

Sterility Testing of Medical Devices: An Overview

Abstract

A sterile medical device is one that is free from viable microorganisms. Medical devices produced under standard manufacturing conditions in accordance with the requirements for Quality Management Systems (QMS) may have microorganisms present on and/or within them prior to sterilization (non-sterile products). The purpose of sterilization is to inactivate the microbiological contaminants to transform the devices from non-sterilized to sterilized. Sterility testing of medical devices is required during the sterilization validation process and routine release testing.

According to ISO 11737-2 (Sterilization of health care products -- Microbiological methods -- Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process), it is important to distinguish between the terms: test for sterility and test of sterility.

Test for sterility: Technical operation defined in pharmacopoeia performed on product following exposure to a sterilization process (release testing). The test can be performed in this case according to methods described in USP <71> and Eur. Ph. 2.6.1.

Test of sterility: Technical operation performed as a part of development,

validation or requalification to determine the presence or absence of viable microorganisms on product or portions thereof (sterilization validation process). In this case ISO 11737-2 must be considered, but is not applicable to:

- Sterility testing for routine release of product that has been subjected to a sterilization process
- Performing a test for sterility
- Culturing of biological indicators or inoculated products

The probability of survival of a pure culture of microorganisms after a physical or chemical treatment used to sterilize medical devices is determined by the number and resistance of microorganisms, and by the environment in which the organisms exist during treatment. This means that there is always a finite probability that a microorganism may survive regardless of the extent of treatment applied. Since the effectiveness of the sterilization cannot be fully verified by subsequent inspection and testing of the product, sterilization processes are validated and the performance of the sterilization process is monitored routinely to ensure the equipment is maintained.

Background

Manufacturers of sterile medical devices must demonstrate the microbiological safety of their

products by means of:

- Knowledge regarding the device's bioburden
- Validation of the sterilization process. An effective sterilization process has a very low Sterility Assurance Level (SAL), meaning that there is an extremely small likelihood (10^{-6}) of any infecting microbes surviving the sterilization process. SAL expresses the probability of a single item in a batch being non-sterile after being subjected to a sterilization process.
- Demonstrating sterility of the products manufactured (each released batch)
- Guaranteeing the sterility assurance during the shelf life of the product.

Regarding the evaluation of sterility on medical devices, manufacturers are required to perform the validation of the sterilization process according to different standards, such as ISO 11137.

Figure 1 shows where sterility testing on medical devices is applied before and during the manufacturing process. Even if the performance of the test is the same, the aim could be different on each step.

Feasibility Study/Evaluation

A feasibility study is a preliminary study that is conducted prior to the execution of the official

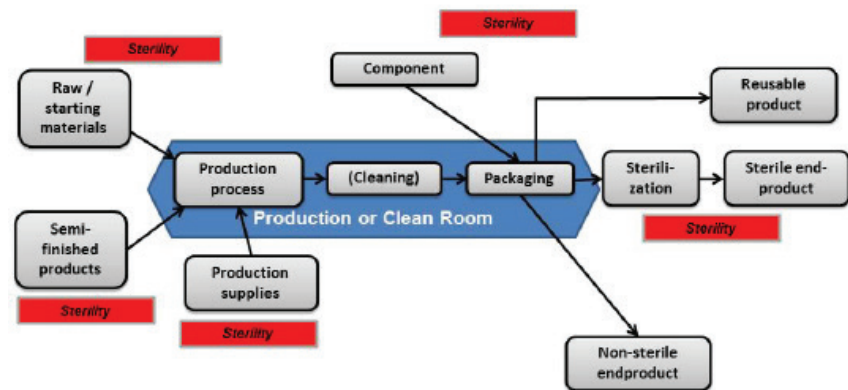
method suitability. The purpose is to examine and execute the steps that are to be taken during the official test method. This study can determine the difficulty of the process and if it needs to be modified for suitability.

