

# **CBER's Perspective on Evaluation and Implementation of Rapid Microbial Methods**

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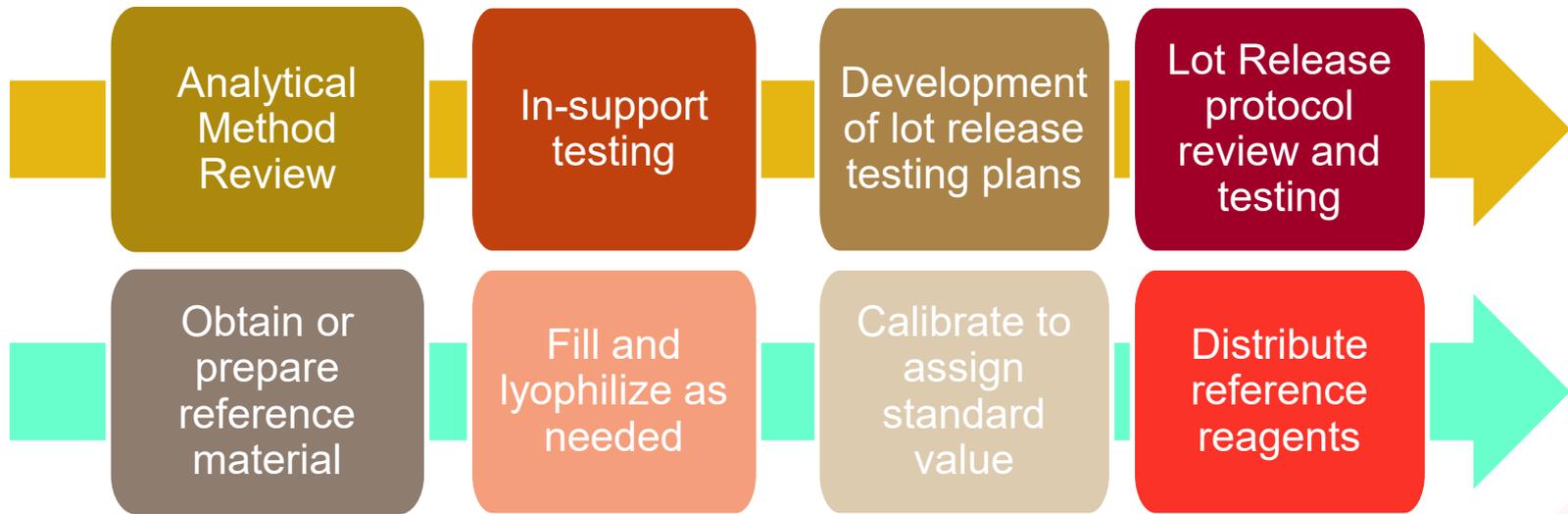
# Learning Objectives

- Understand regulations describing Rapid Microbiological Methods (RMMs)
- Learn about implementing RMMs
- Understand guidances surrounding RMMs
- Hear common RMM deficiencies from case studies

# Division of Biological Standards and Quality Control



To protect public health by ensuring the safety, effectiveness and availability of CBER-licensed products through review of analytical methods, testing of products during licensure and lot release, as well as producing and distributing reference standards.



# Compendial Method Suitability

- Compendial tests are standardized methods for testing different samples
- Regulatory Requirement 21 CFR 211.194(a)(2) Laboratory records
  - Verified the method for suitability under the actual conditions of use
- Components of Method Suitability
  - Reference Standard, Controls, Replicates
- Examples
  - Sterility: Recovery of <100 CFU using compendial method
  - Endotoxin: Positive product recovery and r value of standard curve

# Rapid Microbiological Methods

- Rapid Microbiological Methods (RMMs) allow for test results faster than traditional methods
- Variety of methods for testing diverse products
- Some methods are more straightforward than others
- Alternate methods often require proprietary technology

