

## (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

**Willem E. Saris, Ákos Münnich**



## **Validation Approaches For Alternative Methods:**

Validation of Alternative Methods for Toxicity Testing Chantra Eskes, Maurice Whelan, 2016-09-26 This book provides information on best practices and new thinking regarding the validation of alternative methods for toxicity testing It covers the validation of experimental and computational methods and integrated approaches to testing and assessment Validation strategies are discussed for methods employing the latest technologies such as tissue on a chip systems stem cells and transcriptomics and for methods derived from pathway based concepts in toxicology Validation of Alternative Methods for Toxicity Testing is divided into two sections in the first practical insights are given on the state of the art and on approaches that have resulted in successfully validated and accepted alternative methods The second section focuses on the evolution of validation principles and practice that are necessary to ensure fit for purpose validation that has the greatest impact on international regulatory acceptance of alternative methods In this context validation needs to keep pace with the considerable scientific advancements being made in toxicology the availability of sophisticated tools and techniques that can be applied in a variety of ways and the increasing societal and regulatory demands for better safety assessment This book will be a useful resource for scientists in the field of toxicology both from industry and academia developing new test methods strategies or techniques as well as Governmental and regulatory authorities interested in understanding the principles and practicalities of validation of alternative methods for toxicity testing

The History of Alternative Test Methods in Toxicology, 2018-10-20 The History of Alternative Test Methods in Toxicology uses a chronological approach to demonstrate how the use of alternative methods has evolved from their conception as adjuncts to traditional animal toxicity tests to replacements for them This volume in the History of Toxicology and Environmental Health series explores the history of alternative test development validation and use with an emphasis on humanity and good science in line with the Three Rs Replacement Reduction Refinement concept expounded by William Russell and Rex Burch in 1959 in their now classic volume The Principles of Humane Experimental Technique The book describes the historical development of technologies that have influenced the application of alternatives in toxicology and safety testing These range from single cell monocultures to sophisticated miniaturised and microfluidic organism on a chip devices and also include molecular modelling chemoinformatics and QSAR analysis and the use of stem cells tissue engineering and hollow fibre bioreactors This has been facilitated by the wider availability of human tissues advances in tissue culture analytical and diagnostic methods increases in computational processing capabilities and a greater understanding of cell biology and molecular mechanisms of toxicity These technological developments have enhanced the range and information content of the toxicity endpoints detected and therefore the relevance of test systems and data interpretation while new techniques for non invasive diagnostic imaging and high resolution detection methods have permitted an increased role for human studies Several key examples of how these technologies are being harnessed to meet 21st century safety assessment challenges are provided including their deployment

in integrated testing schemes in conjunction with kinetic modelling and in specialized areas such as inhalation toxicity studies The History of Alternative Test Methods in Toxicology uses a chronological approach to demonstrate how the use of alternative methods has evolved from their conception as adjuncts to traditional animal toxicity tests to replacements for them This volume in the History of Toxicology and Environmental Health series explores the history of alternative test development validation and use with an emphasis on humanity and good science in line with the Three Rs Replacement Reduction Refinement concept expounded by William Russell and Rex Burch in 1959 in their now classic volume The Principles of Humane Experimental Technique The book describes the historical development of technologies that have influenced the application of alternatives in toxicology and safety testing These range from single cell monocultures to sophisticated miniaturised and microfluidic organism on a chip devices and also include molecular modelling chemoinformatics and QSAR analysis and the use of stem cells tissue engineering and hollow fibre bioreactors This has been facilitated by the wider availability of human tissues advances in tissue culture analytical and diagnostic methods increases in computational processing capabilities and a greater understanding of cell biology and molecular mechanisms of toxicity These technological developments have enhanced the range and information content of the toxicity endpoints detected and therefore the relevance of test systems and data interpretation while new techniques for non invasive diagnostic imaging and high resolution detection methods have permitted an increased role for human studies Several key examples of how these technologies are being harnessed to meet 21st century safety assessment challenges are provided including their deployment in integrated testing schemes in conjunction with kinetic modelling and in specialised areas such as inhalation toxicity studies

