Guidelines to Sterility Testing for Biologics

Overview

Guidelines for sterility testing of biologics is addressed in the various worldwide pharmacopeias (e.g., USP, EP and JP), Section 21 of the Code of Federal Regulations (CFR), International Conference on Harmonisation (ICH) and Food and Drug Administration Points to Consider (PTC) documents. This document provides a brief summary of the test methods and sample requirements for the most common types of biological products. The outline below represents existing regulations and current industry practice. Exceptions may exist depending on the product and process from which it was generated.

Sterility Testing Methods

Biological products manufactured under GMP conditions require sterility testing performed under GMP guidelines. There are two common types of sterility test methods:

- **Immersion (Direct Inoculation)**
  The immersion (direct inoculation) method requires the test article be inoculated directly into test media.

- **Membrane Filtration**
  The membrane filtration method requires the test article to first pass through a size exclusion membrane capable of retaining microorganisms. An appropriate amount of rinse fluid is then passed through the filter before transferring the membrane into the test medium.

The pharmacopeias and 21 CFR 610.12 recommend using two media for both the immersion and membrane filtration methods. In both test methods the test article or membrane is incubated for 14 days in the test media.

The majority of biological samples will be tested using the immersion method. However, for test articles with large volumes in which the entire contents must be tested or if there is a substance within the test article that is known or determined to be bacteriostatic or fungistatic (e.g., cell culture containing methotrexate), the membrane filtration method may be required.

WuXi AppTec provides sterility testing protocols that comply with both USP and 21 CFR 610.12 guidelines and can be conducted using the immersion or membrane filtration method. Protocols can be customized for those clients needing to meet EP or JP requirements. Isolator technology is available.

Sterility Method Suitability

It is important to determine if the test article that will be tested for sterility contains elements that will interfere with the growth of microorganisms within the growth media used for the assay. [This testing is commonly referred to as the Bacteriostasis/Fungistasis (B/F) test or sterility test validation, qualification or verification.]

The method suitability test is typically required only once for a given sample type, provided that no additional changes to the product, formulation or manufacturing process occurred. However, suitability testing could be performed on a periodic basis to confirm that no significant changes have occurred to the product or process that may affect the sterility assay results.

See reverse for a guide to sterility testing sample requirements.

Sample Requirements by Test Article Type

Important note regarding samples for sterility method suitability and when to perform the test:
It is ideal to perform the Sterility method suitability (B/F test) prior to performing the sterility test should adjustments need to be made to the sterility test because of the method suitability results. However, the compendia does allow sterility method suitability be performed concurrently with the sterility test using additional samples identical to those submitted for sterility testing. For the immersion method only – in certain situations and at the client's discretion (such as limited amount of product) – once the sterility test is completed, the post-sterility test materials can be used for the method suitability testing so that additional samples are not needed.

Cell & Virus Bank Testing

Cell and virus banks manufactured under cGMP conditions require sterility testing be performed per the appropriate test methods outlined in the pharmacopeias and the 21 CFR 610.12. Sampling requirements for cell and virus banks are not provided within USP, EP or 21 CFR 610.12 guidelines. However, ICH Q5D "Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products" states that sterility testing will be performed on individual containers. It also states that 1% of the bank or no less than 2 containers will be tested. This recommendation has become a standard industry practice. However, since many cell and virus banks are produced under GMP conditions some organizations use the USP/EP/JP Final Product testing guidelines as stated below. Either sampling plan has been accepted by worldwide regulatory authorities.

Unprocessed Bulk Testing (For Protein and Virus Products)

Within the biotherapeutics industry "Unprocessed Bulk" has many other descriptions, including cell/viral harvest, clarified cell/viral harvest, end of production cells or cells at the limit of *in vitro* cell age. Although the pharmacopeias and 21 CFR 610.12 do not reference or provide sterility guidelines for these sample types, the FDA documents ""Points to Consider in the Characterization of Cell lines Used to Produce Biologicals" (1993) and "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (1997) do reference the need for sterility or bioburden testing on each unprocessed bulk lot. These two documents reference 21 CFR 610.12 for guidelines on appropriate sterility test methods to use.

Since there is no reference in the pharmacopeias or 21 CFR 610.12 specifically related to unprocessed bulk material, no sampling guidelines are available either. However, industry practice is to use the sampling guidelines stated for bulk drug substance as detailed in the 21 CFR 610.12. Thus 10 mL/media (for a total of 20 mL) is recommended for sterility testing of unprocessed bulk material. If production-lot volume is limited, contact WuXi AppTec regarding options.

Bulk Drug Substance (BDS) Testing

Sterility testing is required on each manufactured BDS lot. Sampling requirements for Bulk Drug Substance is defined in 21 CFR 610.12, which states that no less than 10 mL will be tested. It is not clear if that is meant for each media or if the amount is to be split into the two test media. Industry practice has taken a conservative approach and 10 mL of BDS is tested in each media. Thus a total of 20 mL is recommended for sterility testing.

Final Drug Product (FDP) Testing

Sterility testing is required on each manufactured FDP lot. Sampling requirements for final drug product are provided in the pharmacopeias. The following tables outline the requirements as stated in the current USP.

Batch Size	Minimum Number to be Tested in Each Media
100 or less	10% or 4 containers, whichever is greater
101 – 500	10 containers
>500	2% or 20 containers, whichever is less
Bulk – Up to 4 containers	Each container
5 – 50	20% or 4 containers, whichever is greater
>50	2% or 10, whichever is greater

Volume / Container	Minimum Quantity to Test in Each Media
Less than 1 mL	The entire contents of each container
1 – 40 mL	Half the contents of each container, but not less than 1 mL
41 – 100 mL	20 mL
>100 mL	10% of the contents of the container, but not less than 20 mL

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For information regarding available sterility assays or to discuss the sterility test method most appropriate for your sample type, please contact a WuXi AppTec Account Manager at (+1) 651-675-2000 or 888-794-0077. Or visit www.wuxiapptec.com