# Method Validation



- Method Validation is a Regulatory Requirement
- 21 CFR 211.165(e) Testing and release for distribution
  - In accordance with 21 CFR 211.194(a)(2)
- 21 CFR 610.9(a) Equivalent methods and processes
  - Assurance equal to or greater than method or process in the general standards

# CBER's Expectations on Rapid Microbiological Methods



- USP <1223> Validation of Alternative Microbiological Methods  
Ph. Eur. 5.1.6 Alternative Methods for Control of Microbiological Quality
- 21 CFR 610.9(a) Comparability Study for Performance Equivalency
- Method Validation Performed in the Presence of Product
- Understand limitations of alternative method and perform studies using worst-case scenarios
- Use microorganisms that are relevant to product and manufacturing environment
- Prior Discussions with CBER Representatives: Type C, Pre BLA or IND Meeting

# Limit of Detection and Specificity

- **Limit of Detection (LOD)**
  - Lowest number of microorganism that can be detected
  - Serial dilution (e.g., 10-fold dilution series; 100 to 1 CFU)
  - Detection limit should not be more than that of compendial method
- **Specificity**
  - Detection of wide range of microorganisms in a sample
  - Microorganisms should be carefully selected
    - Risk to patient or product, manufacturing environment, product failure
    - CBER recommends evaluation with LOD

# Ruggedness and Robustness

- **Ruggedness (Intermediate Precision)**
  - Reproducibility under a variety of normal test conditions
    - Different analysts, different instruments, different lots of reagents, different days
- **Robustness**
  - Method's capacity to remain unaffected by small but deliberate variations in method parameters
    - Sample preparation, incubation conditions

# Equivalence

- Level of agreement in accuracy, precision, specificity, LOD, LOQ, linearity and/or range between methods
- Initially demonstrated using standardized microbiology cultures, later separately using product
- Test samples should be identified that are expected to contain microorganisms to demonstrate new method will provide equal or greater assurance than the existing or established method

# Equivalence



- CBER expects microorganism inoculum at LOD to evaluate equivalency
- Methods should be run in parallel for a specified period or number of product batches or test samples
  - End-user determines the most appropriate strategy for duration and extent of these studies

# USP <1223>



- Infers equivalence of outcome between methods
  - CBER requires detection of low level of microorganism in sample
- Implies that product is not tested during equivalence studies; however, method suitability section states that at least one product type should be assessed during equivalence testing
  - CBER requires product matrices be similar to avoid a repeat equivalency study (e.g., if a rapid method is approved for a product, a new equivalency study for another similar product tested using same rapid method by same manufacturer may not be required)
- Equivalence Demonstration - alternate methods must be validated according to USP <1225> “Validation of Compendial Procedures”. LOD section of USP <1225> states detection limit is the lowest amount of analyte that can be detected in product matrix.

# USP <1223>



- Four options to demonstrate equivalency:
  - Acceptable procedure: infers alternate method measures a signal in presence of product
  - **Performance**: equivalent or better results demonstrated using validation criteria (accuracy, precision, specificity, LOD, LOQ, robustness, ruggedness)
  - **Results**: when two methods give equivalent numerical results; CBER requires RMM demonstrate assurance equal to or greater than general standards
  - Decision: pass/fail result is obtained and includes spiking studies
- **CBER requires Performance and Results Equivalence studies in the presence of product**

# Ph. Eur. 5.1.6



- Describes different rapid/alternate methods in detail
- Risk benefit analysis
  - End-user determines which alternate method to be implemented for the specific product
- Two levels of Validation
  - Primary Validation
  - Validation for Intended Use

# Ph. Eur. 5.1.6



- Primary Validation
  - By equipment/method supplier
  - Without product
  - Covers LOD, specificity, robustness, precision, prerequisite treatment of samples

