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The Sterisart® System Simplifies the Sterility Testing of Therapeutics with Antimicrobial Properties

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Abstract

Membrane filtration-based sterility testing is particularly suited for products with microbial growth-inhibiting properties, such as antibiotics. These compounds need to be purged from the system to mitigate the incidence of false negatives. This is achieved by constructing the sterility testing canisters with materials that exhibit minimal to no non-specific binding. In this study, the recovery of microorganisms following the filtration of ciprofloxacin for intravenous administration and gentamicin for intramuscular injection using the Sterisart® closed system sterility testing device was evaluated. The results demonstrate that the Sterisart® canisters containing regenerated cellulose membranes are optimal for the sterility testing of antibiotics.

Introduction

Pharmaceutical products are routinely manufactured under strict GMP guidelines. Despite these strict codes, as a fail-safe prior to batch release, not all pharmaceutical products undergo stringent sterility testing to identify the potential presence of viable microorganisms. It is crucial that pathogenic microbes, such as bacteria and fungi, are detected in contaminated products before patients come in contact with. There have been rare instances where compromised drugs have been released to the market with devastating consequences for patients, and pharmaceutical companies.

Sterility tests are performed in accordance with the regulatory requirements defined by the International Pharmacopeia (USP <71>, Ph. Eur. 2.6.1., and JP 4.06) and harmonized in ICH Q4B Annex 8. According to these requirements, sterility testing can be performed either by direct inoculation, or by membrane filtration, which is the method of choice. Products are tested for sterility by direct inoculation only when the properties of the product do not permit membrane filtration. The membrane filtration approach typically relies on a closed filtration unit containing a membrane with a pore size not greater than 0.45 µm and that has reliably demonstrated the retention of microorganisms. Other components of the system include a suitable pressure supply (such as a peristaltic pump) that drives the sample across the membrane filter, an appropriate membrane rinsing solution, and growth media. This closed setup is conventionally cleanroom compliant to eliminate any contamination risks and consequent false positives.

Once sample filtration is complete, the closed system is incubated, typically for 14 days, and screened for turbidity as an indicator of microbial contamination. Sterisart® canisters are a closed system for sterility testing based on the membrane filtration method. This closed system excludes the need for physically manipulating membrane filters and thereby mitigates the risk of secondary contamination and false positives.

Being a method based on the evaluation of microbial growth, it is crucial to distinguish between true product sterility and a false negative. Different ingredients of a pharmaceutical formulation can possess innate bacteriostatic or fungistatic properties that can negatively influence the results of a sterility test. Antibiotics possess such growth-inhibiting antibacterial and antifungal properties. It is therefore recommended to use the membrane-filtration method for the sterility testing of antibiotics or use a suitable sterile inactivating agent (such as penicillinase or cephalosporinase) to supplement the growth media.

However, some antibiotics cannot be effectively neutralized. In such cases, non-specific adsorption of inhibitory compounds to the components of the sterility testing system is a major cause for concern. It is therefore critical that the physicochemical properties of the materials used in the construction of the sterility testing canisters must exhibit negligible non-specific adsorption and facilitate thorough rinsing to purge all traces of the antibiotic, and yet deliver on microbial retention.

In this report, a method suitability test was performed using the two antibiotics ciprofloxacin, for intravenous administration, and gentamicin, for intramuscular injection. Ciprofloxacin is a fluoroquinolone antibiotic effective against most Gram-negative bacteria such as *Pseudomonas aeruginosa*. Gentamicin is a type of aminoglycoside used in the treatment of infections mainly caused by Gram-negative bacteria as well as some Gram-positive bacteria such as *Staphylococcus aureus*. The susceptibility of *P. aeruginosa* and *S. aureus* to ciprofloxacin and gentamicin respectively was the main rationale for choosing these antibiotics.

Following dilution and membrane filtration of the antibiotics, the canisters were rinsed, inoculated with microorganisms listed in USP <71>, filled with growth medium and incubated at the prescribed temperature. Uninhibited microbial growth was observed in all the samples well before the prescribed 3-day maximum for bacteria and 5-day maximum for fungi recommended for growth promotion testing. Our results demonstrate that the Sterisart® canisters are optimal for the sterility testing of antibiotics and comply with all pharmacopeial requirements.


SARTORIUS
Pump & Scan
Time
0:00
min:sec
Duration
off
Speed
100
FINISH
SCAN (0 / 7)
START
SARTORIUS
STERISART® UNIVERSAL

Materials and Methods

Product Name	Order No.
Sterisart® Universal Pump	16420
Ampoule breaker for Sterisart® Universal Pump	1ZW---0002
Sterisart® Transfer-Kit for liquids	16472--------GBD
Sterisart® system for liquids in open containers	16467--------GBD
Sterisart® system for closed large volume containers, with septum	16466--------GSD

Table 1 : Equipment and consumables.

