Updates to PDA TR 33

Evaluation, Validation, and Implementation of Alternative and Rapid Microbiological Methods

Josh Eaton
PDA

Current Status

- Three (3) year process
- Team has addressed more than 500 peer-review comments
- Finalizing Peer Review and Prepping for Board Approvals

The Team

Co-Leads:

Amy McDaniel
Bristol Myers Squibb

Michael Miller, PhD
Microbiology Consultants

Jessie Lindner
PDA

Frederic Ayers
INCOG Biosciences Services

Anthony Cundell, PhD
Microbiological Consulting, LLC

Sven Deutschmann, PhD
Roche Diagnostic GmbH

Marc Glogovsky
ValSource, Inc.

Simleen Kaur
U.S. Food and Drug Administration

Anthony Manzano
Aizon

Erika Pfeiler, PhD
ValSource, Inc.

David Roesti, PhD
Novartis

Edwin van den Heuvel, PhD
Eindhoven University of Technology

Olivia Venhuizen, PhD
Amgen

Kevin Wright
Procter and Gamble Technical Centers, Ltd.

Lisa Wysocki
GlaxoSmithKline

The Content

1.0 Introduction
- 1.1 Purpose & Scope

2.0 Glossary
- 2.1 Abbreviations

3.0 Conventional Microbiology and the Move Toward Alternative/Rapid Microbiological Methods
- 3.1 Conventional Microbiological Methods
- 3.2 Introduction to Alternative/Rapid Microbiological Methods
- 3.3 Regulatory Perspectives
- 3.4 Business and Quality Considerations for Implementing Alternative/Rapid Microbiological Methods
- 3.5 Risk Analysis
- 3.6 Automation of Conventional Methods
- 3.7 Method Variability in Microbiology and Alternative/Rapid Microbiological Methods

4.0 Technology Review
- 4.1 Method Choice
- 4.2 Sample Effect Considerations
- 4.3 Categorizing Technologies
- 4.4 Qualitive Detection Principles and Approaches
- 4.5 Quantitative Measurement Principle and Approaches
- 4.6 Identification Techniques, Principles, and Approaches

5.0 The Validation Process
- 5.1 Pre-Validation Activities
- 5.2 Equipment and Software Qualification and Validation
- 5.3 Primary Validation, Method Suitability, Validation for Intended Use, and Comparability Testing
- 5.4 Alternative/Rapid Microbiological Methods for Mycoplasma Detection

The Content

Updated Regulatory Perspectives Section

- FDA; EMA, including Annex 1
- Rest of World (Japan, Australia, China, India, Mexico, LATAM, etc.)
- Compendia chapters on AMM/RMM (USP, Ph. Eur., Japan, India, China)
- Regulatory and compendial perspectives for ATMPs
- How to address potential conflict when an AMM/RMM is approved by one regulatory authority but not another

Automation of Traditional Methods

- May not require full validation as an alterative method
- Extended equipment qualification
- Abbreviated method validation
  - e.g., accuracy and precision of an automated colony when compared with visual CFU counts
  - Confirm time to result

Summary of Scientific Principles Sections

- Quantitative
- Qualitative
- Identification

The Validation Process – Initial Activities

- Review supplier's primary validation
- Assess supplier capabilities

- Perform feasibility or proof-of-concept studies

- Develop user requirements
- Create validation master plan and working procedures

