

## (1223) VALIDATION OF ALTERNATIVE MICROBIOLOGICAL METHODS

### INTRODUCTION

This chapter provides guidance on the selection, evaluation, and use of microbiological methods as alternatives to compendial methods. To properly implement alternative methods, one must consider a number of important issues before selecting the analytical technology and qualifying that method with the actual product. These issues include, but are not limited to, identification of suitable alternative methodology, development of user specifications for equipment selection, demonstration of the applicability of the method as a replacement for a standard compendial method, and qualification of the method in the laboratory.

This chapter outlines:

- User requirements
  - Instrument qualification
  - Validation of alternate technologies
  - Method suitability
- The limitations of the use of CFU as a standard signal for microbiological methods.
- Four novel options for demonstrating equivalence
  - Acceptable procedures
  - Performance equivalence
  - Results equivalence
  - Decision equivalence
- Application of the concept of non-inferiority to method validation
- Guidance on statistical methods that may be employed in method validation

A glossary of the terms used in the chapter is provided at the end of this chapter.

Microbiological methods, other than microbial identification and strain typing methods (discussed in *Microbial Characterization, Identification, and Strain Typing* (11133), described in the compendia fall into two general categories:

1. Qualitative methods (not enumerative) that are used to assess the general microbial quality of compendial articles. This category includes assays that are intended to demonstrate the presence or absence of microorganisms in a compendial article.
2. Quantitative methods that yield a numerical (enumerative) result in terms of the microbial content of a compendial article.

There are inherent analytical factors that must be considered in the implementation of microbiological methods and in the comparison of a candidate alternative method to an existing compendial method. With respect to qualitative ("absence of") analysis, it is critical to consider that in microbiology, the finding of "no microorganisms present" does not mean in absolute terms that zero cells are actually present in the compendial article. A result of "no growth" in a current compendial method is properly interpreted as "no growth was detected in the test sample from the compendial article under the specified conditions".

The actual limits of detection of compendial microbiological methods have never been established quantitatively, and it is understood that many variables can affect the recovery of microorganisms. These variables include selection of growth media, incubation conditions, nutritional requirements of microorganisms that may be present, physical condition of microorganisms, and characteristics of the compendial article under test. Studies on the recovery of microorganisms from potable and environmental waters have demonstrated that traditional plate-count methods reporting cell count estimates as colony-forming units (cfu) may recover 0.1%–1% of the actual microbial cells present in a sample (1). In comparison to alternative methods that use flow cytometry and therefore yield a different signal (cell count), the presence of a greater number of cells based on an alternative method with a signal other than cfu has not correlated with more user risk or a higher likelihood of pathogens being present when there is an established safety record. These results do indicate that in some types of samples, the mean estimated cell count recorded using a growth-based compendial assay may result in a very different mean value than a cell count estimate derived from an alternative microbiological method that relies on a signal other than the cfu. Also, one must consider that in analytical microbiology the concept of false positive or false negative results are both scientifically and conceptually difficult. It would not be appropriate, for example to consider in a comparison between a standard compendial method and a candidate alternative method that a negative result in the standard method meant that positives observed in alternative method were false positives. It is a normal characteristic of a conventional growth-based method to recover some species well and yet to be unable to recover others. While it is not necessary for an alternative method and the conventional method to produce a match in terms of result, what is important is that the candidate method be capable of allowing a microbiologist to make an equivalent decision regarding product quality consistently.

It is extremely important in the application of this chapter that users take into account that microbiology is a logarithmic science. While we can distinguish between 100 and 1000 cfu (a difference of 1 log<sub>10</sub>), it may be not possible to discern smaller differences (less than 0.3–0.5 log<sub>10</sub>). The inherent variability of these methods is substantially greater than analytical chemistry methods. This inherent analytical variability must always be considered in the selection, development, and validation of alternative methods. The expectation of a degree of agreement between alternate microbiological methods and traditional growth-based methods beyond what is technically feasible could complicate the implementation of newer analytical technologies regardless of their specific mode of analysis.

Achieving the level of characterization (variability of the method) that is possible using modern chemical methods (e.g., high-performance liquid chromatography with a precision of 1%–2% relative standard deviation) is not possible in microbiology. It is reasonable to consider that the typical level of precision will typically be on the order of 15%–35% relative

# Validation Approaches For Alternative Methods

**Judd E. Hollander**



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## **Table of Contents Validation Approaches For Alternative Methods**

1. Understanding the eBook Validation Approaches For Alternative Methods
  - The Rise of Digital Reading Validation Approaches For Alternative Methods
  - Advantages of eBooks Over Traditional Books
2. Identifying Validation Approaches For Alternative Methods
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Validation Approaches For Alternative Methods
  - User-Friendly Interface
4. Exploring eBook Recommendations from Validation Approaches For Alternative Methods
  - Personalized Recommendations
  - Validation Approaches For Alternative Methods User Reviews and Ratings
  - Validation Approaches For Alternative Methods and Bestseller Lists
5. Accessing Validation Approaches For Alternative Methods Free and Paid eBooks
  - Validation Approaches For Alternative Methods Public Domain eBooks
  - Validation Approaches For Alternative Methods eBook Subscription Services
  - Validation Approaches For Alternative Methods Budget-Friendly Options

6. Navigating Validation Approaches For Alternative Methods eBook Formats
  - ePub, PDF, MOBI, and More
  - Validation Approaches For Alternative Methods Compatibility with Devices
  - Validation Approaches For Alternative Methods Enhanced eBook Features
7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Validation Approaches For Alternative Methods
  - Highlighting and Note-Taking Validation Approaches For Alternative Methods
  - Interactive Elements Validation Approaches For Alternative Methods
8. Staying Engaged with Validation Approaches For Alternative Methods
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Validation Approaches For Alternative Methods
9. Balancing eBooks and Physical Books Validation Approaches For Alternative Methods
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Validation Approaches For Alternative Methods
10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
11. Cultivating a Reading Routine Validation Approaches For Alternative Methods
  - Setting Reading Goals Validation Approaches For Alternative Methods
  - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Validation Approaches For Alternative Methods
  - Fact-Checking eBook Content of Validation Approaches For Alternative Methods
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
  - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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