Product Name	Order No.
Ciprofloxacin (200 mg / 100 mL)	/
Gentamicin (80 mg / 2 mL)	/
Tryptic Soy Broth (TSB) 100 mL	BD-257247
Fluid Thioglycollate Medium (FTM) 100 mL	BD-257246
Fluid A (peptone water) 300 mL	BD-254979
Tryptic Soy Agar (TSA)	BD-254086

Table 2 : Chemicals, media and rinsing fluids.

Test Strains
Pseudomonas aeruginosa ATCC® 9027™
Bacillus subtilis ATCC® 6633™
Staphylococcus aureus ATCC® 6538™
Clostridium sporogenes ATCC® 19404™
Aspergillus brasiliensis ATCC® 16404™
Candida albicans ATCC® 10231™

Table 3 : Certified microorganisms used in this study.

Sample Preparation

International pharmacopeias, including USP <71>, Ph. Eur. 2.6.1., and JP 4.06, recommend that for liquid antibiotics, a minimum sample volume of 1 mL from 20 distinct containers should be tested when the batch size exceeds 500 units. Accordingly, 20 mL of the respective antibiotic was tested per Sterisart® canister. Ampoules containing gentamicin were opened with the Sterisart® ampoule breaker. Using the Sterisart® Transfer-Kit-for liquids, 40 mL of either gentamicin or ciprofloxacin were aseptically pre-diluted in 200 mL of Fluid A.

Membrane Filration

Step	Description	Pump Speed
1	Pre-wetting with 50 mL Fluid A per canister	50%
2	Filtration of pre-diluted antibiotics	90%
3	Rinse 4x with 100 mL Fluid A per canister	50%
4	Rinse 1x with 100 mL Fluid A spiked with test strain	50%
5	Add either 100 mL FTM or TSB per canister	50%
6	Incubate at 32.5 °C (±2.5 °C) for FTM and 22.5 °C (±2.5 °C) for TSB for 3 to 5 days	/

Table 4 : Schematic workflow for filtration of antibiotics with the Sterisart® Universal Pump.

Two Sterisart® canisters were positioned in the canister holder and the Sterisart® tubing system was threaded through the pump head. The sterile venting filters were left open, the needle was inserted into the septum of the container containing Fluid A and the pump was switched on (see Table 4). Once 50 mL of Fluid A were transferred into the canisters, the sterile venting filters were sealed using the tethered filter plugs. Pre-wetting the membrane with a rinsing fluid limits non-specific adsorption and is strongly recommended. Next, the needle was inserted into the container containing the pre-diluted antibiotics. To reduce the contact time of the antibiotic-containing solution with the membrane, the tethered filter plugs were left on the sterile venting filters. The pump was switched on and the entire content of the bottle was pumped in equal volumes between the two Sterisart® canisters. The pump was switched off and the sterile vent filters were uncapped. To eliminate potential droplets of antibiotics at the canister walls, the needle was inserted into the container with Fluid A and the two canisters were filled with a pre-defined volume of 100 mL, by switching the pump on. The sterile vent filters were capped, and the membrane was rinsed with the contents of the canister. As recommended by the international pharmacopeia, the membranes were washed not more than 5 times with 100 mL per canister and filter.

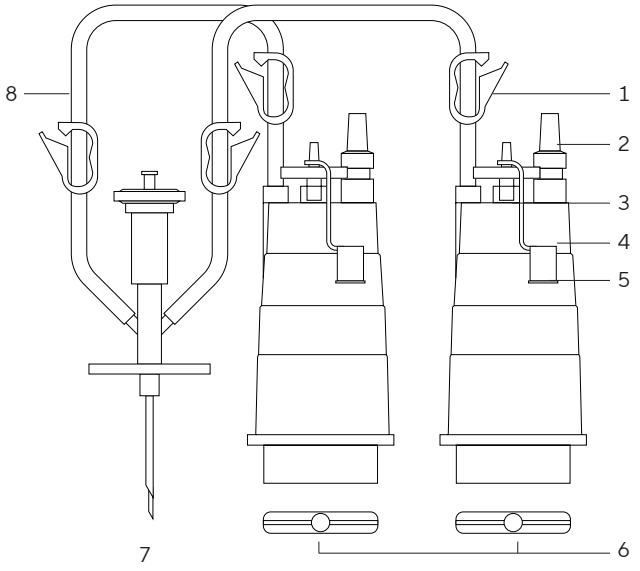
The fifth and final rinsing volume was spiked with <100 colony forming units (CFU) of certified test microorganisms and filtered through the Sterisart® canisters. For cell number quantification, the same amount of the spiked test microorganisms was transferred onto Tryptic Soy agar plates (TSA) using spread plate method and incubated under the same conditions as the respective spiked Sterisart® canisters.

