

APPROACH TO ALTERNATIVE RAPID MICROBIOLOGICAL METHODS

Note: - The following chapter is for information and is not an official requirement.

Introduction

The purpose of this chapter is to provide guidance for selection and validation of methods for use as alternatives to the official compendial microbiological methods. For microbial recovery and identification, microbiological testing laboratories sometimes use alternative test methods to those described in the general chapters of IP for a variety of reasons. Alternative microbiological methods also known as Rapid microbial methods (RMMs) are the technologies that allow the user to get microbiology test results faster compared with traditional culture-plate methods.

In general, rapid methods can be grouped into three distinctive categories in accordance with their application. These categories include qualitative, quantitative, and identification methods. Qualitative rapid methods provide a presence or absence result that indicates microbial contamination in a sample. Quantitative methods provide a numerical result that indicates the total number of microbes present in the sample. Identification methods provide us with a species or genus name for the microbial contaminant in a sample.

Since RMMs may produce sensitive, accurate results, however while using RMMs, acceptance criteria will change in comparison to conventional methods (Quantitative/Qualitative tests), if this is the case, the responsibility lies with the manufacturer/user to produce/show the similarity/equivalence of the results/acceptance criteria while using RMMs. Since the Acceptance criteria mentioned for Microbiological quantitative/qualitative tests in IP is conclusive.

Traditional methods of microbial detection tend to be labor-intensive and take more than a day to yield results. Alternative methods for microbial detection can be sensitive, precise, quick and reproducible test results when compared with conventional, growth-based methods. Rapid methods normally involve some form of automation, and the methods often capture data electronically. Alternative microbiological methods tend to be based on various technology platforms. The more common technologies include nucleic-acid-based detection, which uses DNA or RNA targets; antibody-based detection; biochemical; enzymatic detection such as adenosine triphosphate (ATP) methods; impedance methods; and flow-cytometry-based methods.

The word “rapid” is often used to describe the range of techniques employed, some of the methods included within this collective do not give a more rapid result; they instead provide a more accurate, precise, or detailed result and therefore these methods are termed as “alternative methods”.

These alternative microbiological methods can be applied to a range of microbiological tests, including raw materials, water, intermediate products, final products, and environmental monitoring. There is a sufficient range of RMMS to provide an assessment of the microbiological quality throughout an entire production operation. These can be also used in understanding formulations better in terms of microbial robustness.

These methods can be used as alternatives to four major types of conventional microbiological determinations such as

- i. Qualitative tests for the presence or absence of microorganisms
- ii. Quantitative tests for enumeration of microorganisms
- iii. Quantitative tests for potency or toxicity
- iv. Identification tests

TYPES OF RAPID MICROBIOLOGICAL METHODS

Rapid or alternative methods can be categorized in multiple means. One way is based on technology or application. Such as:

a. Growth-based Methods:

Growth-based methods are those where a detectable signal is usually achieved following a period of subculture. These methods generally involve the measurement of biochemical or physiological parameters that reflect the growth of microorganisms. These methods aim to detect actively growing microorganisms. The methods continue to use conventional liquid or agar media.

b. Direct Measurement:

Direct measurement is where individual cells are differentiated and visualized. These methods generally use viability stains and laser excitation for the detection and quantification of microorganisms without the need for cellular growth.

c. Cell Component Analysis:

Cell component analysis is where the expression of specific cell components offers an indirect measure of microbial presence. These methods generally involve the detection and analysis of specific portions of the microbial cell, including ATP, endotoxin, proteins, and surface macromolecules.

d. Optical Spectroscopy:

Optical spectroscopy methods utilize light scattering and other optical techniques to detect, enumerate, and identify microorganisms.

e. Nucleic Acid Amplification

Nucleic acid amplification technologies are those such as PCR-DNA amplification, RNA-based reverse-transcriptase amplification, 16S rRNA typing, gene sequencing, and other novel techniques.

f. Micro-Electrical-Mechanical Systems:

Micro-Electrical-Mechanical Systems (MEMS) utilize microarrays, biosensors, and nanotechnology to provide miniaturized technology platforms.

