An overview of sterility testing, the impact of Annex 1 and a look into the future of sterility testing

Sterility testing is a process which must be performed as part of the manufacture of sterile products, to provide confidence that they are free of any viable microorganisms which could harm patients. As it is not possible to test every single vial or ampoule of product that is being manufactured, a number of samples representative of the whole batch are taken at different times during the filling operation and tested for microbial contamination. The World Health Organisation adopted requirements for sterility testing in 1973 and today the guidelines for conducting sterility testing are present in various pharmacopoeias worldwide, including the United States Pharmacopeia (USP) and the European Pharmacopeia (EP).

Insourcing vs outsourcing

When conducting this mandatory process, manufacturers of sterile products have two options. Either they can perform the process in house inside a dedicated sterility testing lab or outsource the process to a third-party testing service. Outsourcing is typically more beneficial for smaller manufacturers, as setting up a sterility testing lab can have a high initial cost and high running costs. However, there are downsides to outsourcing such as longer wait times for test results leading to increased storage costs, particularly for products which require cold storage. Also, many testing services charge per test, which can become very expensive, particularly for larger manufacturers who manufacture a lot of product, so require a large number of tests. Finally, outsourcing sterility testing to a third party means there is less control over the process, which can ultimately result in unnecessary scrapping of viable product. This will be explained further on in this article.

Methods

There are two methods for testing the sterility of products - membrane filtration and direct inoculation. By far the most common method is membrane filtration, which involves passing the liquid product through two canisters, each containing a filter capable of retaining viable microorganisms, then filling each of the canisters with different types of growth media - one for growing aerobic organisms and the other for anaerobic organisms. By contrast, direct inoculation involves placing the product directly into the two canisters and is typically used for products that cannot be filtered, such as medical devices. With both methods, the cannisters will then be incubated at the appropriate temperature for 14 days (or 21 days for turbid products) and if there are no signs of growth after this time, the test is considered a pass and the product can be released for delivery to patients. If either canister shows signs of growth (turbidity) then the test is considered a fail.

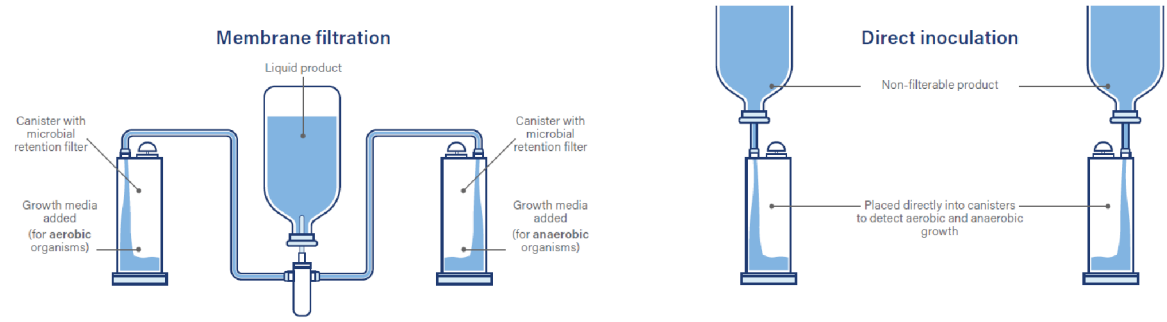


Diagram showing the two sterility testing methods

14 days is a long time for a drug manufacturer to wait to release a product to market, particularly if the product has a short shelf life, such as some cell therapy products. The long wait time can also result in additional storage costs for manufacturers, particularly if the product needs to be stored at a lower temperature. Therefore, industry is looking into ways to reduce the waiting time for results. This is discussed in further detail later in this article.

