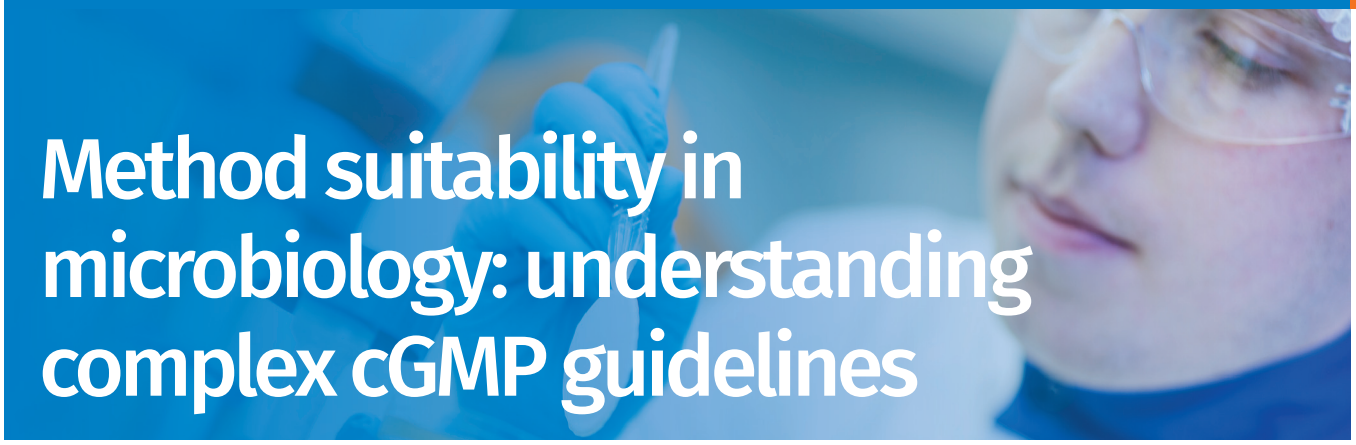

Method suitability in microbiology: understanding complex cGMP guidelines

Particularly in the pharmaceutical industry, microbiological method development and, specifically, method suitability is often overlooked or left until the end of a project. However, it is important to give both method suitability and development due consideration as it can be a complicated process involving wide specifications, vague parameters and the inherent variation that comes from working with living organisms.

Inherent issues when using microorganisms in testing

Microbiological counts are our 'best estimate' based on the widely accepted quantifier known as colony forming units (CFUs). CFUs are arbitrarily defined to come from one single microorganism cell which divides to provide a single colony on an agar medium. This is unlikely to be the case, however, and a CFU may arise from a clump or cluster of a number of cells. Dilution may also introduce variability and determines the limit of detection surrounding the CFU count achieved.

Microorganisms are affected by a number of other factors which may or may not be within the control of the test operator such as environmental stresses, growth phase and nutrient availability[1]. Any microorganisms used should also be within five passages from the original strain as further passaging of the organism can cause phenotypical changes. These are just some of the complications or limitations we face in microbiological examinations from the outset.

Method suitability testing

In microbiological terms, method suitability testing assesses residual antimicrobial activity of the product under test to ensure that the results achieved in recovery test media are truly representative. Ideally you will produce a method of testing that effectively neutralises any antimicrobial effect and will allow growth of control organisms in the numbers expected. Products likely to have this type of effect may contain preservative agents, anti-microbial or bacterial or fungi-static compounds.

The test in principle involves introducing a low inoculum level (usually <100CFU) of microorganisms set out in the USP and Ph. Eur standards into the appropriate stage of the test (after neutralisation) to assess any residual antimicrobial effect. The standard microorganisms used are *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans* and *Aspergillus brasiliensis* as these cover a variety of organisms such as Gram positive and Gram negative bacteria, spore formers, yeast and fungi[2,3,4]. The method of assessing if neutralisation has been successful varies depending on the method employed.

It is imperative that method development is carried out to ascertain the best technique for use and to understand the factors that may have to be considered. Ensuring you have the appropriate controls to compare is vital.

Neutralising

There are three common methods used in the neutralisation of antimicrobial effects: chemical inhibition, dilution and filtering/washing[4]. Often, concentration of a product is directly linked to its effectiveness as an antimicrobial so dilution approaches can be used to negate the antimicrobial effects.

Filtration

Filtration is also commonly used to neutralise the antimicrobial effect of a product, particularly in sterility testing. In membrane filtration, the organism is retained by the filter due to size differential and the antimicrobial agent passes through and becomes the filtrate. Usually, low binding filters or extra washing steps are necessary and employed to reduce the likelihood and effect of residual anti-microbial agent from the product preventing growth of the microorganism on the filter[5,6].

It is important in each case to ensure you are assessing the neutraliser efficacy and toxicity as you do not want the neutraliser itself to have a negative impact on the test microorganisms employed.

Conclusion

It is evident that there is much to consider when conducting any microbiology testing or pharmaceutical method suitability testing and it should be equally considered alongside any other required analyses. Safety testing such as microbiological method validation, verification, development, suitability and the resulting on-going quality control tests are vital in consistently ensuring only safe pharmaceutical products go to market.

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References

1. Approaching Microbiological Method Validation. Tim Sandle. 2015
2. USP General Chapter 61 Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
3. USP General Chapter 62 Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms
4. USP 71 Sterility Tests
5. Method Suitability Control Studies for Microbial Testing: Quantitative Comparisons. Institute of Validation Technology (IVT), Scott Sutton, 2013
6. USP 1227 Validation of Microbial Recovery from Pharmacopoeial Articles