Scientific Advisory Board Review of FDA Division of Microbiology

The National Center for Toxicological Research (NCTR) is a research facility of the Jefferson Laboratories of the Food and Drug Administration (FDA). The Jefferson Laboratories, which include both NCTR and the Arkansas Laboratory (ARL) of the Office of Regulatory Affairs (ORA), are located near Jefferson, a rural community in south-central Arkansas approximately 30 miles from Little Rock.

The mission of the Division of Microbiology is: ***to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science.*** To meet this mission, the Division of Microbiology's projects involve multidisciplinary research approaches that address a variety of FDA issues with special emphasis on: 1) evaluating the impact of antimicrobial agents, food contaminants, food additives, nanomaterials and FDA-regulated products on the microbiome; 2) developing methods to detect and characterize microbial contaminants in FDA-regulated products; 3) determining antimicrobial resistance and virulence mechanisms of foodborne and other pathogens; 4) conducting research to aid FDA in the areas of women's health, tobacco products, and nanotechnology; and 5) improving risk assessments of FDA-regulated products, including integrating systems biology approaches.

The SAB Subcommittee Review took place, virtually, on August 19 and 20th, 2020. The Subcommittee was chaired by Charles Kaspar and co-chaired by Mary Ellen Cosenza. Subject matter Subcommittee member experts were Suresh Pillai and Douglas Rhoads. Key points that the Subcommittee focused on were evidences for the integration of the Division of Microbiology in the overall mission of the FDA and NCTR. The quality of the science was reviewed, and strengths and opportunities were identified as well as emerging technologies or approaches that the Division should consider. The overall relevancy of the work to the FDA public health mission was also considered.

The Subcommittee meeting started with an overview of NCTR by William Slikker, Jr., NCTR Director. After a welcome and introduction of the Subcommittee by the Subcommittee Chair, Charles Kaspar, an overview of the Division by the Director, Carl E. Cerniglia was presented. Steven L. Foley is the Deputy Director and he closed the meeting on Thursday with a presentation/discussion of the Future Research Goals for the Division. Over the two days, several of the principal scientists presented information and data on their key research projects. Emphasis was often placed on how this work aligned with the needs to the FDA Centers. The presentations were grouped into three

(3) topics and this report will summarize the detailed review comments from the Subcommittee in alignment with this grouping. The three topic areas were:

Topic 1: Food Safety and Virology
Topic 2: Microbiome and Biological Interactions
Topic 3: Microbial Contaminants Detection

A virtual Poster Session was also held at the end of the first day. Each of the lead authors for each poster gave an overview of the research being presented in the poster using a few slides. Electronic copies of the posters were also provided to the Subcommittee.

Overarching Comments for the Division of Microbiology Research Program:

The Division of Microbiology has a total staff of 39 comprised of 27 government FTEs and 12 ORISE positions (Post-Docs, graduate students, etc.). The 27 government FTEs include 19 Research Scientists and Staff Fellows, 4 support Scientists and 4 Administrative Staff. Their research expertise lies in the following areas: antimicrobial resistance, host-microbiome interactions, environmental biotechnology, nanotechnology, women's health, virology and *Salmonella* virulence. They also gain expertise by working closely with other NCTR divisions and research groups, including Biochemical Toxicology, Systems Biology, Genetic & Molecular Toxicology, Neurotoxicology, Bioinformatics & Biostatistics, Nanotechnology Core and the Veterinary Services group. They also participate in several Outreach Activities on both a Global and National level. These collaborations keep the scientists and the science relevant and up to date. This was reflected in the ability of the virology group to quickly step up to provide critical support and research on the COVID pandemic. The Division also presented a strong publication record (about 25-30 publications a year) that included peer-reviewed publications, book chapters, symposium and workshop proceedings. Some of the data produced by this Division has been used in the development of FDA regulatory guidance.

The Division's Future Research Strategies are currently focused on prioritizing their research to best meet the FDA mission. This includes increasing engagement with colleagues from the other Centers and enhancing communication channels. The Subcommittee had the impression that the Division (and perhaps other groups at NCTR) are doing a better job of this over the last several years, but it is likely that more frequent interactions can strengthen these alliances. For example, CBER mentioned they hold monthly Science Impact Seminars that could be a good opportunity for scientific exchange. In contrast, there was concern expressed by the Subcommittee and NCTR management that the Division's resources could get "spread too thin" and that this might dilute the value of their work. It was suggested that the Division spend some time prioritizing their work and focus on those projects that are likely to provide the most value to the Agency while remaining true to the primary mission of the Division. Considering these factors and the number of staff, what are the top three research priorities or core competencies for the Division? Projects outside of these priorities can

be conducted, particularly on emerging issues, but it is important for the Division to recognize and establish areas of expertise. Leadership within the division should also examine the ideal balance between Research Scientists and support Scientists. The current makeup appears to be heavy on Research Scientists and lacking in support staff. The Subcommittee doesn't have the necessary information to make a recommendation on the proper balance, but this may be one way to provide additional support to priority areas of research in the future.