**Alternative Testing Methodologies** ,1998 Psychology and Human Performance in Space Programs Lauren Blackwell Landon,Kelley J. Slack,Eduardo Salas,2020-10-08 In Psychology and Human Performance in Space Programs Extreme Application operations experts from multiple space agencies with support from spaceflight researchers outline existing and proposed operations for selecting training and supporting space crews who currently live and work on the International Space Station and who are preparing for future missions to the moon and Mars Highlighting applied psychology in spaceflight whilst acknowledging real world complexities that occur when integrating across an international multi agency collective this volume provides both historical and current perspectives toward spaceflight operations with expert contributions from NASA and international partners such as the Japanese Space Agency Russian space researchers and the Canadian Space Agency Helpfully outlining the progress that has been made so far this book includes topics such as the selection and hiring of astronauts the process of training a crew for a mission to Mars and workload and mission planning Discussing operational psychology in space and on the ground this book looks to the future of research and operational needs for future missions to Mars with an essay from astronaut Dr Don Pettit on his experiences in space and how the Mars mission will challenge us in new ways This second of two volumes will be of interest to professionals in the field of human factors and

psychology in extreme environments     Principles of Toxicology Stephen M. Roberts, Robert C. James, Phillip L. Williams, 2022-04-05 Principles of Toxicology concisely and efficiently presents the scientific basis for toxicology as it applies to the workplace and the environment covering diverse chemical hazards encountered in modern workplaces and natural environments and providing a practical understanding of these hazards for those concerned with protecting the health of humans and ecosystems The work presents not only theory but also practical information regarding chemical hazards to give the student and new professional a working knowledge of the practice of toxicology and the ability to solve problems in environmental and industrial settings Case histories and examples from industrial and environmental exposures to chemicals are included to demonstrate the application of toxicological principles To allow for seamless reader comprehension and further exploration of covered topics the work is supplemented with numerous illustrations to clarify and summarize key points as well as annotated bibliographies In the 4th edition all chapters and references have been updated to account for the latest scientific thinking and new color figures have been added New topics covered in 4th Edition of Principles of Toxicology include Regulatory toxicology including the key regulatory framework in which much of the field of toxicology operates Alternative methods in toxicology including cutting edge approaches to developing new information on the toxicity of drugs and chemicals The dilemma of selecting safe exposure limits guiding readers through practical considerations and pitfalls in developing and using safe exposure limits Ecological risk assessment with detailed discussion of methods and considerations when evaluating the effects of contaminants on plants and animals Providing information on the principles of toxicology and the application of those principles to solve problems in environmental and industrial settings Principles of Toxicology serves as an excellent textbook resource for advanced undergraduate graduate and professional students in a range of environmental and health fields It is also valuable to health professionals who need toxicological information and assistance beyond what is found in an introductory text to general toxicology     *Environmental Health Perspectives* ,1993

*High-Throughput Phenotyping in the Genomic Improvement of Livestock* Fabyano Fonseca Silva, Guilherme J. M. Rosa, Gota Morota, 2021-08-03     Validation Approaches for Alternative Methods Leon Bruner, 1997     **Alternatives to Laboratory Animals** ,2009     In Vitro Skin Toxicology Andre Rougier, Alan M. Goldberg, Howard I. Maibach, 1994

Animal Alternatives, Welfare, and Ethics L. F. M. (szerk.) Zuthpen, Michael Balls, 1997 Hardbound The objective of this conference was to provide a global overview of the present status of replacement alternatives reduction alternatives and refinement alternatives review the progress that has been made with respect to their implementation within the fields of biomedical research testing and education promote the exchange of information on recent developments in animal alternatives and contribute to an ongoing dialogue between the animal protection movement the scientific community the regulatory authorities and industry These objectives were fully achieved and the results have been published in this volume The contents of this book provide an up to date overview of the various aspects dealing with the development validation and

use of animal alternatives In addition current topics on animal welfare and ethical aspects of animal experiments are covered  
*Proceedings of the Indian National Science Academy* Indian National Science Academy,2001    *Euro Abstracts* ,1996  
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Verification of Concurrent Systems Patrice Godefroid,1996-01-24 This monograph is a revised version of the author s Ph D  
thesis submitted to the University of Li ge Belgium with Pierre Wolper as thesis advisor The general pattern of this work is to  
turn logical and semantic ideas into exploitable algorithms Thus it perfectly fits the modern trend viewing verification as a  
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logical issues Patrice Godefroid uses state space exploration as the key technique which as such or elaborated into model  
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presented provide a significant reduction of memory and time requirements for protocol verification    *Women's Therapy*  
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