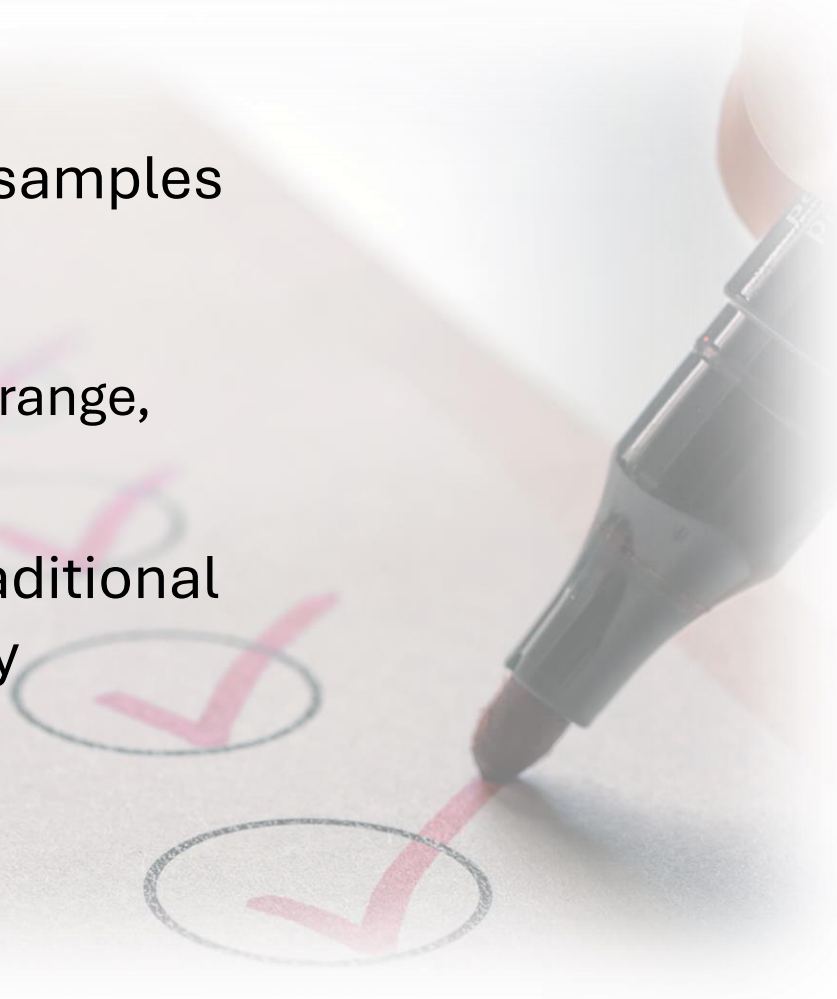
- Perform equipment IQ/OQ/PQ (no test sample)
- Computer system validation
- System integration

The Validation Process – Method Validation

- Primary validation
- Method suitability / Sample compatibility
- Comparability between the methods

Primary Validation

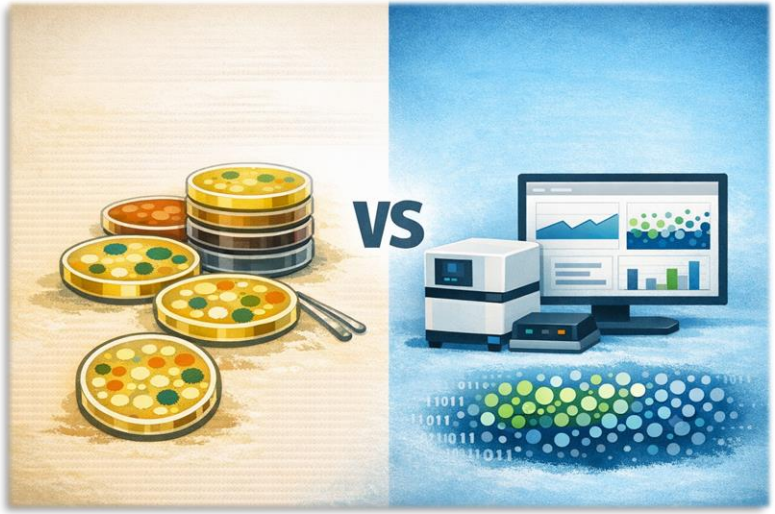
- Usually performed by the supplier absent test samples
  - Qualitative: LOD, specificity
  - Quantitative: accuracy, precision, LOQ, linearity, range, specificity
- May be performed in the AMM/RMM and the traditional method, which can demonstrate non-inferiority
- Robustness and ruggedness



Method Suitability / Sample Compatibility

- Usually performed by the end-user
- Includes test sample using the intended:
  - Sample preparation steps
  - Workflow
  - Instrumentation
- Relevant panel of organisms (<100 CFU)
  - Compendial strains
  - Previously recovered organisms from the test sample or facility
  - May present a risk to the patient
- Evaluate potential for false positives / negatives

Comparability



Performed by the end-user	• With the test sample • Assesses the AMM/RMM and existing method
Assay criteria	• Similar to primary validation
Challenge levels basis	• The intended test procedure, or • The required detection level
Goal	• Statistically demonstrate comparability (e.g., non-inferiority) with the existing method

Comparability with Non-CFU Methods

- Cell counts from non-CFU methods may be different from cell counts obtained from a CFU-based method