It is important that care is taken in choosing an alternative or rapid method for a particular application. The method must determine a product's critical quality attribute and adhere to appropriate good manufacturing practice principles and validation requirements. Alternative methods of analysis may be used for control purposes, provided that the methods used are shown to give results of equivalent accuracy and enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. Automated procedures utilizing the same basic chemistry as the test procedures given in the monograph may also be used to determine compliance. Such alternative or automated procedures must be validated and are subject to approval by the authority competent to authorised manufacturer of substance or product.

In the event of doubt or dispute, the methods of the Pharmacopoeia are alone authoritative and only the result obtained by the procedure given in the pharmacopoeia is conclusive.

In some ways, the process of applying/introducing an alternative method does not differ significantly when compared with implementing a conventional method. The key points of ensuring the method is validated and shows acceptable recovery rates or accurate identification does not differ whether alternative or conventional methods are used.

When choosing to implement an alternative method, it is important to ensure the new method is appropriate for its application.

Any methods that are being adopted need to provide the same results or better than the method which is currently in use that already gives an acceptable level of assurance.

Validation of a microbiological method is the process by which it is experimentally established that the performance characteristics of the method meet the requirements for the intended application, in comparison to the traditional method. Validation of microbiological methods shares some of the same concerns, although consideration must be given to the unique nature of microbiological assays.

Validation of these methods is required and Validation will be centered on two key aspects: the assessment of the equipment and an assessment of the materials that the rapid method will assess to demonstrate that microorganisms can be recovered from the material under test.

The validation strategy should reflect the alternative method selected. Some methods that are based on analytical chemistry will suit validation criteria that include accuracy and precision, specificity, limit of detection, limit of quantification, linearity and range, and ruggedness and robustness. However, microbiology methods do not necessarily lend themselves to this approach to validation. It is important for each validation parameter to be addressed, it may not be necessary for the user to do all of the work themselves. For some validation parameters the RMM vendor helps to perform the validation experiments. Validation studies of alternate microbiological methods should take a large degree of variability into account. When conducting microbiological testing by conventional plate count, by considering many conventional microbiological methods are subject to sampling error, dilution error, plating error, incubation error, and operator error.

Therefore, the following validation strategies may be adopted:

Define the characteristic of the current test that the alternative method is to replace.
Determine the relevant measures that establish equivalence of the RMM to the current method.
This may require statistical analysis.

Demonstrate the equivalence of the alternative method to the current method in the absence of the product sample.

Demonstrate the equivalence of the alternative method to the established method in the presence of the test sample.

It is not the purpose of this chapter to recommend one method/technique over another, nor is it the intention to provide an exclusive or exhaustive list of alternative methods that can be used for pharmaceutical microbiological control.

The information herein may be used, however, in the process of choosing an alternative microbiological method as a supplement or as an alternative to pharmacopoeial microbiological methods and to give guidance on validation of the chosen method.

VALIDATION OF QUALITATIVE TESTS FOR DEMONSTRATION OF VIABLE MICROORGANISMS IN A SAMPLE

Specificity

The specificity of an alternate qualitative microbiological method is its ability to detect a range of microorganisms that may be present in the test article. In conventional microbiological methods it is adequately addressed by growth promotion ability of the media for qualitative methods and can be rely upon growth to demonstrate presence or absence of microorganisms. However, for those methods that does not require growth as an indicator of microbial presence, the specificity of the assay for microbes assures that extraneous matter in the test system does not interfere with the test.

Limit of Detection

The limit of detection is the lowest number of microorganisms in a sample that can be detected under the stated experimental conditions. A microbiological limit test determines the presence or absence of microorganisms, e.g., absence of *E. coli* in 1g. Due to the nature of microbiology, the limit of detection refers to the number of organisms present in the original sample before any dilution or incubation steps; it does not refer to the number of organisms present at the point of assay.

Ruggedness

The ruggedness of a qualitative microbiological method is the degree of precision of test results obtained by analysis of the same samples under a variety of normal test conditions, such as different analysts, instruments, reagent lots, and laboratories. Ruggedness can be defined as the intrinsic resistance to the influences exerted by operational and environmental variables on the results of the microbiological method. Ruggedness is a validation parameter best suited to determination by the supplier of the test method who has easy access to multiple instruments and batches of components.

