Sterility Testing: A Guide to Best Practices

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Sterility Testing verifies the absence of viable contaminating microorganisms in sterile pharmaceuticals and medical devices. Eric Arakel, Global Product Manager for Sterility Testing and Microbial Air Monitoring, and Olivier Guenec, EMEA Business Manager for Microbiology, explore Sterility Testing in a quick 15 minute podcast. Eric and Olivier start with the basics, discuss best practice and also touch upon rapid testing solutions during the course of the discussion. Want to learn more? Read on!

Sterility Testing Podcast Transcript

Olivier Guenec: Eric! We're here to talk about Sterility Testing today. I guess we've got a lot of ground to cover. Where do we begin? Let's perhaps start with the basics. Eric, can you explain what sterility testing is?

Eric Clement Arakel: Simply put, sterility testing ensures that viable microorganisms are not present as contaminants in sterile pharmaceuticals.

O: You said 'sterile pharmaceuticals', how would you describe sterile pharmaceuticals?

E: There are a wide range of sterile pharmaceuticals. Parenterals or injectables, like monoclonal antibodies and vaccines, IV's are some of the most recognised. There are other sterile pharmaceuticals, such as ophthalmic preparations (eye drops), medical implants, sutures and surgical dressing. The list is long.

O: So how would one typically go about testing these sterile pharmaceuticals for sterility?

E: There are two accepted methods. One that is membrane filtration based and the other direct inoculation. In direct inoculation, a certain quantity of each product is transferred directly to two growth media to culture potential viable microorganisms. The growth media enables the culture of viable aerobic and anaerobic microorganisms.
In membrane filtration, a certain quantity of a sterile product is filtered through two canisters, each housing a membrane filter to trap potential viable microorganisms. The canisters are then filled with growth media and incubated at the required temperatures for culture.

O: I assume there must be advantages to the membrane filtration method since it involves a fair bit of preparation prior to culturing, unlike direct inoculation?

E: Absolutely! By filtering large volumes of a sterile product, even a single CFU (colony forming unit) in large volumes, perhaps litres, can effectively be retained on the membrane filter and subsequently cultured. The method also permits for the elimination of compounds with bacteriostatic or fungistatic properties through filtration and rinsing. This is why membrane filtration is the prescribed method for sterility testing.

O: When you say 'prescribed' , do you mean the pharmacopeial chapters?

E: Exactly, the pharmacopeial chapters USP<71>, the Ph. Eur. 2.6.1 and others. The chapters specify that the 'technique of membrane filtration is used whenever the nature of the product permits'. Only if the product is not filterable can direct inoculation be adopted.

O: Can you expand on that? When is a product not filterable?

E: Well, you could say that for products such as some medical devices, like sutures and surgical instruments, membrane filtration is not feasible. However, there are other medical devices, for example, those with tubing and luer connectors, which can be flushed with rinsing fluid directly into a sterility testing canister for membrane filtration. In this case, one would adopt membrane filtration. There are also some products that can precipitate when they come in contact with the rinsing fluid or the membrane, making them unfilterable. The particulate nature of some adjuvants used in vaccine formulations can also create challenges during filtration as well.

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O: You did mention that membrane filtration facilitates the removal of compounds with anti-microbial properties, how does one ensure this is the case for direct inoculation?

E: Irrespective of the method chosen, method suitability has to be demonstrated prior to sterility testing. In direct inoculation, the growth media are challenged with reference strains, following its supplementation with a specified volume of the product. Likewise, in membrane filtration, the growth media are challenged with reference strains, following filtration of the product and rinsing of the membrane.

In either method, one can also supplement the medium with additives that neutralise compounds with antimicrobial activity. This decreases the risk of scoring a false negative, which can have devastating consequences when a contaminated product is released on the market. In a nutshell, there has to be clear rationale as to why direct inoculation is adopted.

O: I assume there may be some difficulties that complicate the process of filtration causing doubt regarding which method to adopt?

E: Sure! But many of these issues can be resolved through trouble shooting during method validation. This can be by:

- Adopting the right membrane filter for filtration.
- Implementing a short pre-wetting step prior to filtration to avoid any non-specific binding or product interaction.
- Rinsing the membrane thoroughly, albeit keeping in mind that the rinsing cycles prescribed by the pharmacopeial chapters are not exceeded.
- Using the right rinsing fluid. Fluid D with Tween or Fluid K with beef extract and Tween can be tested if Fluid A delivers unsatisfactory results during method suitability tests. Surprisingly, some products selectively precipitate in rinsing fluid sourced from one but not the other vendor. This type of product interaction can prove troublesome.
- The filtration of viscous products can also be challenging. This can be remedied by diluting the product in rinsing fluid. Ointments and emulsions can be diluted in sterile isopropylmyristate or mildy warmed to aid filtration.
- Pooling of products that are difficult to filter can also exacerbate the problem. In such cases, it is recommended to spread the sterility test across multiple filtration units. Cost cutting measures can at times lengthen the period of validation. As the saying goes – penny wise pound foolish.