After the last rinsing step, the outlet of each Sterisart®

canister was sealed using the enclosed wing nut plugs. The two sterile vent filters were uncapped. The yellow tube clamp at the outlet of the Y-distributor was opened and the adjacent white tube clamp closed. The needle was inserted into a bottle containing either 100 mL Fluid Thioglycollate Medium (FTM) or Tryptic Soy Broth (TSB) and the Sterisart® Universal pump was switched on. The Sterisart® canisters were filled according to Table 5. For negative controls, 50 mL of fluid A was filtered through Sterisart® canisters and then filled with either 100 mL FTM or TSB. For the positive controls, 50 mL of fluid A were first filtered, then 100 mL of fluid A was filtered with spiked test strain and filled with either TSB or FTM (see Table 5). The tubing was sealed using the two clamps above the inlets of the canisters. The tubing was cut at an appropriate distance away from the clamp and slid onto the slip-connector of the sterile venting filters. Canisters containing FTM were incubated at 32.5 °C ± 2.5 °C while canisters containing TSB were incubated at 22.5 °C ± 2.5 °C. The canisters were visually inspected daily.

Growth promotion tests were performed in compliance with USP <71>, Ph. Eur. 2.6.1. and JP4.06. The experiments were carried out in triplicate with three different lots of Sterisart® canisters.

Please refer to our Sterisart® user manual for a pictorial depiction of the described workflow.



No.	Component
1.	Pre-installed tube clamp
2.	Connector with septum for sterile sampling
3.	Vent filter
4.	Sterisart® container
5.	Tethered filter cap
6.	Wing nut plug
7.	Dual-needle metal spike for closed containers (16466)
8.	Tubing

System	Canister	Test Strain	Medium	Filtered Sample
1	A	/	TSB	/
	B		FTM	
2	A	*C. albicans*	TSB	Gentamicin
	B	*P. aeruginosa*	FTM	
3	A	*C. albicans*	TSB	Ciprofloxacin
	B	*P. aeruginosa*	FTM	
4	A	*C. albicans*	TSB	/
	B	*P. aeruginosa*	FTM	
5	A	*B. subtilis*	TSB	Gentamicin
	B	*S. aureus*	FTM	
6	A	*B. subtilis*	TSB	Ciprofloxacin
	B	*S. aureus*	FTM	
7	A	*B. subtilis*	TSB	/
	B	*S. aureus*	FTM	
8	A	*A. brasiliensis*	TSB	Gentamicin
	B	*C. sporogenes*	FTM	
9	A	*A. brasiliensis*	TSB	Ciprofloxacin
	B	*C. sporogenes*	FTM	
10	A	*A. brasiliensis*	TSB	/
	B	*C. sporogenes*	FTM	

Table 5: Experimental overview of the growth promotion tests (n=3).

Results

Although both gentamicin and ciprofloxacin are highly potent against Gram-negative microorganisms, the canisters containing FTM spiked with *P. aeruginosa* ATCC® 9027™ exhibited clearly visible growth that was no different from the positive control (see Figure 1). Canisters containing FTM medium spiked with *C. sporogenes* ATCC® 19404™ turned fully turbid after 3 days of growth. FTM medium containing *S. aureus* ATCC® 6538™ exhibited clearly visible growth throughout the culture media column within the canister, with colonies growing directly on the membrane.