Robustness

The robustness of a qualitative microbiological method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. Robustness is a validation parameter best suited to determination by the supplier of the test method. As there are no agreed upon standards for current methods, acceptance criteria are problematic and must be tailored to the specific

technique. It is essential, however, that an estimate of the ruggedness of the alternate procedure be developed. The measure of robustness is not necessarily a comparison between the alternate method and the traditional, but rather a necessary component of validation of the alternate method so that the user knows the operating parameters of the method.

VALIDATION OF QUANTITATIVE ESTIMATION OF VIABLE MICROORGANISMS IN A SAMPLE

The plate count method is the most common example of this class of tests used to estimate the number of viable microorganisms present in a sample. The membrane filtration and Most Probable Number (MPN) multiple-tube methods are other examples of these tests. The latter was developed as a means to estimate the number of viable microorganisms present in a sample not amenable to direct plating or membrane filtration

For this validation considerations can be interpreted as:

Accuracy:

The accuracy of this type of microbiological method is the closeness of the test results obtained by the alternate test method to the value obtained by the traditional method. It should be demonstrated across the operational range of the test. Accuracy is usually expressed as the percentage of recovery of microorganisms by the assay method.

Suspensions at the upper end of the expected range and then serially diluted down and testing alongside the compendial method. The alternate method should provide an estimate of viable microorganisms not less than 70% of the estimate provided by the traditional method, or the new method should be shown to recover at least as many organisms as the traditional method by appropriate statistical analysis. It is not dependent on the growth of the microorganisms to form colonies or develop turbidity. This is determined in the *Specificity* evaluation.

Precision:

The precision of a microbiological method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation). However, other appropriate measures may be applied. A statistically significant number of replicates should be used. And the level of variance should generally be within the 10–15% and should not be larger than that found within the pharmacopeia method.

Specificity:

The specificity of a quantitative microbiological method is its ability to detect a panel of

microorganisms suitable to demonstrate that the method is fit for its intended purpose. This is demonstrated using the organisms appropriate for the purpose of the alternate method. It is important to challenge the alternate technology in a manner that would encourage false positive results (specific to that alternate technology) to demonstrate the suitability of the alternate method in comparison to the traditional method.

This is especially important with those alternate methods that do not require growth for microbial enumeration Carried out using a range of microorganisms.

Limit of Quantification:

The limit of quantification is the lowest number of microorganisms that can be accurately counted. As it is not possible to obtain a reliable sample containing a known number of microorganisms, it is essential that the limit of quantification of an assay is determined from a number of replicates. Minimum no. of replicates should be 5 and at each of at least 5 different points across the operational range. The limit of quantification should not be a number greater than that of the traditional method.

Linearity

The linearity of a quantitative microbiological test is its ability to produce results that are directly proportional to the concentration of microorganisms present in the sample within a given range. The linearity should be determined over the range of the test. A method to determine this would be to select at least 5 concentrations of each standard challenge microorganism and conduct at least 5 replicate readings of each concentration.

Range:

The results found in precision, accuracy, and linearity can be used here in order to determine the upper and lower limits of the alternative method's detection.

Robustness:

Different variations of the normal test conditions e.g., different analysts, different instruments, and different reagent lots.

During the course of validation, deviations from the established criteria may occur. The implications of these will depend upon the seriousness of the issue and the degree of drift from established parameters. The deviation may or may not lead to a recommencing of the validation after an appropriate change has been made. In the most serious cases, the deviation can lead to the abandonment of the qualification and the rejection of the equipment or system. All deviations require a deviation report to be generated. Deviation reports must be reviewed by

a competent expert and be accepted by quality assurance.

The validation of alternative method will includes the plan for equipment qualification which contains IQ, OQ and PQ. (Ref: 2.5.10 Validation of Analytical Procedures)

Method Transfer

If a validated method is transferred to another laboratory (including third parties), appropriate change management should be in place. Full validation of the equipment (IQ/OQ/PQ) will need to be carried out. Complete validation may not be required, but, as a minimum, it should demonstrate that the method gives equivalent or comparable results to the original laboratory.