The advantage of performing a feasibility study includes the ability to preview the study procedure to avoid risky or contamination-prone handling. During the feasibility study, if a proposed step requires a change during execution, the effect of the change/deviation is less impactful from a quality perspective. It allows for the opportunity to ensure the method captures the necessary steps that are likely to operate successfully during the official study to avoid false positive results.

The disadvantages of performing a feasibility study may include the cost and resources allocated to the study. In many cases, the cost and resources allocated are justifiable and save money. When an official method is executed, the expectation is that it is to be performed exactly as written. In the event that the test method does not operate as planned, the procedure may require changes, deviation, or even termination. The costs of approving, executing, deviating, and potentially terminating the test method can often exceed the cost of a feasibility study. However, a feasibility study is recommended for the testing of new products, especially if there is no historical data available. Feasibility studies are also recommended for products that are:

- Very large and difficult to handle
- Must be disassembled, cut or destroyed during testing

Figure 1: Scheme of Medical Device Manufacturing Process



- Known to exhibit inhibitory properties like bacteriostasis or fungistasis
- Highly viscous, as this is important when membrane filtration is required
- Colored or have natural turbid appearance, as this is important when the product is to be directly introduced to growth media
- Subjected to extreme pH concentrations

A feasibility study can be performed for any product. As mentioned previously, it provides a level of confidence regarding the successful execution and completion of the official test method. In addition, if the same laboratory conducting the feasibility study is expected to perform the official test method, the experience gained by the lab for managing the product is extremely beneficial as unknowns are minimized and assurances are maximized.

Methods

Sterility testing is a very challenging process that must be performed by trained and qualified laboratory

personnel. Tests for sterility are carried out by:

- **Direct Transfer of the product in suitable growth medium,** which is the preferred method according to ISO 11737-2 as the device is in direct contact with test media throughout the entire incubation period.
- **Membrane Filtration after extraction of microorganisms,** which is applicable for products with antimicrobial activities.
- **Addition of growth medium to the product.**

Depending on the product, additional preparation steps may be necessary like disassembly of the device, or the addition of surfactants to the medium or flushing of the device (e.g. catheters).

Fluid Thioglycollate Medium (FTM) and Soybean-Casein Digest Medium (TSB) are standard media generally used for sterility tests. According to ISO 11737-2, as a standard method, TSB can be used (as a single media) and preparations are incubated at

30+-2°C for 14 days. According to cEP and cUSP, containers are incubated for 14 days at 30 - 35°C (FTM) and at 20 - 25°C (TSB). The conditions are subject to the properties and production of the product.

Growth of microorganisms is determined by examining the turbidity of the media against a clear and transparent negative control after 14 days and several defined times within this period. In some cases turbidity may occur, although it is not attributable to the growth of microorganisms. In these cases, further steps are to be performed to detect any possible microbial growth by macroscopic examination, transferring parts of the cloudy medium into fresh medium and incubating for at least 4 more days, or by applying a subculture of the cloudy medium using commonly accepted microbiological practices. Positive samples are then investigated further, including identification of the contaminant and investigation of the test performance.

Method Suitability

Method suitability is performed to ensure the sterility test method as outlined in the relevant standard is appropriate for a specific device. Each device should have a customised and validated procedure for routine testing. Method suitability may be run concurrently with the sterility test, however should the method suitability fail, the results of the associated sterility test are invalid.

A sterility method suitability study is comprised of two sections:

1. Study Definition (including feasibility if required):

- Preferred methodology is discussed and confirmed, generally direct inoculation, but fluid pathway may also be an option.
- Confirmation of required number of test articles and definition of sample item portion (entire unit or part of, in the case of kits components to be tested, inclusion of packaging, etc.).
- As medical devices come in all shapes and sizes, determination of suitable sample size, method of sterile containment during testing and volume of test diluent also form part of the method development.
- The above information can be documented in a customised, client approved validation protocol.