Test failure

The impact of a test failure can be significant for a pharmaceutical manufacturer as it typically results in:

- Withholding release and potentially scrapping the batch of product which failed the test
- Regulatory involvement
- Performing timely investigations
- Halting production of further product whilst investigations are performed
- Additional cleaning and disinfection of production areas

Ultimately a sterility test failure will result in financial losses to the manufacturer, and potentially drug shortages to patients. A genuine failure prevents contaminated product from being administered to and potentially harming a patient. However, sterility test failures are not always caused by contamination in the product.

False positives

A false positive result is when contamination from a source extrinsic to the product, such as the environment or the operator performing the test, finds its way into the test and causes a failure. This will suggest that the product is contaminated when potentially it is free of microorganisms. In the case of a sterility test failure, the burden of proof is on the manufacturer to demonstrate that the failure is the result of contamination from the operator and/or lab environment. In reality, it is very difficult to prove this, so a false positive result often leads to perfectly safe and effective products being scrapped unnecessarily.

Environment

To reduce the risk of false positives, the sterility testing process should be performed in an aseptic environment. Historically, the common approach to achieve this was by performing the process in a biological safety cabinet (BSC) or laminar air flow (LAF) hood. However, this means the process is still open to the environment and operator, and thus there is still a risk of false positives occurring.

Gowned operator performing sterility testing in a laminar air flow hood

More recently, the industry is shifting towards the use of isolators which provide a physical barrier between the operator/environment and the test, substantially reducing the risk of false positive results. It is important to note that regulators and pharmaceutical advisory committees around the world have requirements and recommendations concerning the environment in which sterility testing should be conducted.

Regulations

EU GMP Annex 1: Manufacture of Sterile Medicinal Products section 10.6 states *"The sterility test should be performed under aseptic conditions."*. The FDA's Guidance for Industry for Sterile Drug Products Produced by Aseptic Processing takes things one step further and states in section XI *"The use of isolators for sterility testing minimizes the chance of a false positive test result".* Furthermore, the pharmaceutical inspection co-operation scheme (PIC/S) provides an entire guidance document (PI 014-3) on isolators used for aseptic processing and sterility testing. So although there is no hard requirement to perform sterility testing in isolators, there is a strong benefit to do so in order to reduce the risk of false positives, which can result in financial losses to manufacturers of products, and drug shortages to patients.

What is an isolator?

According to EU GMP Annex 1, an isolator is *"An enclosure capable of being subject to reproducible interior bio-decontamination, with an internal work zone meeting Grade A conditions that provides uncompromised, continuous isolation of its interior from the external environment".* Isolators have several features to maintain Grade A conditions and isolation from the external environment such as:

An isolator situated in a cleanroom

- Air tight / inflatable seals on all doors
- Interlocks to prevent doors being opened once aseptic conditions have been achieved (following bio-decontamination)
- Unidirectional airflow between 0.36-0.45 m/s (to comply with EU GMP Annex 1 guidance values)
- HEPA filters to cleanup incoming air
- Positive pressure
- Leak/pressure testing of enclosure and gloves separately
- Environmental monitoring systems to confirm that the environment remains aseptic during use
- Airflow and pressure alarms
- Hydrogen peroxide bio-decontamination systems to help inactivate microorganisms on the enclosure surfaces and incoming materials needed for the testing process

Benefits of isolators

Primarily, isolators reduce the risk of false positives occurring during sterility testing, which can save manufacturers of sterile products millions of dollars by minimising unnecessary scrapping of product. As a secondary benefit, isolators can also provide substantial savings through operating costs. Unlike a BSC/LAF which must be situated in a Grade B cleanroom, sterility test isolators can be situated in a lower Grade C or D cleanroom as they are isolated from their surrounding environment. This can result in substantial savings from:

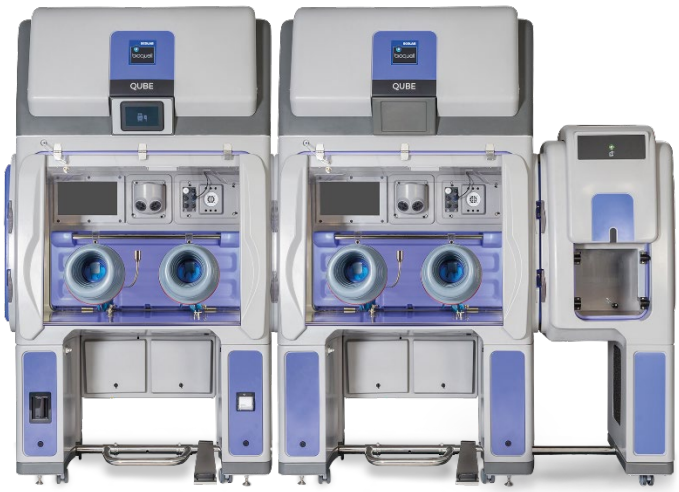
- Reduced energy bills due to lower capacity HVAC system
- Reduced cleaning and disinfection consumable costs
- Reduced labour resource (for cleaning and disinfection)
- Less gowning
- Less maintenance of the cleanroom
- Increased efficiency of operators as lower gowning means they can work for longer periods of time

In fact, one study(1) found that the running costs of a grade B cleanroom can be up to 78% lower than the running costs of a grade C cleanroom. Despite the above benefits, it is important however to note that isolators do have some downsides in comparison to
open LAF/BSC environments:

- They typically have a higher up front / equipment cost. However, this can be offset by the savings from lowering the cleanroom grade and reduced risk of scrapping of product.
- Operators must perform their duties through gloves connected to sleeves, which can be more difficult than gloved hands operating inside a BSC/LAF, so can lead to less efficient work, slowing down a process. However, operators do not have to wear such restrictive gowning in Grade D cleanrooms, meaning they can work for longer periods of time in more comfortable conditions.
- Finally, although automated bio-decontamination of an isolator requires less operator workload than manually disinfecting items into a BSC/LAF, the loading and bio-decontamination process does typically take longer, in some cases taking hours, which can add substantial time to the testing process or requires adjustment to working patterns by loading and bio-decontaminating the isolator overnight ready to start the testing process in the morning. However, there is a way to overcome this issue of long cycle times…

Modular isolators

One of the biggest hurdles to overcome when switching from a BSC/LAF to an isolator is to maintain the same throughput i.e. number of tests, due to the long bio-decontamination cycle. But this problem can be solved by adopting a modular approach to isolator systems. Rather than having one large isolator chamber, which is filled with all required materials needed for a full day of testing and subsequently running a long bio-decontamination cycle, a modular system utilises a multiple chamber principle whereby one small chamber is filled with a small amount of materials to do several sterility tests, and bio-decontaminated. Then these materials are transferred into an adjacent chamber where the pump resides, the interconnecting door is closed, and whilst the testing is being conducted in one chamber, the materials required for the next tests are bio-decontaminated in the adjacent chamber. With a small bio-decontamination chamber and a relatively light material load, bio-decontamination cycle times of as little as 30 minutes can be achieved (subject to quantity and absorbency of load).



An example of a modular isolator system with a bio-decontamination chamber on the left, testing chamber in the middle, and transfer device on the right

Hydrogen Peroxide Vapour and reliability of the sample

When bio-decontaminating materials prior to conducting a sterility test, it is important to ensure that the hydrogen peroxide does not penetrate through packaging and impact the sample. In fact, the recently updated revision of EU GMP Annex 1 has a specific requirement related to this. In section 10.8 it states *"Any process (e.g. Vaporized Hydrogen Peroxide, Ultra Violet) used to decontaminate the external surfaces of sterility samples prior to testing should not negatively impact the sensitivity of the test method or the reliability of the sample."* The concern is that if hydrogen peroxide penetrates into packaging, either of the canisters where the drug product will be filtered into or the vials containing the drug product itself, then there is a risk that it could

result in any microorganisms present being damaged or killed. This could result in a test passing where the product is contaminated i.e. a 'false negative' result. In order to avoid this scenario, proof is required to ensure that none of the vessels, which either the product is in, or will end up in, as well as the consumables the product may pass through or touch, have any hydrogen peroxide in them after being exposed to the bio-decontamination cycle.

