Journal of
Microbial & Biochemical Technology

Sandle, J Microb Biochem Technol 2015, 7:4
DOI: 10.4172/1948-5948.1000209

Short Communication Open Access

Towards a Rapid Sterility Test?

Tim Sandle*
BPL, Dagger Lane, Elstree, Hertfordshire, WD6 3BX, United Kingdom

Keywords: Sterility; Toxins; Microorganisms; Fluid thioglycollate medium

Introduction

Sterility test is an established method for detecting the presence of viable forms of microorganisms in or on finished pharmaceutical products. Sterility, in this sense, means that a product is free from viable microorganisms (although not necessarily metabolic by-products or toxins). The classic form sterility test examines a pharmaceutical product in contact a culture medium, as a way of detecting the possible presence of viable microorganisms. The test is mandatory for all aseptically filled products.

In recent years a number of new technology platforms have emerged. This has been facilitated by a change in policy by the U.S. Food and Drug Administration (FDA), opening the door to alternatives to the pharmacopeia methods. This short review assesses some of these technologies.

Culture Based Sterility Testing

The classic, culture based version of the test first appeared in the British Pharmacopeia in 1932 and later in the United States Pharmacopeia in 1937 [1]. Since then the test, despite modifications to culture media and challenge organisms, has remained relatively unaltered.

The test exists in two forms: for products that can be filtered, the preferred method is membrane filtration. Here a proportion of the products are divided into two and filtered through a 0.45 µm filter. Once filter is incubated in soybean casein digest medium (SCDM) and the other in fluid thioglycollate medium (FTM). The other version of the test is direct inoculation. Here a portion of the product under test is transferred into the two different culture media. The media are incubated for 14 days and then inspected for turbidity as a qualitative indicator of microbial growth. The incubation time is 14 days at 30-35°C for the FTM medium and 20-25°C for the SCBM medium. A test for microbial inhibition is used to verify method suitability [2].

The classic sterility test has a number of weaknesses. These relate to the small number of articles presented for testing (a necessary weakness given the test is a destructive one) together with the vagaries of representative sampling [3]. Another issue falls with the incubation and media parameters: the test is only capable of growing those microorganisms that can be recovered in the particular culture media and at the temperatures selected for incubation and in relation to the incubation time. Furthermore, many microorganisms in pharmaceutical environments are in a stressed or sub lethally damaged state, or they may simply be active but non-culturable, meaning that they would not necessarily be detected in the test even if they were present in the product. Finally, the test is relatively labour intensive and even though protection is offered through isolator technology, the risk of adventitious exists leading to a so-termed false positive.

Rapid Microbiological Methods

Partly due to the weaknesses mentioned above, a number of rapid and alternative microbiological methods have emerged on the market. A further advantage associated with these methodologies, in addition to addressing the described weaknesses, is in improving the time-to-result in that the standard 14 day incubation can be reduced. This allows for a quicker assessment of any microbial contamination risk to be made [4].

The types of technology can be divided into: [5]

a) Growth based technologies

These rely upon the measurement of biochemical or physiological parameters in relation to the growth of microorganisms. For the microorganisms to be detected, the microorganisms must grow and proliferate.

As an example of this technology type, portions of finished product samples are added directly to bottles of liquid culture media and incubated in the system. During microbial growth, CO_2 in the closed container accumulates and this is detected by a fluorometric sensor. These systems can automatically monitor the sensor at set time intervals, and the generation of CO_2 indicates the presence of growing microorganisms.

In a second example, microorganisms can be cultured based on traditional methods. In order to speed-up detection, digital imaging technology can be used to automatically enumerate micro-colonies. With this, illumination with blue light excites micro-colonies to auto-fluoresce [6].

b) Viability based

Viability based systems use viability stains and excitation for the detection and quantification of microorganisms without the need for cellular growth, such as by using flow cytometry and solid-phase cytometry.

