

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

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Learning Objectives

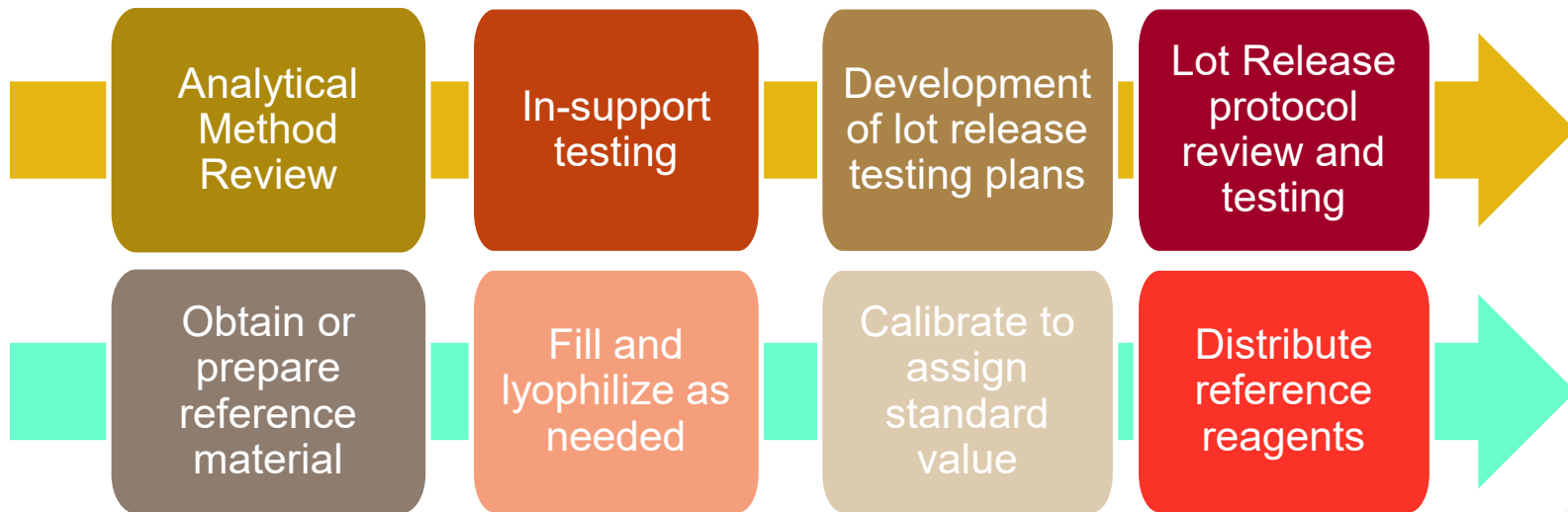


- Understand regulations describing Rapid Microbiological Methods (RMMs)
- Learn about implementing RMMs
- Understand guidances surrounding RMMs
- Hear common RMM deficiencies from case studies

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

A vertical timeline graphic on the left side of the slide, consisting of a black line with six blue circular markers, each corresponding to a point in the list.

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!