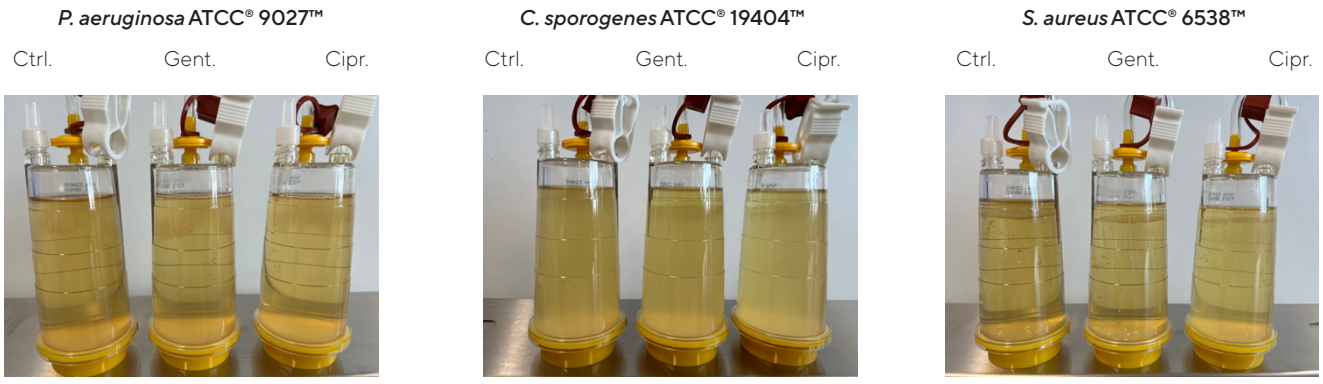


Figure 1 : Exemplary results of test strains grown in FTM after filtration of gentamicin (Gent.) and ciprofloxacin (Cipr.). Growth was always compared to positive control (Ctrl.) where no antibiotic was filtered.

Without prior agitation, all canisters filled with TSB showed clearly visible growth throughout the culture media within the canister or directly above the membranes for *A. brasiliensis* ATCC® 16404™, *C. albicans* ATCC® 10231™, and *B. subtilis* ATCC® 6633™ (see Figure 2).

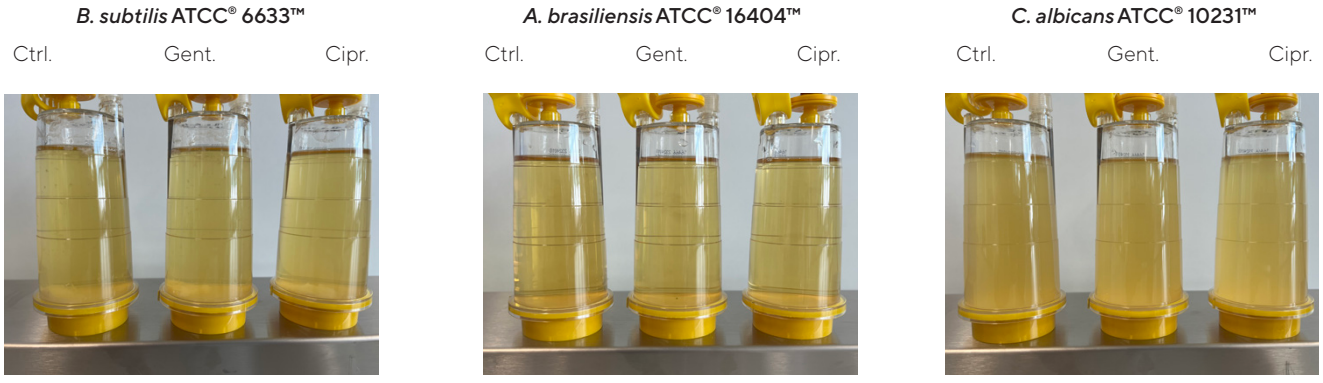


Figure 2: Exemplary results of test strains grown in TSB after filtration of gentamicin (Gent.) and ciprofloxacin (Cipr.). Growth was always compared to positive control (Ctrl.) where no antibiotic was filtered. Samples were agitated for better visibility.

In summary, no growth inhibition was observed in any of the canisters used for filtration of either gentamicin or ciprofloxacin, compared to the positive controls, where no antibiotics were filtered. Growth was not observed in any of the negative controls (see Figure 3).

Figure 3: Exemplary results of negative canisters filled with FTM (left) or TSB (right).

Conclusion

This study demonstrates that Sterisart® canisters are optimal for testing sterile pharmaceuticals with antimicrobial properties, including those products that cannot be effectively neutralized. Rinsing each membrane with 5x100 mL of Fluid A solution guarantees adequate removal of gentamicin and ciprofloxacin. The Sterisart® Sartochem® Regenerated Cellulose Membrane stands as an universal membrane, characterized by minimal to no non-specific binding properties. As a result, there is no imperative need for segregation into products with or without antibiotic properties before conducting sterility tests.

In addition to the tests performed in this study, we have conducted detailed adsorption and desorption tests of compounds with antimicrobial properties using Reverse Phase HPLC. Please see sections 5.1-5.2 of our validation guide for further details.

Our extensive Sterisart® sterility testing portfolio has been designed for simplicity and is fully compliant with every pharmacopeial need. The unique Sterisart® septum port eliminates the risk of false positives and eases aseptic supplementation for antibiotic inactivation or aseptic sampling for identification, sub-culturing or rapid microbial release. **Scan the QR code below** to learn more on a study demonstrating that the integrity of the sterility testing canisters is maintained even after more than 100 repeated septum sampling events via the Sterisart® septum port.

References:

1. US Pharmacopoeia (USP) <71> - Sterility Tests
2. European Pharmacopoeia (Ph. Eur.) 2.6.1. - Sterility
3. Japanese Pharmacopoeia (JP) 4.06 – Sterility Test

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