Sterility Testing

Tepnel Pharma Services

The sterility of a product is defined by the absence of viable and actively multiplying micro-organisms when tested in specified culture media. The test is performed on the end-product and is one of the quality control tests specified for release of a batch of sterile product. The sterility test cannot be used to demonstrate the sterility of the entire batch but it may assist in identifying a non-sterile batch of product.

When selecting samples for sterility testing from a batch manufacture the number of containers tested per batch and quantity tested from each container should be, as a minimum, in accordance with the pharmacopoeia method followed. Samples from aseptic fills should be selected from at least the beginning, middle and end of the batch fill. Additionally, SOPs should define criteria for inclusion and collection of samples immediately after interruptions and operator interventions during the filling process. Samples from terminal sterilisation cycles should be selected from at least the potentially coolest part of the load if such a location was identified during validation studies, and from every load sterilised. If an original test is declared invalid, then any samples used for the repeat sterility test should reflect the original samples in terms of sampling locations or aseptic processing times.

To determine how many samples and what volume of sample will be required for sterility testing, the pharmacopoeias propose the numbers in Table 1 and Table 2 below:

To complete a validation the amount of sample required for testing needs to be multiplied by 3.

Table 1

Minimum Quantity to be used for each Medium Quantity Per Container	Minimum Quantity to be used (unless otherwise justified/authorised)
LIQUIDS Less than 1 ml 1 – 40 ml >40 ml and #100 ml >100 ml	Whole contents of each container Half contents of each container but Not less than 1 ml 20 ml 10% of contents of container but not less than 20 ml
ANTIBIOTIC LIQUIDS	1 ml
Other preparations soluble in water or isopropyl myristate	Whole contents of each container to provide not less than 200 mg
Insoluble preparations (creams & ointments)	Whole contents of each container to provide not less than 200 mg
SOLIDS <50 mg >50 mg and <300 mg 300 mg – 5 g >5 g	Whole contents of each container Half contents of each container but not less than 50 mg 150 mg 500 mg
Catgut & other surgical sutures for veterinary use	3 sections of a strand (each 30 cm long)
Surgical dressing/cotton/ gauze	100 mg per package
Sutures and individually packed single-use material	Whole device
Other medical devices	Whole device, cut into pieces or disassembled

Table 2

Minimum Number of Articles to be tested in relation to Batch Size Number of Items in Batch	Minimum Number of Items to be tested for each Medium (unless otherwise justified/ authorised)
PARENTERAL PREPARATIONS #100 containers >100 and #500 containers >500 containers Large volume parenterals	10% or 4 containers, whichever is greater 10 containers 2% or 20 containers, whichever is less 2% or 10 containers, whichever is less
OPHTHALMIC AND OTHER NON-INJECTABLE PREPARATIONS #200 containers >200 containers If product presented in single-dose containers	5% or 2 containers, whichever is greater 10 containers Apply scheme for parenteral preparations
BULK SOLIDS Up to 4 containers >4 and #50 containers >50 containers	Each container 20% or 4 containers, whichever is greater 2% or 10 containers, whichever is greater
ANTIBIOTIC SOLIDS Pharmacy bulk packages (<5 g) Pharmacy bulk packages ($5 g) Bulks and blends	20 containers 6 containers See bulk solids
DEVICES Catgut & other surgical sutures for veterinary use #100 articles >100 and #500 articles >500 articles	2% or 5 packages, whichever is greater (max. of 20 packages) 10% or 4 articles, whichever is greater 10 articles 2% or 20 articles, whichever is less

The PIC/S Recommendation on Sterility Testing, PI 012-3, specifies that the performance of the sterility test is done in a standard clean room environment. Recommendations involving the use of isolator technology for sterility testing are provided in the “PIC/S Isolators used for Aseptic Processing and Sterility Testing” document.

A clean room environment is one with a low level of environmental pollutants such as dust, airborne microbes, aerosol particles, and chemical vapours. A clean room has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size.

EU GMP guidelines require a clean room to meet particle counts as specified for a particular class at operation and at rest. At operation means during manufacturing or testing process, and at rest means when manufacturing or testing process is not carried out, but room air handling unit is on.

Class	Maximum particles/m3			
	At Rest	At Rest	In Operation	In Operation
	0.5 µm	**5 µm**	**0.5 µm**	**5 µm**
Grade A	3,520	20	3,520	20
Grade B	3,520	29	352,000	2,900
Grade C	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	n/a	n/a

The sterility test should be conducted within a class A Laminar Air Flow cabinet located within a class B clean room, or in a class A isolator within a class D environment. ICH QB4 states that the following sterility testing can be used as interchangeable - Ph. Eur. 2.6.1. Sterility, JP 4.06 Sterility Test, and USP <71> Sterility Tests. The test methodology should be in accordance with the pharmacopoeia method used. Membrane filtration of the product, with either an open or a closed system, is the preferred sterility test methodology. If the product cannot be filtered, then direct inoculation, immersion, in-situ incubation or combination methods as appropriate are acceptable.

Sterility testing should only be performed by personnel who have been trained, qualified and certified to perform the various tasks and procedures related to sterility testing.

The advantage of using a Contract Research Organisation with isolator technology to perform sterility test is:

- They have trained personnel who are dedicated to perform this testing who are continually actively doing it, thus they keep the necessary skills and techniques and manipulations fresh by testing multiple batches of different products daily.
- Isolator technology provides barrier technology in the performance of the test reducing risk of false positives and removing operators from the environment in which products are tested.
- With gaseous decontamination or sanitisation internal to the isolator, the inherent risk of contamination from an external source is reduced, giving additional confidence in the testing.

Tepnel has two Class A isolators in a Class D room within its new sterility suite.

The use of gaseous decontamination or sanitisation within the isolator ensures that the testing is conducted in an environment which is free from microbial contamination and therefore gives increased confidence in the result.

This gives added assurance that the testing is conducted in a completely sterile environment as preferred by Regulatory Authorities and QPs. It also gives Tepnel the ability to handle potent substances such as cytotoxic powders and liquids as the room is kept at positive pressure and the isolator at negative pressure thus reducing operator risk in the handling of carcinogenic or teratogenic compounds.

Useful References

- PIC/S Guide to Good Manufacturing Practice for Medicinal Products Annexes PE 009-14 Annexes, Annex 1, 1 July 2018.
- PIC/S Recommendation on the Isolators used for Aseptic Processing and Sterility Testing, PI 014-3, 25 Sep 2007.
- EudraLex Vol 4 GMP Guidelines to GMP, Annex 1, Manufacture of Sterile Medicinal Products (Corrected Version) 25 Nov 2008.
- IC/S Recommendation on Sterility Testing, PI 012-3, 25 Sep 2007.

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