Here, a sample is filtered through a micro-porous sieve. Any microorganisms found are retained on the membrane and subsequently labelled with a viability stain. After staining the labelled organisms are scanned using digital fluorescent microscopy at specific excitation and emission wavelengths. By using image processing software, a reader unit can analyse fluorescent objects relating to the size, shape and fluorescent signals from most microorganisms. It is possible with this technology to use different fluorescent stains or DNA-probes, in order to screen for specific microorganisms. An advantage with such methods is that they are non-destructive and allow detected microorganisms to be later cultured.

***Corresponding author:** Tim Sandle, Head of Microbiology, BPL, Dagger Lane, Elstree, Hertfordshire, WD6 3BX, United Kingdom, Tel: +44 (0)20 8957 2200; Fax: +44 (0)20 8957 2601; E-mail: tim.sandle@bpl.co.uk

Received June 22, 2015; **Accepted** July 02, 2015; **Published** July 09, 2015

Citation: Sandle T (2015) Towards a Rapid Sterility Test? J Microb Biochem Technol 7:4 217-218. doi:10.4172/1948-5948.1000209

c) Cellular component methods

These systems, such as ATP detection, examine for specific portions of the microbial cell. For example, with one method, after a product has been filtered, any microorganisms present are collected on a membrane. These are then lysed with extractant. Luciferin and luciferase enzyme are added and light is detected with a proprietary luminometer. A photomultiplier tube amplifies the photons and results are reported as Relative Light Units (RLU).

An alternative is to release free fluorochrome into the microorganism cytoplasm and then to expose to the cells to an excitation wavelength for the fluorochrome and enumerate the number of microbial cells using a reader.

The implementation of any of these methods requires an initial risk assessment and method validation. Validation should focus on demonstrating that the test can recover a wide range of different microorganisms and showing that inhibition of microbial growth does not occur [7].

Key validation criteria to consider include accuracy and precision, where different lots of the same product can be challenged with a low level of microorganisms; specificity, in order to demonstrate that extraneous factors are not interfering with the test; limit of detection, which is also based on a low level challenge; and robustness, where certain test parameters can be altered to show that recovery of microorganisms can be achieved in a consistent manner.

Arguably the most important criterion is the level of sensitivity. Here, the selected method must be able to detect down to one microbial cell for all possible types of microorganisms, in relation to the sample size of the product tested.

Summary

This short article has looked at some of the alternative methods for sterility testing. The tests presented offer tangible replacements for the sterility test for aseptically filled products (for terminally sterilised products, the potential to use parametric release in lieu of sterility testing exists [8]). To implement a rapid test to replace a compendial method takes time, considerable validation and a case to a regulatory authority. In addition, selection between the competing technologies requires a careful choice. Should these points be overcome, however, the adoption of rapid microbiological methods for sterility testing can achieve more accurate and faster results.

References

1. Sykes G (1956) The technique of sterility testing. *J Pharm Pharmacol* 8: 573-588.
2. Sandle T (2011) *Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices*, New Delhi.
3. Brown MRW, Gilbert P (1977) Increasing the probability of sterility of medicinal products. *J of Pharmacy and Pharmacology* 27: 484-491.
4. Allison DG (1999) A review: Taking the sterile out of sterility. *Journal of Applied Microbiology* 87: 789-793.
5. Sandle T (2013) *Sterility Testing of Pharmaceutical Products*, DHI /PDA: Bethesda, MD, USA.
6. Moldenhauer J Sutton SV (2004) Towards an improved sterility test. *PDA Journal of Pharmaceutical Science and Technology* 58: 284-286.
7. Miller MJ (2013) The Current State of Rapid Microbiological Methods. *Pharmaceutical Microbiology Forum* 19: 2-14.
8. Haberer K (2004) Terminal Sterilization and Parametric Release. In Williams K L *Microbial Contamination Control in Parenteral Manufacturing*, New York.