Flexibility in sterility testing and managing risks of false positives

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Causes and effects of false positives and false negatives in sterility testing.
This paper reviews the background to false positives and false negatives associated with the sterility test process. It explores the potential causes of such results and suggests methods of mitigation. It also includes an insight into the role hydrogen peroxide vapor bio-decontamination technology can have on false positives and false negatives while indicating how this technology can also be used to eliminate them.
Sterility assurance

Sterility assurance begins at the process design stage. It can be effectively managed by advanced monitoring to detect deviation to predefined specifications risks and by applying quality by design (QbD) principles supported by risk assessed control measures (typically defined as critical quality attributes - CQAs). It is important to set these requirements in the context of quality risk management (QRM), with sterility testing just a final check for major contamination. Single unit sterility test failure may not mean the batch is non-sterile or should be discarded.

Sterility testing methods are useful for detecting major contamination in a batch. However they are analytically and statistically limited in their ability to detect very low-level batch contamination. Contaminated product units may be a result of deviations in contamination control during aseptic processing. The QC sterility test used for batch release only provides a small part of the information needed to evaluate whether a batch is sterile (assuming it has been prepared aseptically without terminal sterilization).

With the increase of medicinal and therapeutic products that have a biological profile which cannot be terminally sterilized, there is an increase in the requirement for aseptic processing where both biological contamination control and test for sterility play a part in batch release.

Sterility testing failures in batch release provide a significant medicinal product supply chain risk (patient risk) and business risk (stock held or discarded). In addition, there can be a huge negative impact on the profile of the business. Such risks reinforce the need to manage and prevent false positive results. The steps in processing samples from the point of fill, or critical process step, to incubation in a microbiological laboratory are many. They are open to environmental deviation that would be reported as a positive growth result, potentially failing the batch.

Within the microbiological laboratory, the challenge is to manage the sterility test process with samples that can vary in closure presentation, unit and batch size. Test samples need processing into a sterility test system. They also need to be removed from the processing environment as a membrane filtration cartridge with growth media that requires incubation. In both cases, there are chances of creating false positives within the processing environment or during the passage through the aseptic connections if the sterility testing environment has become compromised e.g. contaminated during the material transfers procedures.

Risks potentially leading towards false positive sterility test results

The following list identifies some potential risk areas that may lead to false positive sterility test results:

- A compromise of the aseptic state of the sterility test process zone by the operator during any stage of the process, especially due to the tedious nature of some processes where actions are repeated over and over again.
- A compromise of the aseptic state of the sterility test process due to poor ergonomics with bad posture leading to poor handling practice.
- Poor establishment of an aseptic environment before aseptic assembly and connection of the test system / samples. This is typically due to the lack of a bio-decontamination process.
- Poor and ineffective bio-decontamination of test materials and product samples before entry to the test environment. This can allow biological contaminants to be transferred to the sterility test process environment.
- Over reliance on the barrier technology to exclude any biological contamination. This can mean that good aseptic technique is not used in the aseptic connections and during aseptic transfers.
- Poor bio-decontamination of the drain connection in the base of the barrier. This is because, during aseptic assembly, barrier gloves may become contaminated and biological contamination transferred to other critical aseptic connections.
- Poorly sealed closures during the preparation for transfer disinfection leading to sterility compromises of product samples before the test starts.
- Environmental monitoring (EM) sampling interventions can compromise the aseptic process zone if not part of the system design. They can encourage poor technique or lead to open access into the process zone. In addition, monitoring sample recovery has to be secure and not facilitate biological contamination during the recovery or transfer of monitoring samples to the test laboratory. Typically EM plates and membrane filtration sterility test samples are incubated together.

Risk mitigation of potential false positive sterility test results

The starting point for mitigating the risk of biological contamination in a sterility test process environment is the use of barrier separation technology (isolators) with closed system operation and material transfers.

Materials entering the sterility test process zone must use a validated high-level disinfection procedure. To ensure a high degree of assurance and repeatability, a fully automated disinfection process e.g. hydrogen peroxide vapor bio-decontamination, should be used.

The process flow and ergonomic design within the separation barrier technology must facilitate good aseptic technique during connections and in-process sample / media transfers. In addition waste management should not compromise the aseptic process zone or sterile products.

Bio-decontamination processes, sterility test environmental control, sterility test process operations and process compatibilities require control, monitoring and alarm in deviation from specified conditions/parameters. Real time monitoring provides an extra level of risk management to facilitate test suspension (not putting samples at risk) while an environmental deviation is investigated.
Additional risks leading to potential false negative results

• The bio-decontamination process could compromise the test materials. It is essential to avoid chemical disinfectant contamination that inactivates biological contamination in the product during the sterility test process and before incubation of the test media-membrane filtration samples.

• Poor closure of product seals can allow the disinfection agent to mix with the product and inactivate microorganisms before sterility testing. This can be particularly noticeable when exposing the product to the highly effective hydrogen peroxide vapor bio-decontamination process in a transfer bio-decontamination step.

• There can be inactivation of biological contamination that arrives on the growth media plate before culturing / incubation if environmental monitoring plates have a water-condensate layer that is exposed to hydrogen peroxide vapor during transfer disinfection.

Risk mitigation of potential false negative sterility test results

Product sample closures need to permit an effective bio-decontamination process step before entry into the sterility test process zone. Integral closures would eliminate potential biological contamination inactivation before the sterility test (hence would avoid a false negative result).

Test materials including membrane filter cartridges, product and growth media transfer tubes must be validated to ensure that the disinfection agent e.g. hydrogen peroxide, has not been ‘retained’ in its active form as this would potentially impact on fertility of the growth media and potential to support microbial growth. Hydrogen peroxide retention on the filter membrane potentially would inactivate microorganisms on contact before suspension in growth media and subsequent incubation. Such an impact may lead to false negative sterility test results.

Summary

Managing false positives in sterility testing is a critical objective. It reduces the risks in the medicinal product supply chain (risk to patients) and reduces the risks to the business.

There is a clear requirement for a risk based approach to process design, sterility testing and material transfers into the sterility test process zone where there can be a high risk of biological contamination transfer.

The use of barrier separation technology with secure and robust bio-decontamination processes, together with rapid gassing technology for test material transfer disinfection, can provide the optimum balance in false positive risk management and efficient process operations.

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