dissolution model.

Result: The model was initially established and validated through six clinical studies from literature and NDA submissions (Including IV bolus, immediate release tablet and solution) with different strengths. The model works well across different studies, with the prediction error generally within 20%. Only one parameter (CYP enzyme Vmax Scaling Factor) was optimized, which minimized the uncertainty for this model. Moreover, outlier data from one clinical study was successfully identified by this model and cross-validation was performed with data from six generic drugs, which further verified this PBPK model for its validity with high confidence. Preliminary simulation by incorporating dissolution model indicates that this model could be successfully applied to extended-release formulation of Drug X.

Conclusion: A solid mechanistic PBPK model for Drug X has been developed by incorporating drug dependent, formulation dependent and physiology dependent parameters. This model could serve as an important tool for performing risk assessment on the effect of variations of critical quality attributes on drug exposure.

**18. Evaluation of Proton Pump Inhibitor (PPI) Effect for two Formulation of Paliperidone by Utilizing a Multi-pH Dissolution Model and Physiologically Based Pharmacokinetic (PBPK) Absorption Modeling and Simulation Approach**

*Authors: Sharan, Satish, FDA/CDER; Zhang, Xinyuan, FDA/CDER*

Plain Language Synopsis: In this study, we have compared the exposure of hydrophilic matrix tablets and osmotic release formulations to changes in gastrointestinal (GI) transit time and proton pump inhibitor (PPI) effect using a physiologically based pharmacokinetic (PBPK) modeling and simulation approach for risk assessment of pending ANDA applications.

Abstract:
Purpose: Paliperidone is currently approved for treatment of schizophrenia and treatment of schizoaffective disorder. In this study, we have compared the exposure of hydrophilic matrix tablets and osmotic release formulations to changes in gastrointestinal (GI) transit time and proton pump inhibitor (PPI) effect using a physiologically based pharmacokinetic (PBPK) modeling and simulation approach. Dissolution profile is important information out of several in the PBPK models to predict the exposure of the drug. Generally, dissolution is run at one pH medium at a time, which is in contrast to the dynamic pH changes with different transit times in different segments of gastrointestinal (GI) tract. Here we have developed a mathematical model which can integrate multiple dissolution data from experiments conducted at different pH which was further integrated with changing pH in GI segments and corresponding gastrointestinal transit time to ultimately produce a single dissolution profile which can better represent in vivo dissolution condition. This in vivo predictive dissolution profile was then used in GastroPlus software using ACAT model to assess the effect of changes in gastric emptying time and PPI effect for both formulations at extreme conditions.

Methods: GastroPlus (version 9.0, Simulations Plus Inc. Lancaster, CA) based on ACAT (Advanced Compartmental Absorption and Transit) simulation model was used to perform physiologically based pharmacokinetic modeling (PBPK) to mean plasma profile from osmotic pump formulation (RLD) and matrix formulation (TEST) for one of the submitted

abbreviated new drug application (ANDA) under fasting condition. Intestinal transit times were modified to reflect the absorption profile of the formulations. Phoenix 6.4/Matlab R2015a and Berkeley Madonna (version 8.3.23.0) softwares were used to build an integrated dissolution profile that also incorporated changing GI pH and transit time utilizing dissolution data at pH 1.2, 4.5 and 6.8 from respective studies. PBPK model was verified using intravenous data and immediate release oral pharmacokinetic data. The model was then used to predict impact of extreme physiological conditions on both formulations.

Results: Predicted plasma concentrations from intravenous, immediate release oral plasma data under fasting condition for matrix and osmotic release formulations for 6 mg were close to the observed plasma concentration. Our simulation with increased gastric emptying time predicted an increased area under curve (AUC) and maximum concentration (Cmax) for Paliperidone for both formulations which is consistent with label of RLD (Invega) that states "an increase in transit time, e.g., as seen with gastrointestinal neuropathy, diabetic gastroparesis, or other causes, would be expected to increase bioavailability, especially with changes in transit time in the upper GI tract". The T/R ratio was predicted to be 1.13 for AUC and 1.25 for Cmax assuming an extreme situation of gastric emptying time of 3 hrs, which was 1.08 and 0.99 for AUC and Cmax under normal physiological conditions. No significant difference in exposure was observed for PPI effect using pH 7.6 for both formulations.

Conclusion: The proposed mathematical model provides a quantitative tool to integrate multi-pH dissolution data into one profile incorporating GI transit time and changing pH which provides a predictive in vivo dissolution behavior. This model in conjunction to PBPK model provides advanced in-silico tool to test the effects of different physiological situations on BE PK metrics.

Disclaimer: The present work reflects the views of the author(s) and should not be construed to represent U.S. FDA's views or policies.

## 19. Application of Pharmacokinetic Modeling and Simulation Approach to Assess Bioequivalence at Steady State for two Paliperidone Formulations

*Authors: Sharan, Satish, FDA/CDER; Zhang, Xinyuan, FDA/CDER*

Plain Language Synopsis: Paliperidone is an atypical antipsychotic agent approved for treatment of schizophrenia and schizoaffective disorder as monotherapy and other indications. Since bioequivalence studies for ANDA approvals are conducted under single-dose condition, here we compared the performance of hydrophilic matrix tablets (TEST) and osmotic pump formulations (RLD) at steady state using a pharmacokinetic modeling and simulation approach.

Abstract:
Methods: A biphasic drug release pharmacokinetic model was developed which explained drug absorption profile from both formulations using two sequential zero order absorption rate functions. Single dose plasma concentrations after administration of 3, 6, 9, 12 and 15 mg of Paliperidone and steady state concentrations for 3 mg Paliperidone osmotic pump formulation study was collected from literature and used to evaluate the model performance. The model was further applied to predict the mean plasma profiles for both the osmotic pump (RLD) and matrix formulations (TEST) based on the submitted abbreviated new drug application (ANDA) for both fasting and fed conditions. Average pharmacokinetic parameters for the osmotic pump and matrix formulations were predicted using their mean plasma profiles. Steady state plasma profiles for both formulations were simulated and key PK parameters were evaluated. The mean percent peak to trough fluctuation (PTF) was defined as, PTF = 100×(Cmaxsteadystate – Cminsteadystate)/Caveragesteadystate. Phoenix 6.3 and Berkeley Madonna (version 8.3.23.0) software were used for parameter estimation and prediction of steady state concentrations for both formulations.

Results: We developed a PK model which successfully explained and compared the biphasic drug release of Paliperidone from

both osmotic pump and matrix formulations. Our model explained the observed data after single dose of both formulations and the steady state plasma profile for the osmotic pump formulation (RLD). The model predicted that approximately 31% and 69% of Paliperidone were absorbed sequentially by a zero order process for the osmotic pump formulation of RLD under both fasting and fed conditions. The initial absorption rate for the matrix formulation was observed to be faster than the osmotic pump formulation. Based on simulation, the mean percent peak trough fluctuation for steady state for the osmotic pump formulation (6 mg) was predicted to be lower than the matrix formulation (6 mg) (6.90 % vs 27.00 % (fasting) 8.71 % vs 20.35 % (fed)).

Conclusion: We have developed a reliable PK model which adequately captures the absorption kinetics of both osmotic release and matrix formulations which can be used to assess the steady state PK profiles for both formulations.

### 20. Variation in RF Heating Characteristics of Insulated vs. Bare Metal Stents

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Plain Language Synopsis: This work aims to characterize the RF induced heating of two stent variations - conformally coated and externally covered stents - and to compare the results against traditional bare metal stents. These thin coatings have not previously been explored due to computational limitations, recently overcome by modern computing tools.

Abstract:
The magnitude of radiofrequency (RF) induced heating of elongated medical implants such as stents is typically characterized as a function of the length and diameter of the device with a peak temperature increase occurring for a particular set of resonant dimensions. This work aims to characterize the RF induced heating of two stent variations - conformally coated and externally covered stents - and to compare the results against traditional bare metal stents. Numerical finite element method (FEM) simulations were performed to calculate the induced 1g-averaged SAR (SAR-1g). Devices were modeled in a tissue-mimicking gel ($\sigma$ = 0.47 S/m, $\varepsilon r$ = 80) and excited by a homogeneous 128 MHz electric field incident along the length of each device. Conformally coated stents were modeled with a conformal 0.1mm coating of PTFE insulation around all surfaces. Externally covered stents were modeled with a 0.1mm layer of PTFE insulation around only the external surface. The peak value of SAR-1g located within a region 1cm surrounding the implant was used as a metric to compare across the full range of design variations. The magnitude of the resultant SAR-1g was normalized to the peak value across all variations. Simulations varying the implanted device's parameters were performed for 30 values of length (i.e., 5 mm to 150 mm, step = 5 mm) and 20 values of diameter (i.e., 1 mm to 20 mm, step = 1 mm) for a total of 600 variations for each of the three cases. The RF heating characteristics for the bare metal stent were as expected with the resonant length approximately equal to the half wavelength of the incident wave in the dielectric gel. The addition of the conformal insulation increases the resonant length of the device and the addition of the external insulation increases both the resonant length and overall magnitude of SAR-1g. These results indicate that once dielectric insulation is added to stent structures the RF heating characteristics are no longer simply characterized by the length and diameter and further analysis of insulation thickness and relative dielectric constant must be explored.

### 21. Optimization of an In Silico Cardiac Cell Model for Proarrhythmia Risk Assessment

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Plain Language Synopsis: We present an optimized in silico model for prediction

of Torsade-de-Pointes risk as part of the comprehensive in vitro proarrhythmia assay. Our model incorporates multi-channel pharmacology data and dynamic drug interactions with the rapid delayed rectifier potassium current and is able to accurately predict TdP risk of the training compounds.