The Subcommittee acknowledges the personnel actions made since the last review that include filling the Deputy Director, Program Specialist, Management Analyst, and Support Scientist positions. It is also our understanding that some positions were converted to FDA staff fellows. There was also a discussion on the current challenges of hiring scientists in the current environment (an issue for NCTR in general as well). The Subcommittee recommends that scientists from the Division communicate with faculty at institutions more broadly than the current communication that is primarily with local universities/colleges. Increased visibility and interactions beyond the State of Arkansas with doctoral Universities should expand the applicant pool. Division scientists should make sure they are more visible to graduate students and post-docs from some of these other institutions. Each scientist should build a pipeline to multiple universities. Another potential venue for recruitment of scientists is at national meetings, most have a placement service where NCTR positions could be posted and potential candidates interviewed . During the overview presentation, it was noted that approximately 42% of the Research Scientists in the group will be eligible for retirement within the next 5 years. Therefore, the Subcommittee agrees that hiring and succession planning should be a key area of focus.

Topic 1: Food Safety and Virology

Overarching Comments: This is a broad area of research evaluating bacteria associated with antibiotic-coated medical devices, improvement of databases and tools for the identification of virulence and antimicrobial resistance genes in bacterial pathogens, development of detection methods for pathogens in FDA-regulated products, and more recently the study of viruses. It is clear from these projects that NCTR scientists are communicating with other centers and collaborating in a number of important areas. The lead scientists have identified significant areas on which to focus and are making excellent progress. The recruitment of Dr. Azevedo and the addition of her expertise in the area of virology demonstrates "forward thinking" by NCTR administration and scientists. Food Safety and Virology contains several core disciplines of microbiology that could be stand-alone areas of research; bioinformatics, microbial virulence, and now virology. The Subcommittee suggests that NCTR leadership carefully balance the breadth of research and emerging priorities with the potential overextension of the Division's capabilities.

COVID-19: Ongoing Studies to Address Coronavirus Data Gaps

It is exciting to see that NCTR is playing strong roles in the surveillance of SARS-CoV-2, identification of appropriate reagents to study virus-host interactions, and the development of experimental models to study spike protein-induced inflammation. The significant scientific talent both within the Microbiology Division and the rest of NCTR, as well as the enviable animal test facilities, can play a major role in assisting with this pandemic, especially as the country and the rest of the world recovers from this pandemic. Specifically, NCTR is ideally suited to carry out experiments with the actual virus and inactivation/decontamination strategies as well as defined QMRA studies.

Development of* Salmonella *Virulence and Plasmid Databases.

This project uses a variety of contemporary tools to develop and utilize DNA sequence databases. The analysis tool for predicting the presence of virulence genes from whole genome sequence data and comparing virulence gene profiles is a major accomplishment. The impact of this work is significant considering that these work products may ultimately end up on the NCBI sequence analysis pipeline. Additionally, a tool to predict virulence and/or antimicrobial resistance will be valuable for regulatory science involving recalls, epidemiological investigations, risk assessment, and pre-harvest risk factors.

Role of Plasmids in Increased* Salmonella *Virulence.

Salmonella continues to be a leading cause of foodborne illness in the U.S. and therefore, the focus on virulence plasmids is important. The food industry combines treatments at critical control points during processing with intrinsic and extrinsic factors of foods to control pathogens such as *Salmonella*. Do such treatments actually promote sub-lethal kill and therefore lead to increased lateral transfer of virulence plasmids? Due to the carriage of virulence and antibiotic-resistance genes by plasmids, understanding the factors (food matrix related, processing technologies and consumer handling) that contribute to transfer, or persistence of virulence plasmids may be of value. Identifying pre- and post-harvest production practices and medical devices that contribute to reservoirs of plasmid-harboring, pathogenic pathogens is an important area of research. A challenge with the prediction of gene function from sequence data is that the gene function or phenotype must be confirmed to validate the prediction. It is possible the multidimensional tissue culture work that is being developed will be useful to make some of the gene function confirmations; however, investigators should utilize the animal facilities available to confirm virulence when needed.

Method Development and Validation for Improved Detection and Isolation of* Salmonella *in Spices.