Directly compare the numerical values from both methods

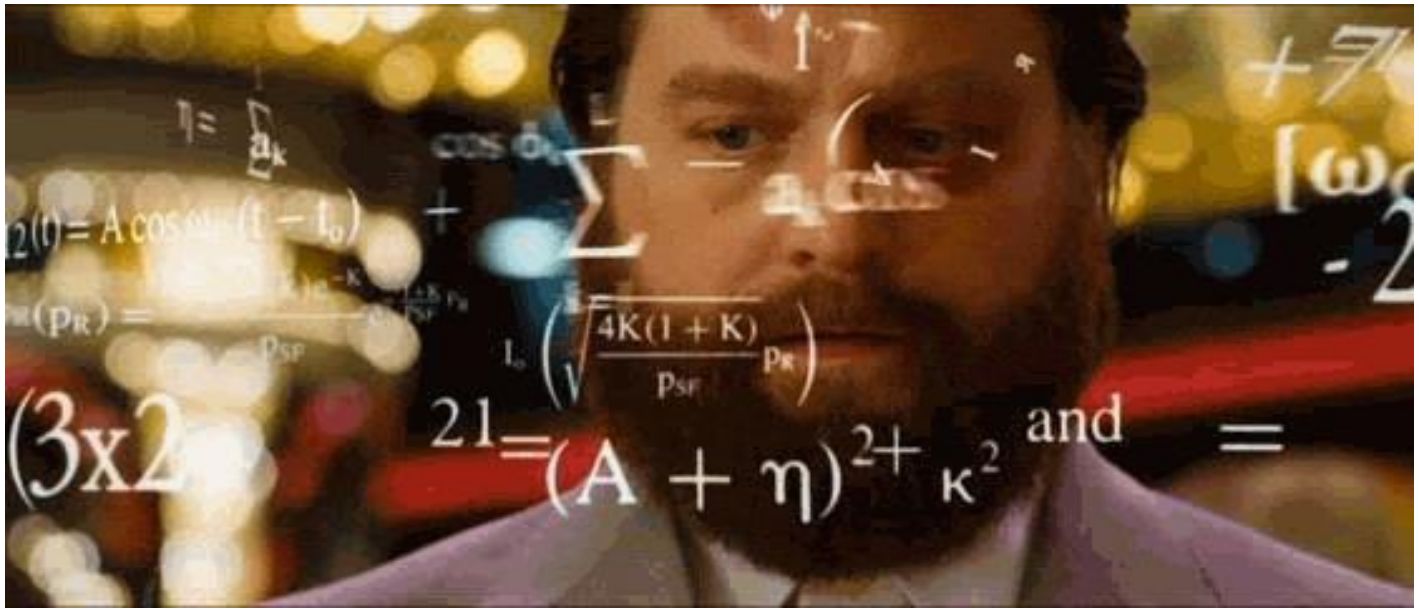
- **Use a non-inferiority statistical test**
- **Demonstrate comparability via a quality decision**
  - e.g., linear trends, ratio of counts, comparison of detectable events

Changing Acceptance Levels

- Where possible, establish a correlation between the two methods (e.g., see Annex 1)
- This correlation may be used to set new alert and action levels based on the new AMM/RMM signal
- Alternatively, establish an AMM/RMM upper limit that would indicate an excursion with the existing method

How to do this?

Statistics



Statistics

- Options for demonstrating comparability
- Recommendations for each validation criterion
- Comprehensive appendices
  - How to select a statistical test
  - Examples with simulated data
  - Formulas for use in Excel, SAS, R
  - Or use statistical software

9.0 Appendix I: Validation of Alternative and Rapid Microbiological Methods: Statistical Analysis–Qualitative Methods

9.1 Qualitative Measurement Principles and Approaches
9.2 False-Positive Rate for Qualitative Alternative/Rapid Microbiological Methods
9.3 Comparability of the Limit of Detection of Qualitative Alternative/Rapid Microbiological Methods
9.4 Estimation of the Detection Limit

10.0 Appendix II: Validation of Alternative and Rapid Microbiological Methods: Statistical Analysis–Quantitative Methods

10.1 Quantitative Measurement Principles and Approaches
10.2 Accuracy of Quantitative Microbiological Methods
10.3 Linearity and Accuracy
10.4 Limit of Quantitation
10.5 Precision

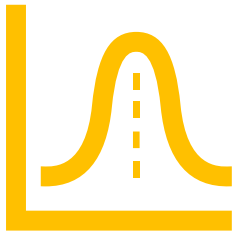
11.0 Appendix III: Validation of AMM/RMM: Statistical Analysis–with Non-CFU Signals