Abstract:
Drug-induced Torsade-de-Pointes (TdP) has been responsible for the removal of many drugs from the market and, therefore, is of major concern to global regulatory agencies and the pharmaceutical industry. The Comprehensive in vitro proarrhythmia assay (CiPA) was proposed to improve prediction of proarrhythmia risk, with in silico model prediction of drug TdP risk levels based on in vitro multi-channel pharmacology data forming an integral part of this initiative. Previously, we reported that combining drug and rapid delayed rectifier potassium current (IKr) dynamic interactions and multi-channel pharmacology is important for TdP risk classification and we presented a modified version of the O'Hara Rudy cardiac model that includes a Markov model of IKr to represent dynamic drug-IKr interactions (IKr-dynamic ORd model). In this study we further optimized this model by adjusting the model parameters based on published human cardiomyocyte experimental data in control and drug block conditions. We then simulated the cellular effects of the 12 drugs designated for model training in the CiPA initiative using the optimized model and manual patch clamp experimental data and screened a range of candidate metrics to categorize drugs according to their TdP risk. We demonstrated that a novel metric quantifying the net electronic charge carried by major ionic currents during steady state action potential shows the best separation of drugs according to their TdP risk levels across a wide range of concentrations and pacing rates. These findings provide a solid foundation for developing in silico models for regulatory assessment of cardiac safety under the CiPA paradigm.

## 22. Enhancing Regulatory Review of Modeling and Simulation (M&S) for Regenerative Medicine (RM) Products by Developing Best Practices and Test-Case Models

*Authors: Ortega, Ryan, FDA/CBER/OTAT; Morrison, Tina, FDA/CDRH/OSEL*

Plain Language Synopsis: Modeling and simulation may be used to provide support for regulatory submissions for regenerative medicine products, but there is no precedent for assessing model validity and applicability at CBER/OTAT. This work aims to develop best practices for reviewing modeling by leveraging expertise throughout the FDA and assessing test-case models.

Abstract:
Traditional approaches to non-clinical testing for drugs and medical devices may have limited utility for regenerative medicine (RM). Due to advances in computing technology and in the basic science of RM, there exists an opportunity to adapt existing modeling and simulation (M&S) methodologies for generating evidence that might be included in regulatory submissions for RM products.

Regulatory review of M&S for RM products requires a combination of techniques taken from the extensive modeling expertise at the FDA (there are approximately 200-250 scientific and regulatory personnel with direct modeling experience) and from recent verification and validation (V&V) paradigms. A set of best practices is being developed to aid the review of M&S for RM products in CBER's Office of Tissues and Advanced Therapies (OTAT). This will occur from interactions with the FDA's new M&S Working Group, V&V methods described in the literature, interviews with regulatory reviewers in CBER/OTAT and CDRH Office of Device Evaluation (ODE), and from regulatory review experience in CBER/OTAT and CDRH/ODE. These knowledge streams will be combined to develop a job aid that will be iteratively improved-upon following utilization of the aid by OTAT personnel to review test-case models of RM products.

There is no precedent in CBER/OTAT for assessing the validity and regulatory applicability of M&S for RM products. In order to generate the test-case models, we are partnering with engineering senior design

groups at undergraduate institutions. As part of their senior project, students are developing models of RM products selected from a list generated by experts in CBER and CDRH. Two student groups are developing a model of cells flowing through a delivery catheter to determine the effects of varying catheter parameters on cell viability. One student group is developing a model of a cartilage tissue scaffold in order to determine how in vivo degradation may affect product durability. These models will be reviewed using the job aid in order to develop best practices in CBER/OTAT. Of note, this novel method of student engagement requires minimal resource allocation and serves to educate our future stakeholders about the regulatory process, particularly as it pertains to M&S.

### 23. Biokinetic modeling of nickel released from cardiovascular devices

*Authors: Saylor, David, FDA/CDRH; Chandrasekar, Vaishnavi, FDA/CDRH; Sussman, Eric, FDA/CDRH; Craven, Brent, FDA/CDRH; Simon, David, FDA/CDRH; Fisher, Jeffrey, FDA/NCTR; Hood, Alan, FDA/CDRH; Brown, Ron, FDA/CDRH*

Plain Language Synopsis: Many cardiovascular devices contain nickel, which can lead to adverse health effects if released in sufficient quantities. However, patient exposure to nickel from these implants is not well established. We have developed models to help assess risk by predicting the release and accumulation of nickel in patients with these devices.

Abstract:
Many alloys used in cardiovascular device applications contain nickel, which if released in sufficient quantities, can lead to adverse health effects. While nickel release from these devices is typically characterized through the use of in vitro benchtop immersion tests, it is unclear if the rate at which nickel is released from a device during in vitro testing is representative of the release rate following implantation in the body. Likewise, the systemic and local biodistribution of nickel released from cardiovascular devices cannot be determined from in vitro tests. The uncertainty in the extent of nickel exposure to patients is one of the largest knowledge gaps to estimating the toxicological risk of nickel from implanted devices. To address this uncertainty, we have developed a novel multi-scale biokinetic model that combines a traditional toxicokinetic compartment model with physics-based models for nickel release from the device and accumulation and dispersion in local tissue. Thus, the model links the rate of nickel release from a cardiovascular device not only to local tissue concentrations, but also serum and urine nickel concentrations in patients, which are easily measured endpoints. The framework was parameterized using a combination of in-vitro nickel release test results, ex-vivo tissue characterization, and clinical observations. The model-derived predictions are consistent with levels of nickel measured local to implants in animal models and in serum and urine of patients following treatment with the atrial occluders and the optimized parameters in the model are physiologically plausible. The congruity of the model with available data suggests that it can provide a framework to specify both local and systemic exposure limits for nickel released from cardiovascular devices and interpret post-market biomonitoring data.

### 24. Surveying the 2.4 GHz ISM spectrum in a hospital environment and its application in wireless coexistence testing of medical devices

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Plain Language Synopsis: Medical device manufacturers are integrating wireless local area networks (WLAN) and Bluetooth technology into medical devices to spur innovation in healthcare. Accordingly, this work makes an important contribution to approximating the probability of a wireless medical device coexisting in its intended environment by providing a framework for modeling the environment.

Abstract:
Medical device manufacturers are integrating wireless local area networks (WLAN) and Bluetooth technology into medical devices to spur innovation in healthcare. These wireless technologies use the 2.4 GHz industrial, scientific, and medical (ISM) unlicensed band, which shares spectrum with other wireless devices, increasing the likelihood of communication loss and errors. Unlike spectrum bands that are dedicated for medical use, such as the wireless medical telemetry service (WMTS) and the medical implant communications service (MICS), the ISM band was intended for a broad range of applications that generate and use locally radio frequency (RF) energy. The fact that the ISM band is unlicensed, coupled with the availability of technologies it accommodates such as WLAN and Bluetooth, made it a popular choice for an increasing number of wireless-enabled medical devices.

The increasing use of shared, unlicensed spectrum bands by medical devices and nonmedical products highlights the need to address wireless coexistence to ensure medical device safety and effectiveness. This work makes an important contribution to approximating the probability of a medical device coexisting in its intended environment by providing a generalized framework for modeling the environment. The application of this framework is shown through an 84-day spectrum survey of the 2.4–2.48 GHz ISM band in a hospital environment in the United States. A custom platform was used to monitor power spectral density and record received power. Channel utilization of three nonoverlapping channels of 20 MHz bandwidth—relative to IEEE 802.11 channels 1, 6, and 11—were calculated and fitted to a generalized extreme value (GEV) distribution. Low utilization of channels was observed (<10%) in the surveyed environment, with sporadic occurrences of higher utilization of channels (>50%). Reported findings can be complementary to wireless coexistence testing. This work provided input to the development of a consensus standard for wireless device coexistence test methods and a consensus document focused on wireless medical device coexistence risk management.

### 25. Monte Carlo modeling of a-Se Full-Field Digital Mammography (FFDM) detector Modulation Transfer Function (MTF) resolution performance using ARTEMIS

*Authors: Cheng, Yu-Han, CDRH/OSEL; Lin, Ja-An, CDRH/OSB; Fang, Yuan, CDRH/ OIR*

Plain Language Synopsis: We developed a Monte Carlo computational model (ARTEMIS) for simulation of medical x-ray detector resolution performance for breast imaging applications, and validated the model with previously published experimental measurements. ARTEMIS can be used for detector performance simulations and potentially incorporated in virtual clinical trial studies to improve/streamline trial design.

Abstract:
In this work, we study the a-Se x-ray detector resolution limits for breast imaging applications (FFDM) using ARTEMIS - a detailed Monte Carlo computational model for simulation of direct x-ray detectors. We have focused on MTF resolution performance because for breast imaging applications, detectability of micro-calcifications and small lesions in mammograms has driven the development of high spatial resolution imagers with small pixel pitch. Monte Carlo methods can be used to model complex x-ray and secondary particle interactions in semiconductor detector materials. The model takes into account generation and re-absorption of characteristic x rays, spreading due to Compton scattering and high-energy secondary electron transport, and drift and diffusion of electron-hole pairs under the applied external electric field. The transport of electron-hole pairs is achieved with a spatiotemporal model that accounts for recombination and trapping of carriers and Coulombic effects of 3D spatial charge distribution. The location information for each detected electron and hole over millions of simulation histories are used to build the detector point response.

The ARTEMIS computational model is validated with previously published experimental measurements. ARTEMIS can be used for

simulation of FFDM detector performance and potentially incorporated in virtual clinical trial studies to improve and streamline trial design.

### 26. The Applications of Physiological Based Pharmacokinetics (PBPK) Absorption Models in Biopharmaceutics Assessment for Pharmaceutical Development

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Plain Language Synopsis: PBPK absorption modeling and simulation is a promising approach to promote clinically relevant risk assessment and specifications by linking product quality and in vivo performance. A model drug, venlafaxine, has been selected to explore the feasibility of using PBPK modeling to aid in biorelevant dissolution specification setting and IVIVC/R establishment.

Abstract:
The advanced modeling approach, namely PBPK absorption model, which integrates anatomical and physiological parameters of the gastrointestinal (GI) tract as well as the physicochemical properties of drug substances and products, has been increasingly used as an important tool in drug development and regulatory decision making for various designed purposes. Despite the promising perspectives, the applications of PBPK absorption models in biopharmaceutical assessment, e.g., in vitro-in vivo correlation/relation (IVIVC/R) establishment and clinically relevant product specifications have not yet been systematically explored and evaluated. This project focused on exploring the potential role of PBPK approach for promoting clinically relevant product specification towards patient-centric product quality. Using venlafaxine as a model drug, a clinically meaningful and scientifically sound PBPK model was developed using in vitro dissolution data as input and verified using available clinical data from intravenous and oral administrations (including oral solution, immediate release and extended release products). The PBPK base model was utilized for establishing IVIVC using mechanistic deconvolution approach for an extended release product of venlafaxine with an osmotic pump design. The mechanistic IVIVC was developed and internally validated with three different release rates. The prediction error for all three release rates are within the range of 0.2 % – 10 %. Finally, the IVIVC model was used to evaluate the clinical relevance of the proposed dissolution acceptance criteria. The experience obtained from this research demonstrated the advantage of PBPK absorption modeling for mechanistic IVIVC/R development, as well as the great potential for setting clinically relevant product specification. Future research dedicated to PBPK modeling for linking critical quality attributes with in vivo performance via IVIVC/R, especially in the Quality by Design (QbD) paradigm would be highly valuable for promoting patient-centric quality system in pharmaceutical industry.