2. Bacteriostasis & Fungistasis (B&F) Study:

A B&F study challenge of the defined test method by the addition of prescribed microorganisms (representative of the main bacterial groupings, yeast and mould) is shown in Figure 2. The suitability of the method is confirmed by the successful recovery of the challenge organisms at the completion of the test. This study can be performed singularly or in triplicate (per client requirements). A successful B&F study confirms that the device itself will not inhibit the recovery of any viable organisms should they be present in the test sample. Where inhibition (related to the test article) is observed, there are several ways in which the test method may be modified to reduce this antistatic effect. Some of the method

customisations to be explored are the addition of an appropriate substance to the specified test media, diluents or solvents, or to the preparation prior to testing.

Per ISO 11737-2, use of *Clostridium Sporogenes* is not required as it is not an aerobic or facultative organism.

Client-supplied isolates may also be included in validation studies if required.

Laboratory, Equipment, Environment for Sterility

According to ISO 11737-2, sterility tests should be conducted under aseptic conditions in controlled environments as indicated in ISO 14644, such as under a Laminar flow hood or bio safety cabinet in an assigned room. If a clean room environment is used, grade B/ISO 7

Organism	Culture Type	Nutrient Media
<i>Staphylococcus aureus</i>	ATCC 6538 or equivalent	FTM/ TSB
<i>Bacillus</i>	ATCC 6633 or equivalent	TSB
<i>Pseudomonas aeruginosa</i>	ATCC 9027 or equivalent	FTM/ TSB
<i>Candida albicans</i>	ATCC 10231 or equivalent	TSB
<i>Aspergillus brasiliensis</i>	ATCC 16404 or equivalent	TSB

areas are usually entered through personnel airlocks (grade C/ISO 8 and D/ISO 9) to minimize microbial and particulate contamination of protective clothing. According to GMP guidelines, limits for particles and microbial counts are defined. A regular environmental monitoring is established according to ISO 14698 for each clean room grade.

Figure 2: Challenge Organisms

All required equipment, consumables, growth media and test items are aseptically introduced into the clean area under an elaborated sanitization process. These transfers and tests are conducted by highly trained personnel skilled in aseptic techniques to ensure the minimization of false positive results.

Investigation of Positive Sterility Test Results & Sterility Test Failure

The investigation of sterility test failures is a process that requires attention to environmental data as well as many other factors, including training and sample difficulty. Moreover, data related to the product manufacturing and sterilization process should be considered during an investigation as the evaluated product may be not sterile.

False positive results could be due to laboratory contamination from the testing environment or technical error. Even the best handling and method conduction will show false positive results in 1:1000 cases. For this reason, when microbial growth is detected in a sterility test of a medical device the laboratory should start an investigation to determine the cause of the positive result.

The main potential root causes that must be investigated to determine if a positive sterility test is the result of a laboratory error are:

- Equipment (isolator/biosafety cabinet/clean room):
 - Malfunction or not working properly
 - Variability on microorganism reduction by decontamination

procedures (chemical disinfectant and UV) and sterilization processes (steam heat processes, cold peracetic / H₂O₂ for isolators).

- Disinfection procedure of the test sample:
 - Packaging configuration that is difficult to disinfect
 - Compatibility of packaging materials with decontamination agents
- Analyst
 - Qualification of the analyst
 - Microbial count of personnel monitoring
- Environmental conditions
 - Microbial count of the environment such as surfaces, settle plates, or Dynamic Air environmental control
 - Negative Controls (investigation on any peculiarities)
- Analytical Method
 - Unplanned deviation during the analysis
 - Level of handling required for the execution of the test
 - Dimension of test sample requiring the use of devices such as cut or forceps
 - Type of vessels used; closure integrity

Evaluation of investigation results should be performed in a case by case basis due to the diversity of products, clients and laboratories. Eurofins Medical Device Testing has more than 40 years of experience in

sterility testing of medical devices, encompassing simple items such as fluids, lenses or implants, and more complicated instruments like catheters, infusion applicators or immunoadsorbents.

References

ISO 11737-2: Sterilization of health care products -- Microbiological methods -- Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

ISO 11137-2: Sterilization of health care products -- Radiation -- Part 2: Establishing the sterilization dose

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“OECD Principles of Good Laboratory Practice”