O: So what next? What happens after filtration of the product?

E: The containers with growth media are placed in an incubator and periodically checked for microbial growth over a 14 day period.

O: Isn't 14 days a long wait? Why wait that long?

E: Yes, it can be quite the wait. The 14 day period of incubation was introduced to ensure the cultivability of slow growing microorganisms such as Cutibacterium acnes to name one. The pharmaceutical product may create an inhospitable environment for potential contaminating microorganisms, creating a lag in their growth recovery. The 14 day incubation was established to ensure that such dormant forms recover effectively.

O: So is sterility testing in its current form rate limiting?

E: That is a difficult one. To my knowledge, no.
There was growing frustration that the release of vaccines was stalled pending the completion of the safety tests. Understandably the tests cannot be rushed.
There are other analytical tests in the QC release process that take longer that the sterility test. Batch release can take between 21-28 days. So for the moment, no. Sterility Testing is not rate limiting.

O: So, Eric, what about rapid methods? Are they the methods the future?

E: I will play it safe and say, perhaps! I don't want my comment to age poorly. As you know, the principle of the test remains largely unchanged since the mid-1930's. Our reusable sterility testing systems were launched in the late 1960's and the test has remained largely unmodified since, save the fact we have moved from reusable to single-use systems in the interest of quality and safety. So, like I said, I will answer that with a 'perhaps'.

O: Are you aware of rapid methods being implemented for sterility testing?

E: I am aware of rapid methods being adopted for the testing of short-shelf life products, such as ATMPs, or cell and gene therapies, and radiotracers used in PETs.
Some of these products are meant for immediate use. In the past, some of these therapies have been administered without the completion of a sterility test, given that these products were administered to terminally ill patients who would not survive without treatment.

So yes, rapid methods based on ATP-bioluminescence, CO2 detection and nucleic acid amplification are now being adopted to test such short shelf life products.

O: We are maybe looping back here, but do you see these methods being adopted for the release of – let's call it – traditional sterile pharmaceuticals for want of a better word?

E: Again, I will say perhaps! We have partnered with Charles River and have paired the traditional test with a rapid method. Products are filtered, the canisters filled with growth media and incubated for a period of 5-6 days. A sample is then drawn aseptically via the septum port, featured on our canisters and transferred to the Celsis platform for testing. This has shortened the period of incubation and release. However, the method still relies on membrane filtration and microbial culture. There is perhaps no side stepping this requirement and I would like to highlight this. Membrane filtration facilitates the testing of large volumes, eliminates, or at the very least reduces the presence of compounds with antimicrobial properties, and the growth step ensures recovery of slow growing microorganisms. By staying growth-based, the method also ensures that only viable microorganisms are reliably detected. This avoids any undue concern on background or noise, leading to false positives.

O: I'd like to get back to something you mentioned before, that specified quantities must be tested for sterility. Perhaps... I missed picking up on this at that point. Could you elaborate?

E: Correct! The volume to be tested and the number of containers to be tested per batch have been specified in the pharmacopeial chapters on sterility testing. For

instance, a fixed volume, depending on the final volume, from 2% or 20 containers, whichever is less, is tested for sterility from a batch of 500 containers. So, 20 containers from a batch of 1000 containers or a similar 20 containers from a batch of 100,000 containers.

There is therefore discussion on whether the results of a sterility test, either by the traditional method or by rapid methods, are statistically reliable. However, let's not get caught up in this. The industry has developed other means of ensuring sterility through improved manufacturing standards with the help of guidelines such as the EU-GMP Annex 1. The sterility test remains an important check for the foreseeable future and serves to monitor possible gross contaminations.

O: We've discussed false negatives in the presence of antimicrobial substances, what about the opposite, false positives? What happens if a contamination is introduced during the sterility test?

E: If a sterility test failure is detected a thorough root cause analysis is initiated. Every step of the process is revisited to determine the cause of the failure. Corrective action must be implemented before the test is repeated. If the sample fails, the entire batch is considered contaminated and the batch must be destroyed. To avoid such a time consuming and expensive hold of release, sterility tests are performed in controlled environments, similar to those adopted in aseptic manufacturing. There is increased adoption of barrier technologies such as isolators for sterility testing in recent years.

O: Being a pivotal test, I assume there is guidance available on how to validate a sterility test?

E: There are several guidance documents, besides the pharmacopeia, on sterility testing. The PIC/S, TGA, PDA and the FDA have issued guidance on sterility testing. Most of the guidelines are harmonised, similar to the pharmacopeial chapters.

O: Is there anything we may have overlooked during our discussion?

E: I don't quite think so. We've covered a wide range of topics today. Maybe the only thing I would like to mention is that most guidelines on sterility testing recommend that validation be repeated annually and not necessarily only where there is a change implemented. This may not be a pharmacopeial requirement, but it is considered good practice.

O: I guess that covers everything then. That was quite the discussion. Thank you very much, Eric.

E: Thank you, Olivier.

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