### 27. Effect of Actuation Force on Simulated Regional Nasal Spray Deposition in a Healthy Nasal Cavity

*Authors: Delvadia, Renishkumar, FDA/CDER; Walenga, Ross, FDA/CDER; Geng, Tian, FDA/CDER; Saluja, Bhawana, FDA/CDER; Schroeter, Jeffry, Applied Research Associate; Kimbell, Julia, University of North Carolina; Sheth, Poonam, Cirrus Pharmaceuticals (now Recipharma Laboratories)*

Plain Language Synopsis: We developed a 3D computer model to study if changes in actuation force when using nasal sprays would change the location where medication deposits inside the nose. We found that the medication deposition pattern inside the nose was not affected by the actuation force for the nasal spray we studied.

Abstract:
Nasal sprays are often delivered by hand-actuated pumps, where manual actuation introduces variability in actuation force. Variability in actuation force introduced by manual use of spray pumps may have an effect on target site deposition of the emitted drug. The goal of this study was to use in

vitro measurement of nasal spray duration and droplet size distribution (DSD) with computational fluid dynamic (CFD) methods and a nasal reconstruction based on a healthy individual's computed tomography (CT) scan, to test the hypothesis that regional droplet deposition is affected by different actuation forces. Fluticasone propionate suspension nasal spray (FP) was selected as representative of commonly prescribed nasal suspension sprays for the simulations. A nasal reconstruction based on de-identified nasal CT scan images (0.7-mm resolution) from a 37-year-old female (56.7 kg) with no radiological evidence of nasal abnormalities was previously created. A nasal spray bottle reconstruction was virtually positioned in the left nostril of the nasal reconstruction according to FP instructions for use. The left nostril and nasal vestibule were manually distended slightly around the nozzle, and the nozzle was subtracted from the distended airspace. The respiratory region was subdivided into a general target region for treatment of rhinitis, a septal respiratory region between the squamous and target regions, anterior and posterior respiratory regions, and the nasopharynx. Steady-state, inspiratory airflow was simulated at an estimated resting breathing rate of 15.7 L/min using Fluent™ (v.14.5, ANSYS, Inc.). An estimated spray orifice diameter of 0.3 mm and measured spray durations and actuation forces of 34.3N, 56.9N, and 84.3N (N, Newton) were simulated. Simulations predicted that no FP droplets deposited on the right side after delivery into the left nostril, and that particles deposited almost exclusively in the most anterior regions of the left side, including the squamous-lined area, the target site in the nasal valve area, and the septal respiratory region. The results showed no significant differences in deposition fraction in these regions across the different actuation forces used. This study suggests that variability in hand-actuation of spray pumps may not significantly affect anterior nose target site deposition of nasal spray pump medications.

## 28. A Multiscale Modeling Approach to Optimize Pulmonary Drug Delivery

*Authors: Delvadia, Renishkumar, FDA/CDER; Walenga, Ross, FDA/CDER; Geng, Tian, FDA/CDER; Kannan, Ravi, CFD Research Corporation; Przekwas, Andrzej, CFD Research Corporation*

Plain Language Synopsis: We developed a 3D computer model of the lungs to predict distribution of a medication dose administered by an inhaler to the lungs and throughout the body. Our preliminary results showed that the model could be efficiently used to predict distribution of inhaled medication in the body.

Abstract:
Pulmonary drug delivery via oral inhalation is being increasingly used for both treatment of lung diseases and for delivering drugs to the systemic circulation. Efficacy and safety of such orally inhaled drugs is dependent on deposition and absorption of drugs in targeted regions of the lung. However, analyzing inhaled pulmonary drug disposition is experimentally challenging, as it involves complex mechanisms, such as regional drug deposition, dissolution, transport through lung barriers and mucociliary clearance. The goal of the project was to develop, evaluate, and improve multiscale computational physiologically-based absorption and pharmacokinetic models of pulmonary (inhaled) drugs. The framework employed Typical Path Lung (TPL) & Computational Fluid Dynamics (CFD) models to calculate lung depositions. Results presented here are based on TPL models. To evaluate dissolution, we employed a Noyes-Whitney type equation in any compartment based on dose, solubility, diffusivity, size, and mono/poly-dispersability of the selected drug. An experimental data-based equation is used to account for the loss of dissolved drug due to mucociliary clearance. The lung-barrier transport/absorption model from Yu et al. is used to predict drug retention/transport across lung tissue from 'epithelial-to-blood'. Finally, a whole-body human physiology-based pharmacokinetic model (PBPK) is used to connect the pulmonary blood to gut (CAT) model to predict lung and blood PK of the drug. The model provided good prediction of inhaled PK values for mometasone furoate

(MF), budesonide and fluticasone propionate (FP) when compared to literature values from clinical studies. The results showed that our comprehensive multiscale modeling approach could be efficiently used to predict orally inhaled drug product PK profiles at multiple lung sites.

### 29. The Effects of Formulation Factors on the Aerosolization Performance of Metered Dose Inhalers

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Plain Language Synopsis: The effects of drug-to-excipient interactions on MDI product performance are not well understood. Using a systematic approach, we found that excipients amounts and drug particle size distributions may impact the MDI aerosol characteristics. This work allows evaluate the extent to which the formulation factors govern MDI product performance.

Abstract:
Introduction: The product performance of metered dose inhalers (MDIs) depends on a multitude of factors including, but not limited to, the physicochemical properties of drug(s), device geometries, and nature and amount of inactive ingredient(s) [1]. Although much is known about the effects of changes in device geometry on the aerosolization performance of MDIs, [2,3] the effects of changes in formulation factors on MDI product performance are not widely understood. Therefore, the purpose of this work is to provide a better understanding of the effects of different levels of inactive ingredients and drug particle size distribution (PSD) on aerosolization performance of MDIs.

Methods: Three commercial MDIs (albuterol sulfate and mometasone furoate suspensions, and beclomethasone dipropionate solution) were chosen as model systems, reverse engineered, and the aerosolization performance was characterized by measuring delivered dose (DD) and aerodynamic particle size distribution (APSD). A reduced factorial statistical design of experiments (DoE) approach was used to develop a MDI batch manufacturing plan to vary the levels of inactive ingredients (ethanol and oleic acid) and drug PSD (D50, the median diameter of the PSD). MDI batches were manufactured, characterized, and the data was statistically analyzed. Multivariate mathematical models and design spaces were developed to predict the MDI aerosolization performance according to the different levels of formulation factors.

Results: The changes in drug PSD had statistically significant effects on the APSD of suspension MDIs studied, but not on DD. The changes in concentrations of inactive ingredients (ethanol and oleic acid) showed, in some cases, statistically significant effects on DD and APSD of suspension and solution MDIs studied. However, several cases without effects were also found. The possible effects of varying these must be studied on a case-by-case basis.

Conclusions: The outcomes of this study allowed defining design spaces for DD and APSD according to the different levels of ethanol and oleic acid concentrations, and drug PSD. The systematic approach utilized in this work can contribute as a quality by design (QbD) tool to evaluate the extent to which the formulation factors govern the aerosolization performance of MDIs, helping to design MDI formulations with desired in vitro product performance.

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[3] Smyth, H., et al., Spray pattern analysis for metered dose inhalers: Effect of actuator design. Pharmaceutical Research, 2006. 23(7): p. 1591-1596.

### 30. Mathematical Modelling of hERG Channel Kinetics at Physiological Temperature

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Plain Language Synopsis: Drug block of cardiac ion channels can disrupt normal electrical functioning. We present an optimized mathematical model of hERG channel kinetics. As many drugs block hERG it is important to represent hERG kinetics accurately within mathematical models of cardiac electrical activity which will be incorporated within routine drug safety assessment.

Abstract:
A key component of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative is to incorporate in silico assessment of the cardiac risk liability of drugs within routine pharmaceutical safety assessment strategies. Many pharmaceutical compounds interact with the ion channel encoded by the human ether-a-go-go-related gene (hERG) and block of this channel has been linked with increased proarrythmic risk. Consequently, drug interactions with the hERG channel are a major focus of routine pharmaceutical cardiac safety assessment strategies. It is therefore important that drug interactions with the hERG channel are accurately represented within mathematical models of cardiac electrophysiology which are to be used within the in silico component of the CiPA paradigm.

We present an optimized mathematical model which describes hERG channel kinetics observed at physiological temperature. We calibrated the model by fitting the parameters describing transition rates between distinct conformational states to data derived from manual patch clamp experiments. The patch clamp experiments were performed on HEK-293 cells at physiological temperature in response to a series of voltage-step protocols exploring activation, deactivation and inactivation gating kinetics. The model structure was selected to ensure that all model parameters could be accurately estimated from the experimental data used to calibrate the model, while also providing a reasonable description of observed gating kinetics. We then validated the model by predicting the current responses to a series of action potential protocols recorded at different pacing frequencies.

After constructing and validating the model in the absence of any drug compounds we then extend this approach to determine drug-specific models of drug-hERG channel interactions. More accurate representations of ion channel kinetic models within cardiac action potential models constructed in this way may lead to enhanced predictive ability of in silico predictions of cardiac safety risk within the CiPA paradigm.

### 31. Utilization of Informatics Tools to Mechanistically Analyze Drugs Associated with Serotonin Syndrome

*Authors: Rebecca Racz, FDA/CDER/OTS/OCP/DARS Keith Burkhart FDA/CDER/OTS/OCP/DARS*

Plain Language Synopsis: Serotonin syndrome is often a drug interaction that may be linked with different drug protein targets/pathways. Informatics tools were used to explore the relationship between several drugs, their targets and mechanisms, and serotonin syndrome. Second generation antipsychotics, benzodiazepines, cholinesterase inhibitors, and pregabalin were found to be associated with serotonin syndrome.