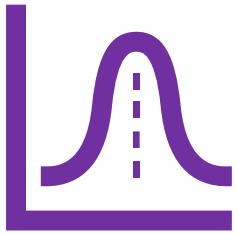
11.1 Qualitative Microbiological Methods with Non-Colony Forming Unit Signals
11.2 Quantitative Microbiological Methods with Non-CFU Signals

Example of TR33 Recommendations: Accuracy

- Different options provided
- Recovery of each method against a predicted challenge
- Comparison of the recovery between both methods
  - “RMM” = Rapid microbiological method
  - “PCM” = Plate count method

Accuracy: Recovery against a predicted challenge





- For each challenge level, the mean recovery and its confidence intervals were determined using a normal distribution
- The recovery counts were log transformed
- Determined non-inferiority when the lower 95% confidence limit (LCL) for each challenge level is above the non-inferiority margin of 70%

Accuracy: Recovery against a predicted challenge

- Formula for the mean recovery at one challenge level is:

$$\boldsymbol{\theta_A = \exp\{\mu_{log}\} \equiv \exp\{\sum_{i=1}^{n}[log(r_i)/n]\},}$$

where $\mu_{\log}$ is the mean of the log transformed recoveries, r_i the recovery (i.e., the count divided by the spiked concentration level), log the natural logarithm, and n the number of counts for the specific challenge level

Accuracy: Recovery against a predicted challenge

- The lower 95% confidence limit is obtained by:

$$\boldsymbol{LCL = \theta_A \cdot exp\left\{-t_{n-1}^{-1}(0.95) \cdot s_{log}\right\}}$$

where $s_{\log}^2 = \sum_{i=1}^{n}[(\log(r_i) - \mu_{\log})^2/(n-1)]$ is the variance of the logarithmically transformed recoveries and $t_{n-1}^{-1}(0.95)$ is the 95% upper quantile of the t-distribution with $n-1$ degrees of freedom

- All calculations were performed in Excel

Accuracy: Recovery against a predicted challenge

B. subtilis & *C. albicans* Mix (CFU/sample)	RMM		PCM	
	Mean % Recovery	95% LCL	Mean % Recovery	95% LCL
5	248.2	**160.7**	137.7	**99.0**
25	167.2	**129.5**	147.0	**128.2**
50	126.0	**107.9**	131.7	**114.5**
100	112.5	**101.2**	117.0	**104.4**
300	108.5	**97.8**	106.9	**98.6**

- Both methods demonstrated non-inferiority (i.e., the 95% LCL was above 70%)

Accuracy: Compare recovery between the methods

- For each challenge level, the ratios of the two means and the lower confidence limits were established using a Poisson distribution
- Determined non-inferiority when the lower 95% confidence limit (LCL) for each challenge level is above the non-inferiority margin of 70%

Accuracy: Compare recovery between the methods

- The LCL for each challenge level is obtained by:

$$(\bar{x}_L/\bar{x}_C) \cdot \exp\{-1.645 \times \sqrt{1/(n_L \cdot \bar{x}_L) + 1/(n_C \cdot \bar{x}_C)}\}$$

where $\bar{x}_L/\bar{x}_C$ is the ratio of RMM/PCM CFU × 100, n_L = # RMM replicates, $\bar{x}_L$ = RMM mean recovery, n_C = # PCM replicates, and $\bar{x}_C$ = PCM mean recovery

- All calculations were performed in Excel

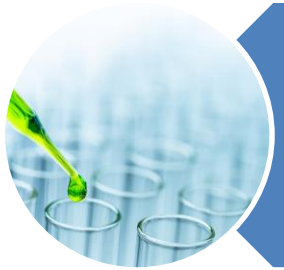
Accuracy: Compare recovery between the methods

B. subtilis & *C. albicans* Mix (CFU/sample)	Mean counts (CFU/sample)		Ratio RMM/PCM × 100	95% LCL
	RMM	PCM		
5	14.6	7.4	196.2	**148.2**
25	44.3	37.3	118.8	**103.4**
50	64.3	66.9	96.2	**86.3**
100	113.6	118.1	96.1	**88.6**
300	328.4	322.3	101.9	**97.1**

- The recovery in the RMM was non-inferior to the PCM at all challenge levels

Summary

PDA TR33 will be published soon (August-ish)



Offers many options for using statistics to demonstrate comparability between an AMM/RMM and an existing microbiology method

The statistics appendices provides detailed, step-by-step strategies for choosing an appropriate model with example data and calculations

Thank You!