The contamination of low-moisture foods, like spices and flour, by *Salmonella* and enterohemorrhagic *E. coli* is a problem facing the U.S. food industry. The impact of NCTR research in this domain is noteworthy especially since the method developed for the detection of *Salmonella* in spices will be added to the Bacteriological Analytical Manual and is relevant to the FDA mission. The future research mentioned focuses on

the role of the multidrug-resistant (MDR) *Salmonella* and the role of efflux pumps in resistance as well as the prevalence and types of antibiotic-resistance genes in MDR *Salmonella*. There is no doubt that MDR pathogens are important. Are spices, low-moisture foods, MDR pathogens, or *Salmonella* the focus of this research program? If spices are the focus of this research program, then the Subcommittee suggests that it is important to look at pathogens beyond just *Salmonella*. We know very little about enteric viruses in spices and even less about protozoan parasites. NCTR can have a major impact in this area. In several aspects, this is a model project for the future in that it satisfies an agency need and makes use of multiple areas of strength within the Division; microbiological methods, *Salmonella*, and antibiotic resistance.

Topic 2: Microbiome and Biological Interactions

Overarching Comments: This area of research evaluates the impact of xenobiotics and nanoscale materials on the microbiomes inhabiting areas of the human body contacted by the materials, i.e., gastrointestinal tract. This is a reasonable approach to assess potential impacts of these materials but there needs to be a link between changes in microbiome with established human toxicity markers; like immune dysfunction, DNA damage, etc. Alternatively, some of these materials may increase susceptibility to infection due to alteration of the normal flora and could be evaluated using animal models. Overall, this section is interacting well with the other centers and collaborating in a number of important areas. There could be stronger interactions amongst the NCTR lead scientists to maximize the return on investments.

Approaches to Assess Xenobiotics Interaction with the Gastrointestinal Tract using Animal and Non-animal Models.

Dr. Khare presented quite a bit of data from four different ongoing projects. Most involved transcriptomics and microbiomes. All have potential and fit within the scope. Changes are being catalogued for corn oil as a vehicle, arsenic, BPAF and silver nanoparticles, but understanding what changes are significant (good or bad), is not as clear.

Preclinical Safety Evaluations of Nanoparticles in Vaginal Products.

Dr. Wagner presented detailed work on host responses to nanoparticles, with and without *C. albicans* infection. Much progress is being made and very detailed responses are being found. The *in vitro* work is with human vaginal epithelial cells and is producing very nice results. However, there is concern with the mouse vaginal model as there is no good, pH-matched, animal model for the human vagina. There has been concern for years about the lack of a pH matched animal model for the human vagina. Findings on

the changes in mouse vaginal microbiota associated with silver nanoparticles may have little bearing on a vaginal microbiome in humans.

The Impact of Nanoscale TiO2 and ZnO Used in Sunscreens on Bacteria that Colonize the Skin.

Dr. Chen presented on establishing a model to look at the interactions of nanoparticles in sunscreen with skin microbiome with different wavelengths of UV light. The effort is to understand how the skin microbiome is altered by environmental treatments. The approach has been to coat bacterial growth media with nanoparticles, then inoculate with bacteria, followed by UV light treatment. Although some interesting results are being obtained, there is concern that the system does not adequately replicate the conditions of use. The bacteria are tested individually and not in concert, and are being tested under growth-stimulating conditions. Where is the corresponding epidermis component? Is the epidermis/hair follicle milieu a high growth area? Bacterial response to UV treatment is modulated by growth rate and the phase of growth (i.e., stationary phase). Some introspection on improving the model for more relevant findings is recommended.

Evaluation of Toxicity of Nanocrystal Drug Formulation using Intestinal Epithelial Permeability and Immunotoxicity.

Dr Gokulan presented early work on drug interactions with human intestinal epithelial cells and tissue, especially in relevance to different drug forms. They are clearly developing strong collaborations/interactions with other centers. Exciting results are sure to be realized as they develop this system further.

Topic 3: Microbial Contaminants Detection

Overarching Comments: This research area focuses on the detection of microbial contaminants in FDA-regulated products such as tattoo inks, pharmaceutical products, and fecal transplant specimens. Some researchers are still relying on conventional culture-based methods to characterize microbial populations while other groups are using contemporary NGS approaches. It may be useful for the researchers focusing on detection of microbial contaminants to use NGS tools to understand the genetic diversity of the samples/organisms that they are studying. Overall, this section is interacting well with the other centers and collaborating on a number of important areas.

Microbial Survey of Commercial Tattoo Inks, Permanent Makeup Inks, Microblading Inks, and Ink Diluents Available in The United States.

Dr. Kim presented data from looking at microbial contamination in inks for the tattoo/makeup market. This is very important work and appropriate for applied FDA research. With the growing tattoo industry and the global supply chains for tattoo inks, a

deep understanding of the microbial contamination in these products is important. The research conducted so far has been solid and built on good scientific principles. The Subcommittee feels that relying on culture-based methods to date is definitely warranted and defensible. However, as was indicated by Dr. Kim, the use of culture-independent methods such as NGS should be performed in parallel. Moreover, it may be prudent to perform such studies with some more information about the source(s) of these samples (beyond just country of origin) being tested. The planned future studies include microbiological surveys. One suggestion would be to obtain samples that are in some way linked to previous samples. This could provide some information on whether the microbiological quality is deteriorating or improving.