Abstract:
Background: Serotonin syndrome (SS) is often a drug-drug interaction that results in enhanced serotonin neurotransmission by acting through different drug protein targets/pathways. Second generation antipsychotics (SGAs) and concomitant drugs highly associated with SS and their molecular protein targets were evaluated to explore molecular protein targets and mechanisms for developing SS.

Methods: SGAs were data mined in FAERS to identify the concomitant drugs taken with SGAs and disproportionally associated with SS. Bioinformatics and cheminformatics tools were used to further mechanistically evaluate disproportionality of four classes of drugs. A bioinformatics tool data mines public FAERS for individual drugs, drug combinations and drug targets for disproportionality using Proportional Reporting Ratio (PRR) scores. A cheminformatics tool evaluates potential unknown off-target binding. Literature searches and case analyses followed to further analyze signals, associations, and mechanisms.

Results: SGAs are disproportionally associated with SS (N: 1075, PRR: 4.59). Many serotonergic receptors were associated with SS; 5-HT2A antagonism (N: 916, PRR: 7.90), 5-HT1A agonism (N: 600, PRR: 8.73), and 5-HT2C antagonism (N: 186, PRR: 15.07). Benzodiazepines were highly associated with SS (N: 1188, PRR: 4.83). Alprazolam and clonazepam were two of the top 20 concomitant medications with SGAs in 1075 SS cases (alprazolam concomitant N: 66, PRR: 4.28; clonazepam concomitant N: 133, PRR: 10.75). Cholinesterase inhibition (N: 120, PRR: 3.56) was found to be significantly associated with SS. N-methyl-D-aspartate (NMDA) antagonism was associated with SS (N: 219, PRR: 5.07). Pregabalin was predicted to bind to the NMDA receptor.

Discussion: Strong evidence exists for an association of SGAs and SS likely via two potential mechanisms of action found in literature: 5-HT1A upregulation via 5-HT2A antagonism and partial agonism at 5-HT1A. Some benzodiazepines may enhance serotonin activity via several mechanisms, including decreasing serotonin metabolism and increasing 5-HT receptor reactivity, causing concern over their use for SS treatment. By increasing acetylcholine, cholinesterase inhibitors may have opposing serotonergic action through the muscarinic (decrease serotonin) and nicotinic (increase serotonin) receptors, while predicted pregabalin NMDA binding may increase serotonin neurotransmission.

Conclusions: SGAs, benzodiazepines (alprazolam and clonazepam), cholinesterase inhibitors, and pregabalin were found to be disproportionately associated with SS via various mechanistic pathways/targets.

### 32. Is Type I error control for multiplicity really "out of the picture" in pharmacoepidemiology?

*Authors: Zhao, Yueqin, FDA/CDER/OB; Rima Izem, FDA/CDER/OB; Mark Levenson, FDA/CDER/OB*

Plain Language Synopsis: Through simulation, we illustrate that Holm's method and Benjamini-Hochberg method for multiplicity comparisons can control Type I error rate while retaining good powerand sensitivity.

Abstract:
Multiplicity of research hypotheses is common in epidemiologic studies investigating drug safety. However, multiple test procedures are underutilized in these studies because controlling the type I error inflation for null associations of drug with risk reduces the power to detect those non-null associations of drug with risk. Through a simulation study, we show that type I error could be seriously inflated even when there are only a small number of hypotheses to test with rare safety events in large databases. By comparing Holm's method and Benjamini-Hochberg method with methods without Type I error adjustment, we illustrate that the Holm's method and Benjamini-Hochberg method can control Type I error rate while retaining good power and sensitivity.

### 33. Modeling and Simulation Tasks Parallelization on HPC

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Plain Language Synopsis: Innovative techniques for migrating scientific applications to massively parallel supercomputers will be presented.

Abstract:
To implement FDA's mission of protecting and promoting public health, FDA scientists rely more and more on modeling and simulation (M&S) techniques implemented on powerful supercomputers with thousands of CPUs to conduct innovative scientific research. Because of its scalability, this approach broadens the range of potential experiments that can be simulated in silico; has the potential to improve confidence in medical devices and drugs through improved data; and can drastically reduce the cost and increase the speed of innovation. However, traditional M&S techniques have been based on single CPU implementation which exhibits two major drawbacks: (1) increased computation time and (2) limited ability to deal with large data. As an example, a project investigating immunogenicity assessment for therapeutic protein products would require 28 years of computations on a single CPU workstation – an infeasible task. After migration of the project to the CDRH High Performance Computing (HPC) platform it takes only about four days to complete. In addition, data generated from massive simulations cannot be processed using traditional desktop applications (e.g., Excel) because of its large size, but this data can be parsed and then recombined such that it can be processed efficiently with the HPC architecture. The above mentioned project generates a data set of 121 million of records which is practically impossible to process using Excel. A special "divide-and-conquer" parallelization technique processes this data set within minutes. This poster will present innovative techniques to address both of these drawbacks such that FDA's ability to perform sophisticated, large scale in silico modeling and simulation is greatly enhanced.

## 34. Application of Hydrodynamic Modeling to Predict Viral Impacts from Wastewater Treatment Plant Discharges Adjacent to Shellfish Growing Areas

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Plain Language Synopsis: Hydrodynamic modeling was conducted to predict the extent of sewage impacts from wastewater treatment plant (WWTP) and combined sewer overflow (CSOs) failures and bypasses on shellfish harvest areas to better inform classification decisions and management of harvest areas to reduce the risk of enteric pathogenic microorganisms in shellfish.

Abstract:
Since the inception of the National Shellfish Certification Program in 1925, now the National Shellfish Sanitation Program (NSSP), dilution analysis has been used as a means to minimize the presence of enteric pathogenic microorganisms in shellfish growing areas. Today failures and bypasses at wastewater treatment plants (WWTPs) and combined sewer overflows (CSOs) that release untreated or partially treated sewage into shellfish receiving waters are of particular interest to shellfish control authorities and public health officials. Additionally, events that cause high flows into the WWTP resulting in a degradation of efficiency to reduce enteric viruses are also a significant concern. Since 2008, the U.S. Food and Drug Administration (FDA) has conducted numerous hydrographic dye studies assessing WWTP impacts to shellfish growing areas supplemented with the testing of shellfish sentinels for enteric viruses and their surrogates (Goblick, 2008, 2015). The findings from these studies demonstrate that achieving a steady-state 1000:1 dilution level in waters adjacent to WWTP discharges established as prohibited (no shellfish harvest allowed) appears to be adequate for mitigating the impacts of viruses on shellfish when

WWTPs have typical treatment and disinfection practices and when operating under normal conditions. However, these studies also indicate that when a WWTP is operating outside of normal operation WWTP efficiency may be reduced and additional shellfish growing area may need to close in a timely manner during these events. To assess these events, which may be triggered by wet weather or high flow, FDA has used hydrodynamic models to predict the extent of sewage impacts on receiving waters under a various range of conditions not achieved through dye studies.

### 35. Predicting Opiod Binding Affinity using Molecular Docking

*Authors: Ellis, Christopher, FDA/CDER; Kim, Marlene, FDA/CDER; Kruhlak, Naomi, FDA/CDER; Hawkins, E. Gregory, FDA/CDER; Stavitskaya; Lidiya, FDA/CDER*

Plain Language Synopsis: Clandestine laboratories produce large quantities of new street drugs that are trafficked into the United States. Despite identical modes of action, slight structural modifications render the new drugs legal. Therefore, the OCP/DARS Chemical Informatics Program has developed computer models to assist with the legal classification of newly identified street drugs.

Abstract:
Opioids represent a class of drugs commonly used to treat moderate to severe pain. However, the therapeutic utility of opioids may be limited due to the development of tolerance and dependence and their potential for abuse. The large influx of new synthetic opioids permeating the street-drug market has generated the need for a fast and effective method to evaluate the risk of a substance to public safety. Therefore, a molecular docking procedure was developed to predict the binding affinity of uncharacterized drugs to the three main opioid receptor subtypes (mu, delta, and kappa). The model was validated by correlating the docking score of structurally diverse opioids with experimentally determined binding affinities to the three receptors. In particular, the binding concentration regime of fentanyl derivatives was accurately predicted, and the binding score was strongly correlated to the experimental binding affinity (r=0.9). Fentanyl derivatives with sub-nanomolar binding affinity (e.g. carfentanil and sufentanil) have significantly lower binding scores (dG < -10 kcal/mol), while less potent fentanyl derivatives have increased binding scores (dG > -8 kcal/mol). The strong correlation between the predicted binding scores and the experimental binding affinities suggests that this approach can be used to accurately predict the binding strength of opioid substances in the absence of in vitro data.

### 36. Population PK/PD Model Optimization and Parameters Estimation by SAS

*Authors: Hezhen Wang; Yaning Wang*

Plain Language Synopsis: SAS scripts to call NONMEM and PsN to get the PK/PD model parameters such as theta, eta, omega, sigma, OFV, VPC, bootstraps, draw diagnostic plots, optimization the model parameters and form the consistent reports.

Abstract:
There are several softwares for the population PK/PD model simulation and parameters estimation. However, there are several shortcomings among them. As well known, the SAS software is well maintained and very stable. Therefore, it is necessary to program a SAS script for the population PK/PD model optimization and parameter estimation as well as formation of consistent reports, which will be more convenient for reviewers.

The programed SAS script could call NONMEM to obtain datasets, which could be used to set up the diagnostic plots and read all the parameter estimation from NOMMEM output such as theta, eta, omega, sigma, OFV, RSD and shrinkage, rounding errors, boundary errors and minimization results and parameter description.

Moreover, the script could call PsN to get VPC, bootstrap datasets and plot all the graphs. And it could identify significance of the parameters to optimize the population PK/PD model and form a consistent word reports.

### 37. Deriving a provisional tolerable intake for intravenous exposure to silver nanoparticles released from medical devices

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Plain Language Synopsis: Nanotechnology shows great promise in biomedicine, including medical devices. The health risks of nanoparticles released from medical devices to patients is unknown. Our work establishes a safe exposure level, an amount released from a device that does not pose appreciable harm to human health, for nanoparticles in specific biomedical products.