This can be tested for by running de-ionized water through the whole process in replacement of the drug product and then measuring the levels of hydrogen peroxide (if any) in the de-ionized water. Using test methods that Ecolab have developed, levels in the water as low as 15ppb can be detected. If there is no presence of hydrogen peroxide detected at these low levels, this then provides evidence in support of there being no ingress of hydrogen peroxide during the bio-decontamination process. Thus the product or the sterility test are highly unlikely to be detrimentally affected. Ecolab can offer this ingress testing service, which is conducted at an Ecolab facility using a Bioquell Qube isolator to accurately simulate the process.

Ingress testing being conducted

The future of Sterility Testing

As previously mentioned, a number of companies specialising in sterility testing are developing various rapid sterility testing methods in order to reduce the wait time for results, which improves operational efficiency for the drug manufacturer and ensures patients receive their therapy quicker. This is of particular importance to cell therapy based products where the patients are often critically ill and the product can have a limited shelf life. Developing innovative rapid testing methods is also aligned with EU GMP Annex 1 Section 9.28 *"The adoption of suitable alternative monitoring systems such as rapid methods should be considered by manufacturers in order to expedite the detection of microbiological contamination issues and to reduce the risk to product."* This is a clear message that manufacturers should look to implement new rapid methods.

There are a number of different companies offering a variety of rapid sterility testing methods with alternative modes of action including real-time PCR (qPCR), ATP- Bioluminescence and one system which detects microorganism growth by tracking CO_2 produced as a living microorganism metabolises the substrate in a culture medium. These methods all have different accuracies and speed of results. The more established rapid systems still tend to use the 'traditional' method of processing the drug product through a retaining filter in a canister and filling with growth media. The 'rapid' part is conducted following this stage to analyse the sample for microbial contamination. The more novel technologies which don't use the 'traditional' membrane filtration method, tend to be at an earlier stage in development.

Summary

As has been made clear in this article, sterility testing is a mandatory process to ensure sterile products are free from microorganisms, and if the process is done incorrectly, it can result in unnecessary scrapping of compliant product. Therefore, it is advised to consider the advantages and disadvantages of the various approaches to conduct sterility testing, outlined in the table below. To learn more about how the Bioquell Qube can benefit your sterility testing operation, please click here. Bioquell Qube

Approach	Advantages	Disadvantages
Outsourcing	◥ No initial investment required for lab and equipment	◥ Longer result wait times ◥ Higher running costs

		◥ Less control and potentially higher risk of false positives
In house with a LAF/BSC	◥ Lower initial cost ◥ Easier to work in ◥ Quicker material transfer process	◥ Higher risk of false positives ◥ Higher running costs from Grade B cleanroom ◥ Operators must wear more restrictive gowning
In house with a traditional isolator	◥ Reduced risk of false positives, resulting in less scrapping of uncontaminated product ◥ Reduced running costs from lower cleanroom grade	◥ Higher up-front cost ◥ Long bio-decontamination cycle times reducing throughput ◥ Can be hard to work in
In house with a modular isolator, the Bioquell Qube	◥ Reduced risk of false positives, resulting in less scrapping of uncontaminated product ◥ Reduced running costs from lower cleanroom grade ◥ Higher testing throughput ◥ Quicker bio-decontamination cycle times than other larger isolator systems	◥ Higher up-front cost which can be offset by lower cleanroom running costs

(1) Costing a cleanroom per square foot, Cleanroom Technology, 28 February 2018, https://www.cleanroomtechnology.com/news/article_page/Costing_a cleanroom_per_square_foot/139470