***Burkholderia cepacia* Complex in Pharmaceutical Products.**

Dr. Ahn presented on developing methodology relevant to understanding the possible fate and the detection of Burkholderia complex in drug production/formulations. This is important work but there were some gaps in the presentation and the Subcommittee encourages publication in high-visibility journals. The finding that *B. cenocepacia* can survive for 40 days in distilled water and antiseptics is noteworthy. One of the outputs of this research program is the development of an oligotrophic medium for the U.S. Pharmacopeia. The proposed research on this topic appears to be focused on evaluating different molecular detection tools for this organism. The Subcommittee feel that understanding how these bacteria are able to survive in oligotrophic conditions will also be scientifically valuable. Understanding the molecular basis for their extended and robust survival may provide some insight into strategies that could be used for control. The NCTR Microbiology Division is well suited to delve deep into the science of such microbial survival strategies given the scientific talent and the instrumentation both within the Division and elsewhere at NCTR. A variety of contemporary molecular tools can be brought to bear to understand the metabolic state of these persistent organisms.

Establishment of Standardized Methods for Sporicidal Efficacy Assessment - Optimization of Spore Preparation Methods for* Bacillus *spp..

This is a very focused and needed research project of importance to both the food and pharmaceutical industries. Given that bacterial spores are of interest to a variety of federal agencies, it may be of value for the NCTR Microbiology Division to build collaborations with other Federal Laboratories that also spend considerable resources to understand bacterial spore formation, persistence, and inactivation.

Poster Session Feedback

The scientists within the Microbiology Division provided the Subcommittee with a short overview and posters of on-going research. The posters covered topics such as antimicrobial resistance mechanisms, effects of antimicrobials (e.g., tetracycline) on the gastrointestinal microbiome, the risks associated with *C. difficile* multiplying in FMT

samples, and the development of in vitro vaginal tract models. Although the short summaries and posters were presented on-line rather than in-person, they were well done with summaries of key findings presented in a concise manner. The findings that antibiotic impregnated catheters enhance the over expression of virulence proteins in *P. aeruginosa* is very interesting and timely. A number of commercially available household articles are now coated with antimicrobials. Do these promote the expression of virulence genes of opportunists such as *P. aeruginosa* and possibly others? The studies on fluoroquinolone resistance related mutations in uropathogenic *E.coli,* fecal transplant specimen characterization and storage, in vitro culture of human intestinal microbiota for fecal transplantation, and factors impacting plasmid transmission are all timely topics that are of importance to the mission of the FDA.

Future Research Goals for the Division of Microbiology: Serving FDA's Needs.

There is no doubt that the NCTR's Microbiology Division is fulfilling a national need on a variety of topics. There is significant scientific talent and the Division administration has done a very good job in attracting additional talent at all levels. However, there is concern about future challenges in hiring top talent and support staff, as well as providing adequate facilities to support the growth of these programs. As mentioned earlier, the Subcommittee feels that the NCTR Microbiology Division should build bridges with Research universities across the country to help with recruiting efforts. The Asst. Division Director commented that bench scientists lack the necessary bioinformatic skills that are needed to deal with large datasets. Universities are similarly dealing with this challenge by strategic cluster hiring of scientists with different domain expertise that can complement each other with individual skills in bench science, computation, and modeling. Could NCTR hire teams of scientists and technical staff across multiple projects that encompass bench science, modeling and computational skills? Such an approach could help position NCTR to address complex, cross-cutting issues. Also, the Microbiology Division should continue to expand its collaboration with the talented bioinformatics group at NCTR. The Division administration should also consider the proper mix of Principal Investigators and support staff when developing future staffing plans. The Division appears to communicate well with other FDA centers and other divisions at NCTR. The Subcommittee encourages Division scientists to continue or increase their participation in appropriate FDA working groups like methods development, the microbiome, antibiotic-resistant microbes, nanoparticles, etc. Communication within the Division is hampered because labs and offices are spread among different buildings at NCTR. To address this challenge, the Subcommittee encourages leadership and scientists within the Division to coordinate regular meetings, both formal and informal, to facilitate or continue communication and interactions. We acknowledge that this may already occur, but want to emphasize the importance of communication and interactions among Division staff when they are spread across the NCTR campus. A challenge for the division looking at the future is balancing on-going efforts with emerging priorities as well as defining core areas of strength and emphasis to prevent over-extension across an ever-expanding list of challenges, technologies,

and disciplines. It is not possible to cover all of the needs of the FDA and its Centers with the existing number of staff and space.