Abstract:
Silver nanoparticles (AgNP) are incorporated into medical devices used intravenously (i.v.) for their anti-microbial characteristics and are produced with varying physicochemical particle properties including size, shape, coating and agglomeration state. The potential exposure and toxicity of AgNPs to patients is unknown due to lack of toxicological data. A stringent battery of biological tests for each nanomaterial produced would be costly and time-consuming. The aim of this safety assessment is to derive a provisional tolerable intake (pTI) value for AgNPs released from blood-contacting medical devices. A comprehensive literature review of in vivo studies investigating critical adverse health effects induced from i.v. exposure to AgNPs was reviewed and evaluated by the Annapolis Accords principles. Key studies were further analyzed by the Toxicological Data Reliability Assessment Tool (ToxRTool) to determine the critical study for use in derivation of the pTI. The lowest statistically significant dose-dependent adverse health effect reported in the critical study served as the point of departure (POD). The POD was based on a 28-day i.v. repeated AgNP (20 nm) dose toxicity study that reported an increase in relative spleen weight in male and female rats with a lowest 5% lower confidence bound of the benchmark dose (BMDL) of 0.14 mg/kg bw/day. An increase in spleen weight after AgNP (<20 nm) has been reported in other studies including in other species, and the spleen has been reported to be a primary target for Ag accumulation and toxicity after AgNP exposure. The POD was extrapolated to humans by a modifying factor (MF) of 1,000 determined with scientific basis to account for uncertainties in intraspecies variability (uncertainty factor (UF1 = 10), interspecies differences (UF2 = 10) and lack of long-term toxicity data (UF3 = 10). The pTI for long-term i.v. exposure to 20 nm AgNPs released from blood-contacting medical devices was determined to be 0.14 µg/kg bw/day. This pTI may not be appropriate for nanoparticles of other physico-chemical properties or routes of AgNP administration. The methodology presented is deemed appropriate for deriving pTIs for nanoparticles in general.

### 38. Scientific and Regulatory Considerations on Particle Size Analysis for Nanomaterials

*Authors: Wang, Chiaochun Joanne, FDA/CDER; Sun, Zhigang, FDA/CDER*

Plain Language Synopsis: Although numerous commercially available instruments have been used extensively for particle size analysis of nanomaterial drug products, selecting suitable particle sizing methods and establishing appropriate particle size specifications for nanomaterial drug products is still a big challenge for quality control of nanomaterial drug products manufactured at large commercial scales.

Abstract:
While the defining characteristic of nanomaterials is its nanometer size range, currently there are no standardized methodologies or regulatory protocols for characterizing particle size distribution (PSD) of nanomaterials. As a result, both industry and regulators have been confronted with challenges when selecting the appropriate particle sizing methods and establishing particle size specifications. Based on information collected from drug applications containing nanomaterials submitted to the

Agency, this work aims to provide an overview of the most frequently used particle size measurement methods for quality control and discuss regulatory considerations to determine their adequacy.

Currently, majority of nanomaterial-containing drug product falls into two dosage forms: oral solid or parenteral suspension. Oral dosage formulation predominantly leverages nanotechnology as a solubilization enhancement strategy. By reducing particle size to the nanometer range, surface-to-volume ratio is increased, which in turn increases the dissolution rate of poorly soluble drug substance. Nanomaterial-containing drug products for parenteral use include iron-carbohydrate complex drugs, liposomes, nanosuspensions, lipid complex and emulsion. These nanomaterials are drug delivery systems designed to optimize bioavailability at particular location over a period of time. Their complex structures pose unique challenges to particle size characterization, motivating the need of applying complementary sizing methods to provide a complete picture of the particle population (i.e. microscopic and spectroscopic techniques).

However, in many NDAs and ANDAs, it is common to see incomplete particle size specification with no meaningful or adequate control of PSD. We will discuss the information that should be included in the PSD specification for meeting regulatory requirement (analytical procedures, method validation, and acceptance criteria), and how to establish appropriate limits. We will also illustrate how to use a risk assessment approach as advocated by the Quality by Design (QbD) paradigm for developing particle sizing methods as well as establishing particle size specifications for nanomaterial drug products to ensure reproducible commercial manufacturing for consistent drug product quality.

### 39. Screening and formulation design of carbopol loaded Testosterone gel using various permeation enhancers

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Plain Language Synopsis: Understanding Quality of Transdermal Testosterone Gel Products

Abstract:
Introduction: Transdermal gels of therapeutics have the advantages of extended systemic drug exposure, simplified dosing regimen, and avoidance of first-pass metabolism. However, complex formulation designs and special delivery route present a tremendous challenge in evaluation of product performance.
The objective of the current study was to understand the effects of various formulation variables on critical quality attributes of Transdermal carbopol loaded Testosterone gel.

Methods: Placket–Burman (PB) screening design was employed to screen the effect of six formulation factors, namely Testosterone loading, type and percentage of permeation enhancers and concentrations of alcohol, gel forming agent and cross linking electrolyte (0.1N NaOH). Fifteen formulations were prepared according to PB design with three replicates of central point. Three concentrations (0.5, 2.75 and 5% w/w) of either isopropyl myristate (IPM) or isopropyl palmitate (IPP) were employed to prepare the gels loaded with either 1% or 2% of Testosterone. The gels were characterized for morphological and rheological properties such as viscosity and yield stress based on Herschel–Bulkley (HP) and Power Law analysis models. Kinetics of alcohol evaporation and in vitro permeation of Testosterone through human cadaver skin from carbopol- gels under occlusive conditions were also evaluated.

Results: Significant positive influences of carbopol, NaOH and Testosterone concentrations on the resultant gel viscosity were detected. However, only carbopol concentration was the significant factor affecting the yield stress value based on HP model. Kinetic parameters of alcohol

evaporation were dependent on the incorporated percentage of alcohol with minor contributions from other formulation parameters. Interestingly, IPP and IPM were equivalent for their influences on the permeation parameters, namely Testosterone flux, permeability and diffusion coefficients through cadaver skin. However, all permeation parameters were significantly enhanced by increasing loading concentrations of either IPP or IPM, alcohol and Testosterone. Percentage employed of alcohol was found to have a synergistic effect with either IPM or IPP in enhancing Testosterone permeation. Mass balance analysis showed that the recovered amounts of Testosterone from the skin compartment were dependent on the percentage employed of permeation enhancer. To maximize Testosterone permeation while minimizing the gel viscosity, the optimized formulation would be prepared with 2% testosterone, 5% IPM, 70% alcohol, 0.5% carbopol and 9% 0.1N NaOH.

Conclusion: The concentrations of penetration enhancer and alcohol in Testosterone gels should be controlled with minimal variation during manufacturing to ensure consistent product performance.

Learning Objectives:

(1) Development of in vitro permeation methods of testosterone through human cadaver skin from carbopol gels should be done under occlusive conditions.

(2)Concentration of penetration enhancers should be controlled to assure consistent product performance.

(3)Alcohol evaporation form Testosterone gels should be minimized during product manufacturing and shelf life.

Disclaimer: The views expressed are those of authors and do not necessarily represent the official position of the Agency (FDA).

## 40. Asymmetric Flow Field Flow Fractionation Hyphenated with Inductively Coupled Mass Spectrometry for the Determination of Size Distribution of Gold Nanoparticles in Dietary Supplements

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Plain Language Synopsis: For polydispersed nanoparticle solution, determination of size and size distribution is problematic. In this method nanoparticles are separated based on size and determine size of each fraction.

Abstract:
Engineered nanoparticles are available in large numbers of commercial products claiming various health benefits. Nanoparticle absorption, distribution, metabolism, excretion, and toxicity in a biological system are dependent on particle size, thus the determination of size and size distribution is essential for full characterization. Number based average size and size distribution are major parameters for full characterization of the nanoparticle. In the case of polydispersed samples, large numbers of particles are needed to obtain accurate size distribution data. Herein we report a rapid methodology for the characterization of gold nanoparticles in dietary supplements using asymmetric flow field flow fractionation coupled with visible absorption spectrometry and inductively coupled plasma mass spectrometry. Our method demonstrates improved nanoparticle recovery and excellent size resolution. A linear relationship between gold nanoparticle size and retention times was observed and the method was used for characterization of unknown samples. The particle size results from unknown samples were compared to results from traditional size analysis by transmission electron microscopy, and found to have less than a 5% deviation in size for unknown product over the size range from 7 to 30 nm.

## 41. Non-invasive solution NMR methods for measurement of particle size distributions in oil emulsion products

*Authors: Chen, Kang, FDA/CDER; Patil, Sharadrao, FDA/CDER; Keire, David, FDA/CDER*

Plain Language Synopsis: Complete oil emulsion drug products can be analyzed by new DOSY-NMR method for particle size distribution. The method is sensitive, reproducible and non-invasive. It differentiates similar drug products from different manufacturers and is expected to be of regulatory use.

Abstract:
Oil emulsion drug products are classified as complex drugs due to their formulation. The products contain particles of oil droplets stabilized by surfactant and micelles composed of extra surfactant molecules. The particle size distribution is a critical quality attribute (CQA) in evaluating ANDA products' bioequivalence to an RLD. For example, the reference drug product, Restasis®, from Allergan, contains 0.05% Cyclosporine A (CsA) and is used as a topical immunomodulator with anti-inflammatory effects. OTR/DPQR has manufactured similar CsA oil emulsion formulations and used cryo-electron microscopy (cryo-EM) and analytical ultracentrifugation (AUC) to measure the particle size distributions of these oil emulsion products. However, cryo-EM and AUC require extensive sample preparation steps that may lead to disruption of the original emulsion. Another approach is dynamic light scattering (DLS) which is non-invasive, but may require a 10-fold dilution of the drug product to reduce interference from excipients. Thus, analytical methods with minimal sample handling are desired. Here, to address the ANDA review needs, solution NMR methods were developed to non-invasively and directly assess the particle size distribution using a 2-dimensitonal (2D) Diffusion Ordered SpectroscopY (DOSY) method with a water suppression technique, which can be applied directly on emulsion drug products without dilution. The results showed the RLD product Restasis® has a highly homogenous distribution of oil droplet size in the range of 70+/-2 nm in diameter, while the ANDA products had different size ranges and were less homogenous. The same NMR DOSY method has also been applied to compare another ophthalmic product Durezol® and its ANDA product, and between IV emulsion products Diprivan® and Propofol®. The DOSY NMR method developed is a sensitive and non-invasive analytical method for particle size distribution and chemical component analysis which shows promise as a new analytical tool oil in water emulsion products.