# Ph. Eur. 5.1.6



- Validation for Intended Use
  - By User (sponsor, testing facility, etc.)
  - Covers user requirement specification such as LOD, time to detection/result, specificity, number and type of samples
  - Design qualification: design of equipment is suitable for performance of method
  - Installation, Operational and Performance Qualification

# Ph. Eur. 5.1.6

- **Performance Qualification**
  - Verification of primary validation data by supplier (CBER has accepted supplier validation data)
  - Verification for intended use: suitability testing, LOD, specificity, ruggedness, robustness, equivalence
  - LOD: “limit test determines the presence or absence of microorganism in a defined quantity of sample under test”
  - Equivalence testing: comparison testing of methods directly on validation parameters at low levels of inoculation (e.g., less than 5 CFU)

# Guidance for Validation – Risk Based Approach



- USP <1071> “Rapid microbial tests for release of sterile short-life products: a risk-based approach”
- Ph. Eur. 2.6.27 “Microbiological examination of cell-based preparations”
  - Refers to Ph. Eur. 5.1.6 for method validation
- 21 CFR 211.165(a) allows early release of product (i.e., negative to date)
  - Additional in-process controls may be needed

# Guidance for Validation – Risk Based Approach



- Short Shelf-Life Products: usually non-cryopreserved preparations infused into patients before completion of test
- Limited Sample Size: patient specific products, limited manufacturing quantities
- Number of samples and volume for testing for short shelf-life products
- Focus is on method suitability, but validation is required too

# Case Studies



- Limited Sample Size
  - Sample to media ratio
    - Ph. Eur. 2.6.27 - 1% of preparation 10-1000 mL
  - Additional in-process tests may be needed

# Case Studies



- Incubation conditions - *most common issue noted*
- Compendial Sterility
  - FTM at 30-35°C, TSB at 20-25°C
- Rapid Microbial Technology
  - Aerobic at 20-25°C and anaerobic at 30-35°C
  - No aerobic incubation 30-35°C
  - Slow growers do not grow timely at 20-25°C
- For RMMs, CBER expects three incubation conditions to support growth of variety of microbes

# Case Studies



- Lack of clarification between Primary Validation and Validation for Intended Use
- Primary Validation
  - Data published in literature can be used for Primary Validation
- Validation for Intended Use
  - Performed by end-user in the presence of product
  - Data published in literature not acceptable for Validation for Intended Use

# Case Studies



- Evaluate environmental isolates in validation studies
  - Environmental monitoring, sterility failure contaminants
  - Slow growing microbes
- Compendial microbes may not always represent real-world scenarios

# Challenge Question #1



## What is the difference between primary validation and validation for intended use?

- A. Primary validation is done in the presence of product, validation for intended use is not
- B. Validation for intended use is done in the presence of product, primary validation is not
- C. There is no difference, both are done in the presence of product
- D. There is no difference, neither are done in the presence of product

# Challenge Question #2



**What regulation requires equivalence testing between current official standards and new rapid microbial methods?**

- A. USP <1223>
- B. Ph. Eur. 5.1.6
- C. 21 CFR 610.9(a)
- D. USP <1071>

# Resources



- USP <1223> Validation of Alternative Microbiological Methods\*
- Ph. Eur. 5.1.6 Alternative Methods for Control of Microbiological Quality\*
- USP <1071> Rapid microbial tests for release of sterile short-life products: a risk-based approach
- Ph. Eur. 2.6.27 Microbiological examination of cell-based preparations

\*Both chapters include description of different rapid methods

# Summary



- RMMs allow users to get test results faster than traditional methods
- Method suitability demonstrates the test is suitable under the actual conditions of use
- Method validation is a regulatory requirement for alternate methods
  - LOD, Specificity, Ruggedness, Robustness, Equivalence
- Guidances describing validation of RMMs include USP <1223> and Ph. Eur. 5.1.6

# Closing Thought



If you are interested in implementing a rapid microbial method, do not hesitate to contact CBER for guidance if needed!