## 42. Application of cryo-electron microscopy (cryo-EM) for morphological characterization of complex nanoscale drug products in support of bioequivalence

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Plain Language Synopsis: Differences in manufacturing processes may cause changes in the morphological structures formed in drug products. The purpose of this project is to evaluate cryo-electron microscopy as a highly sensitive regulatory tool for comparing the morphological properties of various complex drug products containing nanomaterials.

Abstract:
A variety of complex drug formulations including emulsions, suspensions, and liposomes have been developed to enhance the bioavailability of active pharmaceutical ingredients. The complex nature of these formulations presents significant challenges to the development and approval of generic drug products.

Differences in manufacturing processes may cause differences in morphological characteristics of products that are formulated qualitatively (Q1) and quantitatively (Q2) the same. These morphological differences may affect drug release behavior and bioavailability. Therefore, for certain drug products, comparative morphology should be included as a part of physicochemical characterization studies used to demonstrate

product sameness. In order to demonstrate comparable morphology, reliable and suitable characterization techniques need to be identified, developed and validated. In this study, cryo-electron microscopy (cryo-EM) was evaluated as a potential regulatory tool for the morphological characterization of various complex drug products to support demonstration of product sameness and ultimately bioequivalence.

A wide variety of complex drug products including intravenous iron injections, ophthalmic emulsions, anesthetic emulsion/liposome injections, anticancer liposomal injections and protein-bound paclitaxel nanoparticles were investigated using cryo-EM. Cryo-EM directly revealed the globule/particle size distribution, shape, internal structure, agglomeration tendency and surface features. For example, under cryo-TEM, intravenous iron formulations (sodium ferric gluconate, iron sucrose, low molecular weight iron dextran and ferumoxytol) exhibited a narrow particle size distribution, with an averaged iron core size of 2 nm for all four intravenous iron products tested. Compared to traditional room temperature sample preparation, cryo-TEM did not show artifact of agglomeration for the intravenous iron nanoparticles. Cryo-TEM also provided evidence of coexistence of nanoemulsion droplets and liposomes in an anesthetic injection product and found the composition of complex oil and lipid structures can vary between approved products. As conventional electron microscopy is inherently associated with various degrees of artifacts during sample preparation, cryo-EM methods will be instrumental for reliable visualization of drug products at the nanoscale, which will facilitate both the development and regulatory review of generic complex nanoscale drug products.

### 43. Changes in Glycolic Acid Human Skin Penetration by Altering Dendrimer Surface Chemistries.

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Plain Language Synopsis: Dendrimers are highly branched stable polymeric nanoparticles capable of binding chemicals and may potentially increase delivery of chemicals into skin. Skin penetration studies were conducted to determine the effectiveness of nanoparticles facilitating topical delivery of chemicals and improve CFSAN's ability to assess exposure and risk for ingredients with safety concerns.

Abstract:
Poly(amidoamine) (PAMAM) dendrimers are highly branched stable polymeric nanoparticles that may be synthesized to specific sizes, shapes, and surface chemistries for targeted delivery of chemicals. There is concern that dendrimers may be used for targeted delivery of cosmetic ingredients into skin, and may increase the skin absorption of ingredients currently considered safe in cosmetics, such as glycolic acid. We evaluated the skin penetration of radiolabeled G4 dendrimer conjugated with glycidol (Gly) and the G4 dendrimer bound to glycolic acid (GA), to determine if enhanced skin penetration of GA could occur. The skin penetration of a radiolabeled glycolic acid G4-Gly dendrimer mixture and a GA solution and were also evaluated. Treatments were applied (0.2% concentration) in aqueous solutions onto human cadaver skin assembled in flow-through diffusion cells. After a 24 hour exposure the extent of skin penetration was determined by liquid scintillation counting for quantification. From the results, most of the radioactive G4-Gly dendrimer penetrating skin remains in the stratum corneum (4.7%), with less than 1% absorbing into the epidermis and dermis. When the G4 dendrimer was bound to GA, penetration into the stratum corneum increased to 29%, and to about 4% in the epidermis and dermis. The stratum corneum level (29%) of the G4 dendrimer bound to GA was also significantly increased compared to the GA treatment alone (4.6%) and the radiolabeled glycolic acid G4-Gly dendrimer mixture (3.4%). There was an increase in radioactivity in the receptor fluid with the glycolic acid G4-Gly mixture (4.6%), compared to just the G4-Gly dendrimer (0.5%), the G4 dendrimer bound to GA (0.25%), and glycolic acid treatment alone (2.7%). It was

found that the G4 dendrimer may increase the penetration of GA; demonstrating that dendrimer chemistry may alter skin absorption of associated chemicals.

### 44. Hyperspectral Imaging Coupled to Enhanced Darkfield Microscopy to Enable High Throughput Characterization of Complex Formulations

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Plain Language Synopsis: The Cytoviva enhanced darkfield mMicroscope offers superior detection of nanomaterials due to improvement within the optical system. The microscope is coupled to a detector which allows different materials to be chemically differentiated from each other. This tool is being evaluated to characterize complex formulations such as creams and emulsions.

Abstract:
Submissions of complex drug products containing nanomaterials have increased in numbers. These drug products often have microstructures with nanoscale features which are difficult to simultaneously characterize both in size and chemical structure. The Cytoviva enhanced darkfield microscope (EDFM) has improved the alignment of the optics of the traditional cardiod darkfield condenser, enhancing the signal to noise and improving the limit of detection. Furthermore, the available short-wave infrared (SWIR) and visible near infrared (VNIR) detectors enable spectroscopic characterization of materials, even those with nanoscale features. These features coupled together make this instrument attractive for rapid and non-destructive characterization of complex drug formulations such as emulsions and creams. We are evaluating this instrumentation to assist in the quality evaluation of complex drug product submissions. We are testing the robustness of the technique, by doing cross-validation to Raman spectroscopy, atomic force microscopy and dynamic light scattering (DLS) for both sizing and chemical characterization. We find that EDFM is a complimentary technique to DLS, offering insight to particle shape and aggregation that would otherwise be unknown. We also are using the hyperspectral imaging (HSI) capabilities in both VNIR and SWIR wavelengths to assist in the chemical identification of different phases within complex drug product formulations. In this poster, we present a critical analysis of the instrument capabilities, including strengths and shortcomings as well as how it performs against other lower-throughput methods.

### 45. Using nanoscience in characterization of submicron particles in biologics: Analysis of extracellular membrane micro- and nanovesicles in platelet transfusion products

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Plain Language Synopsis: Blood transfusion products contain a wide-spectrum of microscopic membrane vesicles and protein particles which may affect the product safety and efficacy. Current developments of nanotechnology bring significant progress to nanoscale analytical methods. Our results demonstrate that these methods can be successfully used for analysis of membrane vesicles in platelet products.

Abstract:
Blood transfusion products contain a wide-spectrum of submicron phospholipid membrane and aggregated protein particles which may affect the product safety and efficacy. Current developments of nanotechnology bring significant progress to nanoscale analytical methods. We have employed different high resolution techniques to analyze platelet extracellular vesicles (PEVs) in liquid stored and cryopreserved platelets (PLTs): a) nanoparticle tracking analysis (NTA); b) flow cytometry (FC); c) asymmetrical-flow field-flow fractionation (AF4) with light scattering (LS) detection; d) atomic force microscopy (AFM); and e) cryo-

electron microscopy. We have shown that >90% of PEVs released from activated or stored platelets are in the nanoscale range of 40 nm to 300 nm, and thus below the reliable detection limit of conventional FC. Due to high polydispersity, PEVs are unsuitable for direct analysis by LS techniques. In 6% DMSO cryopreserved platelets (CPP), the AFM and NTA size distribution of PEVs indicated a peak diameter of 100 nm, corresponding to exosome-size vesicles. Thrombin generation based in vitro procoagulant activity (TG-PCA) of CPPs was 2- and 9-fold higher per PLT and per volume, respectively, compared to liquid stored platelets (LSPs). In addition, 26 % of TG-PCA of CPP products was associated with the exosome-size PEVs present in the CPP supernatant. In a study of PEVs released from activated PLTs, the extracellular vesiculome included ectosomes, exosomes, free mitochondria, mitochondria-containing vesicles and exhausted PLT ghosts. We found that PEVs chemical anatomy: lipid profile, surface biomarkers and packed biomolecules differs between the small exosome size PEVs (S-PEVs) and large vesicles (L-PEVs). Interestingly, S-PEVs have 5.7-fold higher TG-PCA per unit of surface area compared to the L-PEVs. Our results demonstrate that the advanced nanoscience analytical and imaging methods can be successfully used for analysis of membrane micro- and nanovesicles in platelet products. This is particularly important for characterization of platelet derived hemostatic biologics, such as cryopreserved or freeze dried platelets, where PEVs represent a major component with a marked contribution to procoagulant activity of these products. The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

## 46. Developing Quantitative Metrics for Safety and Performance Evaluation of Pulsed Laser Interactions with Plasmonic Nanoparticles in Emerging Medical Products

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Plain Language Synopsis: There is currently insufficient knowledge regarding laser-nanoparticle interaction processes and resultant bioeffects, including particle photodegradation and photothermal and photomechanical tissue damage. We are developing innovative test methodologies to evaluate the safety and performance of emerging medical products that combine pulsed lasers and nanoparticles.

Abstract:
The use of plasmonic nanoparticles (PNPs) in biophotonics for diagnostics and therapeutics is a rapidly growing area with many implications for clinical use. PNPs have unique optical properties that allow them to serve as contrast agents for optical diagnostic applications such as breast cancer detection and therapeutic procedures such as precision cellular nano-surgery. The unique properties of PNPs are due to their surface plasmon resonance (SPR), a collective electron oscillation on the particle surface that occurs when PNPs are excited by the appropriate wavelength of light. By changing particle size, shape, and composition, the spectral range of SPR absorption and scattering peaks can be tuned from the ultraviolet to near-infrared.

When PNPs absorb laser pulses on the order of 1-10 nanoseconds in duration – such as those used for photoacoustic imaging and laser ablation, energy is dissipated through rapid thermal diffusion and pressure wave generation. While such effects can enhance diagnostic or therapeutic procedures, they can also result in unintended side effects. Laser-induced PNP melting and fragmentation, as well as photothermal and photomechanical tissue damage have been noted in the literature, but are not well understood. Since current optical safety standards do not account for these effects, it is currently difficult to evaluate the safety and performance of diagnostic/therapeutic techniques that utilize PNPs.

The purpose of this research is to establish

a rigorous understanding of laser-tissue interactions involving PNPs, and to develop test methods and metrics for characterizing safety and performance. A key component of our experimental approach is an advanced pump-probe microscopy system for nanosecond-scale, time-resolved imaging of micro-bubble dynamics, with simultaneous measurement of acoustic transients and in-line optical spectroscopy. We present preliminary results obtained using gold nanospheres irradiated at 532 nm, including highly dynamic and concurrent optical, thermal and acoustic phenomena. Additionally, we evaluate the effect of PNP size on laser-induced damage thresholds and resultant optical property and morphology modifications – as measured by spectrophotometry, electron microscopy, and dynamic light scattering. The results of this study will provide independent quantitative data that can play a pivotal role in the evaluation of safety and efficacy of laser-based diagnostic/therapeutic nanotechnology products.

### 47. Quantification of Impurities in Carbon Nanotubes: Development of ICP-MS Sample Preparation Methods

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Plain Language Synopsis: The quantification of metal impurities in Carbon Nanotubes (CNTs) is essential to conduct environmental and health risk assessments. This study aims to develop a method which can be used effectively in laboratories to quantify multi-element impurities in CNTs using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Abstract:
Carbon nanotubes (CNTs) are one of the prospective nanomaterials to be used in industrial fields and consumer products due to its unique electrical, optical, physical, and chemical properties. The widespread use of CNTs can generate the potential for release of CNTs into the environment and increase human exposure from its final products or product line. Safety assessment including potential impacts on human health and the environment along with, several toxicity tests and releasing scenarios have been reported. However, additional work related with the quantification of metal impurities still needs to be accomplished to understand the biocompatibility, toxicity, and risk assessment of CNTs. Herein this study aims to develop a method which can be used effectively in laboratories to quantify multi-element impurities in carbon-based structures such as graphene, graphite, and carbon nanotubes using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Prior to the analysis, samples were prepared by conventional microwave acid digestion (CMAD) and microwave oxygen combustion (MOC). In addition, the ICP-MS method was validated by determining multiple analytical figures of merit such as linear calibration range, system stability, method detection limit (MDL), limit of quantitation (LOQ), and accuracy. Both of the developed CMAD and MOC methods worked well to digest metal impurities in carbon nanotubes. The ICP-MS results were in good agreement with each other and with the certified values. In comparison of two methods, CMAD is a simple and straightforward two-step procedure with safe experimental conditions, and requires only a few milligrams of sample. Moreover, CMAD overcomes the disadvantage of the pre-existing CNT digestion methods such as the requirement of multi-step acid digestion and drying processes. We expect that the developed methods can assist in the assessment of health risk and science-based regulation of products containing carbon nanotubes.

### 48. Comparative Evaluation of Particle Counting Techniques to Improve Regulatory Review of Complex Drug Products Containing Nanomaterials

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Plain Language Synopsis: New single particle analysis techniques, which measure particle size and number (concentration), are potentially powerful regulatory tools to ensure nano-

scale drug product quality. To assess their regulatory potential, we have compared the accuracy, precision, and analytical range of these techniques using particle standards and four propofol nano-emulsion/liposome drug products.

Abstract:
The concentration (number) of particles in complex drug products containing nanomaterials can affect the quality, consistency, and safety of the product as well as be used as a critical quality attribute for determining generic product sameness. Historically, due to the lack of appropriate analytical techniques, concentration has only been assessed through a bulk measurement of the amounts of each compound present in the formulation. This has recently changed as advances in single particle counting techniques now enable the accurate measure of individual particles and their concentrations. Although promising, their capability as a regulatory tool has not yet been examined. Herein we tested the analytical capabilities and limitations of two commercially available instruments, tunable resistive pulse sensing (TRPS) and particle tracking analysis (PTA), for measuring particle concentration. The analytical range, accuracy, precision, and robustness of the two techniques were assessed using a series of NIST traceable particle size standards. Since these instruments utilize different fundamental properties to measure concentration, the impact of concentration extremes (1.0E+6 – 1.0E+11 particles/mL), particle size (50 nm – 1,000 nm), and particle composition (polystyrene and emulsion) were also examined. Compared to the higher accuracy, resolution, and larger analytical range of TRPS, PTA nanoparticle concentration measurements were more precise and reproducible. To assess their potential regulatory use and to gain a better understanding of acceptable inter and intra-variability present in approved generic products, the lot-to-lot variation and generic-to-innovator concentrations of four propofol emulsion products were measured. The lot-to-lot variation in particle concentration was not statistically significant, indicating reproducible product sameness for each manufacturer. Interestingly, a higher variability was observed among drug products from different manufacturers although they are qualitatively (Q1) and quantitatively (Q2) equivalent to the reference (innovator) product. These findings indicate that particle concentration may provide a highly sensitive measure to assess product characteristics in vitro. However, the reproducibility and significance of these findings as a measure of bioequivalence is part of ongoing research. This study represents the first comprehensive evaluation of particle concentration measurement techniques and their potential regulatory use for FDA-regulated nano-scale products.

### 49. Determination of Globule Size Distribution of Cyclosporine Ophthalmic Emulsion using Asymmetric Flow Field Flow Fractionation

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Plain Language Synopsis: Asymmetric flow field flow fractionation has been demonstrated to be a viable high resolution technique to determine the globule size distribution within a complex drug product such as cyclosporine ophthalmic emulsion. Advanced characterization techniques such as AF4 may be useful in the evaluation of bioequivalence of cyclosporine ophthalmic emulsion.

Abstract:
Cyclosporine ophthalmic emulsion is a complex formulation, with drug that may be distributed across aqueous, oil, and micelle phases. Globule size distribution is one of the physicochemical characteristics recommended if the in vitro approach is taken to demonstrate the bioequivalence for a generic version of this drug product. Cryogenic transmission electron microscopy (cryo-TEM) and dynamic light scattering (DLS) methods have suggested that cyclosporine emulsion has a relatively broad size distribution ranging from tens of nanometers to a few hundred nanometers. The aim of this study is to develop an online

method using asymmetric flow field flow fractionation (AF4) coupled with multiple online detectors to separate and determine the globules size distribution of cyclosporine ophthalmic emulsion. Restasis®, the reference listed drug (RLD) of cyclosporine ophthalmic emulsion 0.05%, was used as a model drug product for method development. Experimental factors including mobile phase composition and concentration, focus flow, focus/injection duration and elution cross flow profile were investigated to achieve optimal separation efficiency of oil globules present in the cyclosporine emulsion. Multiple online detectors including UV, MALS, DLS and RI were coupled to the AF4 to acquire information on geometric and hydrodynamic size and globule concentration of the emulsion drug. Several in-house formulations with different size distribution but with same qualitative (Q1) and quantitative (Q2) composition as the Restasis® were prepared and analyzed by the AF4 method. The results were compared with cryo-TEM and DLS data to determine the sensitivity and resolving power of the developed AF4 method. It was observed that majority of the globules in the emulsion ranged from 20 to 60 nm in diameter which agreed with the cryo-TEM data. The online and batch-mode DLS results showed a close match which support the utility of using online DLS for the accurate determination of hydrodynamic size. The difference in the size distribution and relative globule concentration of in-house formulations could be discriminated by the AF4 method. The results provided an evaluation of suitability of the AF4 technique for the size based characterization of ophthalmic emulsions

### 50. In vitro Toxicological Evaluation of Ultrasmall Superparamagnetic Iron Oxide Nanoparticles on Human Coronary Artery Endothelial Cells

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Plain Language Synopsis: Medical devices enabled with nanotechnology represent an emerging area for novel biomedical products. Understanding the potential adverse health responses of nanoparticles used in devices is critical to establish product safety. We are investigating the effect of iron oxide nanoparticles on cells to support device safety evaluation.

Abstract:
Ultra-small superparamagnetic iron oxide nanoparticles (USPIONs) possess reactive surfaces, are easily metabolized, and show unique magnetic properties. These properties are desirable for designing novel theranostic biomedical products; however, their toxicity mechanisms are not completely elucidated. Therefore, the goal of this study was to evaluate cell interactions (uptake and cytotoxicity) of USPIONs by employing human coronary artery endothelial cells (HCAECs) as a vascular cell model.

PVP-coated USPIONs were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) to determine their morphology, size, and zeta potential. To examine cell uptake, cell imaging was conducted using live-dead confocal microscopy and TEM. To estimate cell viability, alamar blue and real-time CES electrical impedance assays were performed after 24 h exposure to 0 (control), 25, 50, 100 or 200 µg/mL of USPIONs suspended in EGM-2 culture medium. Reactive oxygen species (ROS) production was assessed using the DCFDA dye assay in cells exposed to USPIONs for 6 and 24 h.

USPIONs exhibited an average diameter (TEM) of 17 nm (range 7-37 nm) and average hydrodynamic diameter (DLS) of 44 nm. Zeta potentials were -31 and -42 mV and supports USPIONs are stable in colloidal solution. Cell microscopy imaging showed significant USPION internalization, even as early as 6 h at the lowest concentration. Dose-dependent cytotoxicity was observed with cell viability as 81%, 72%, 56% and 53% of control at 25, 50, 100 and 200 µg/mL, respectively. No evidence of ROS production was observed; therefore, the mechanism of cell injury could not be attributed

to oxidative stress. Additional experiments are ongoing to elucidate other mechanisms of cytotoxicity caused by PVP-coated USPIONs to better understand iron oxide nanoparticle toxicity.

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## 51. Screening formulation and process parameters affecting in vitro performance of acyclovir topical cream

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Plain Language Synopsis: The aim of current study was to understand the effects of various formulations and processing variables on critical quality attributes of acyclovir topical cream.

Abstract:
Purpose: The aim of current study was to understand the effects of various formulations and processing variables on critical quality attributes of acyclovir topical cream.

Methods: Fifteen DOE formulations were prepared according to Plackett-Buman (PB) screening design with 5% acyclovir loading and varying percentages of propylene glycol, poloxomer and sodium lauryl sulfate as formulation variables. The pH value and acyclovir particle size were also varied as processing parameters. Acyclovir powder was milled to the specified size range using jet-mill before incorporating into the formulations. The resultant particle size distribution was further assessed by the HELOS laser diffraction technique. The rheological properties and particle size of dispersed acyclovir in the creams were evaluated using a stress-controlled hybrid rheometer and polarized light microscopy, respectively. After separating the aqueous phase of the prepared creams by centrifugation, the percentage of acyclovir distributed into the aqueous phase was determined. The in vitro release of acyclovir from the 15 formulations was evaluated using immersion cell model fitted to USP apparatus II. The release rates of acyclovir were calculated based on Higuchi diffusion model. The in vitro permeation of acyclovir through human cadaver dermatomed skin was performed using vertical diffusion cell assembly. The amount of acyclovir retained per surface area of skin within 6 hours was also assessed as a dependent parameter.

Results: The results of multiple regression analysis revealed that none of the investigated variables were significant for their influences on the rheological properties of the prepared creams. The processing parameters of the jet milling process were well correlated with the resultant particle size of acyclovir powder with R-square value more than 0.8234. Non-significant differences existed between the particle size of acyclovir powders (D90) and those obtained by particle size analysis (D99) from cream specimens, confirming that the final particle size of product was solely dependent on the employed API particle size with minimal contribution by formulation and processing parameters. A significant positive effect of percentage incorporated of propylene glycol was found on the percentage of acyclovir recovered from the aqueous phase. The in vitro release rates of acyclovir from the 15 formulations were significantly affected by both pH value and percentage incorporated of propylene glycol with p-values of 0.0135 and 0.0006, respectively. On the other hand, increasing percentages incorporated of sodium laurel sulfate and propylene glycol resulted in significant increases in amount of acyclovir retained by the skin within 6 hours. The predominant effects of these ingredients on drug distribution between the aqueous and oily phases of the cream as well as on skin integrity would explain these results.

Conclusions: The pH value and concentrations of propylene glycol and sodium laurel sulfate in

acyclovir topical creams should be controlled with minimal variation during manufacturing and shelf-life to ensure consistent product performance.

### 52. Influence of Silica Nanoparticulates on Biologics

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Plain Language Synopsis: Glass delamination is a drug product quality concern. This study is focused on the influence of silica nanoparticulates on biologics. Here we evaluated the hydrodynamic size of FDA approved biologics in presence of silica nanoparticles of different sizes.

Abstract:
According to USP <1660> glass delamination is a product quality issue that might affect both safety and efficacy of a biologic drug. A number of drugs in the recent past have been recalled due to glass delamination. Studies reported have tried to address this issue from a macro scale dimension. Recent publications have emphasized the importance of evaluating nanoparticulates that can have a negative influence on the quality of drug product. In the event of glass delamination occurring during manufacturing or post marketing, nanoparticulates can come in contact with the drug product. Here in this study, we are addressing the impact of glass nanoparticulates on the biologics, which has not been addressed until now. Almost all the glass storage vials for biologics contain silicon dioxide (silica, 60 – 80 wt%), having a silica-rich inner surface. In this study, the impact of Silica nanoparticles (SiNPs) on clinically approved biologics was evaluated under various storage conditions such as in-use stability and thermal stress condition. Indeed the hydrodynamic size of the biologics got influenced in the presence of SiNPs of different sizes. The current study demonstrates that biologics can get heavily influenced by small amounts of Silica nanoparticulates and for the first time we show that each biologic behave differently to SiNPs and need to be addressed on a case-by-case basis.

### 53. Interference of Steroidogenesis by Gold Nanorod Core/Silver Shell Nanostructures: Implications for Reproductive Toxicity of Silver Nanomaterials

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Abstract:
Silver nanomaterials are widely used in personal care products. Recent studies have indicated that these nanomaterials may penetrate the blood-placental barrier and gain access to the ovaries. It is largely unknown how silver nanomaterials influence ovarian physiology and functions such as hormone production. This study examines the in vitro toxicology of silver nanomaterials, focusing especially on cytotoxicity and steroidogenesis while exploring their underlying mechanisms. In this study, primary rat granulosa cells were exposed to gold nanorod core/silver shell nanostructures (Au@Ag NRs), which were compared to cells exposed to gold nanorods only. The Au@Ag NRs generated more reactive oxygen species (ROS), reduced mitochondrial membrane potential, and decreased production of adenosine triphosphate. Au@Ag NRs promoted steroidogenesis, including progesterone and estradiol, in a time and dose-dependent manner. Chemical reactivity and transformation of Au@Ag NRs were then studied by electron spin resonance spectroscopy (ESR) and X-ray absorption near edge structure, which identified the generation of free radicals and intracellular silver species. These results suggested that both particle-specific activity and intracellular silver ion release of Au@Ag NR contribute to the toxic response of granulosa cells.

## 54. Platinum nanoparticles inhibit antioxidant effects of vitamin C via ascorbate oxidase-mimetic activity

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Abstract:
The development and application of nanomaterials as consumer products including food, drugs, and cosmetics, are rapidly expanding. However, interactions between these novel materials and other chemical components of consumer products have not been thoroughly studied. Here, by using electron spin resonance techniques, we compared the effects of Au, Ag, and Pt nanoparticles (NPs) on the antioxidant activity of vitamin C (sodium L-ascorbate, NaA). Chemical studies showed that Pt NPs exhibit ascorbate oxidase-like activity, thereby oxidizing NaA but Au and Ag NPs do not. This ascorbate oxidase-mimetic activity of Pt NPs results in a dramatic loss of antioxidant activity of NaA for scavenging hydroxyl radicals and superoxide radicals. Further study suggested that the ascorbate oxidase-mimetic activity of Pt NPs is critically dependent on particle size. Finally, in vitro cell studies demonstrated that Pt NPs with ascorbate oxidase-mimetic activity inhibit the cytoprotective effect of NaA on cells challenged by oxidative stress. Our findings enable a better understanding of enzyme-mimicking NPs interaction with naturally-occurring antioxidants and should guide future applications.

## 55. Exploring the activities of ruthenium nanomaterials toward reactive oxygen species

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Abstract:
Reactive oxygen species (ROS), including hydrogen peroxide (H2O2), hydroxyl radicals (•OH), superoxide radical (O2•-) and singlet oxygen(1O2), are continuously generated in biological systems and their levels must be controlled to maintain redox homeostasis in cells and tissues. Research on nanomaterials able to scavenge ROS has undergone a tremendous growth in recent years. Because of the excellent catalytic activity and good biocompatibility, considerable effect has been made to the noble metal-based nanoparticles, particularly gold, platinum, palladium, and iridium. To the best of our knowledge, the interactions between ruthenium nanoparticles (Ru NPs) and ROS have never been systematically explored thus far. Here, we use electron spin resonance (ESR) spectroscopy to provide direct evidence for the activities of Ru NPs with ROS. To study the ROS scavenging ability of Ru NPs, a series of ROS-generating models was established using chemicals and light irradiation. Our experiments show that Ru NPs possess CAT-like activity of decomposing H2O2 into H2O and O2 under acidic, neutral and alkaline conditions. Mimicking SOD, Ru NPs catalyze the reaction of superoxide anions under acidic and neutral environments. Our results also verified that Ru NPs can efficiently quench •OH and 1O2. All the scavenging activities of Ru NPs performed in a dose-dependent manner. These findings may shed new light on the mechanisms and applications of Ru NPs mimetic enzymes and could provide valuable guidance for the biological effects of Ru NPs under relevant physiologically conditions.

## 56. Continuous Monitoring of Albumin-Bound Paclitaxel Particle Dissolution Profiles Using Dynamic Light Scattering and In Situ UV/Vis Fiber-Optic Probes

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Plain Language Synopsis: Particulate protein-bound paclitaxel dissolves too rapidly for conventional methods to capture a drug release profile for comparison with generic

formulations. To address this, we evaluate several novel approaches correlating change in particle size and concentration to drug dissolution at concentrations similar to those found in the human body during administration.

Abstract:
To ensure the bioequivalence of a proposed generic albumin-bound paclitaxel (Ab-Ptx) drug product, FDA recommends that products be qualitatively (Q1) and quantitatively (Q2) similar and have comparative physicochemical characteristics, including particle size, morphology, and in vitro release kinetics. Conventional dissolution tests under sink conditions lead to rapid dissolution rates for Ab-Ptx, which undergoes a rapid burst release below the amorphous paclitaxel solubility concentration of approximately 35 µg/mL. Dissolution analysis techniques that can discern between particulate and solubilized drug in near-real time are, therefore, desired. One approach is to first obtain dissolution data with UV-Vis and correlate it with particle sizing techniques such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), in order to compare paclitaxel release rate to a reduction in particle size in near real-time. In our study we found that Ab-Ptx dissolution with fiber optic UV-Vis probes could not distinguish between dissolved and suspended particulate paclitaxel as both forms absorbed at 240 nm. Despite the overlapping UV spectrum of HSA protein, paclitaxel UV absorbance was found to follow Beer-Lambert's law up to saturation at paclitaxel concentrations of 70 ug/mL. Over 33 µg/mL, the particle diameter remained stable at 114-134 nm. At 17-20 µg/mL (below solubility), a linear decrease in particle diameter occurred, reducing the average diameter to 16-22 nm after 1 hr. Further diluting Ab-Ptx to a paclitaxel concentration of 13 µg/mL made the particle size decrease rapidly to 23 nm within 5 min of dispersing. An NTA time course experiment also revealed a gradual decrease in particle number at 15 to 35 µg/mL. Cryo-TEM imaging of Ab-Ptx showed the main size group as irregularly shaped roughly spherical particulates with a diameter ranging from 40-150 nm as well as smaller fragmented particulates <20 nm in diameter. DLS measurements provided an indirect indication of paclitaxel release as a change in particle size with time that was dependent on the paclitaxel concentration. These findings confirm that particle sizing methods such as DLS are capable of correlating size with possible drug release at sink and non-sink conditions, though they are not sufficient for providing a definitive stand-alone dissolution profile.

# Acknowledgements


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