

TECHNOTES

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A Study Monitoring **70% Isopropyl Alcohol** Sterility in Use ►

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Summary

Isopropyl alcohol – It is used throughout the life science cleanroom to sanitize work surfaces, gloves, and tools. It is sprayed ubiquitously. It is also commonly accepted that IPA spray bottles can disperse biological contaminants. Logically, if spores exist in an environment and the trigger mechanism of the bottle pulls in contaminated air, those spores can be dispersed all over your cleanroom.

But does that really happen?

This study disproves that widespread misconception.



Introduction

Peering into a life science cleanroom you will see varying forms of sanitizers, the most commonly used is 70% Isopropyl alcohol, also called isopropanol or simply IPA⁴. “IPA is a sanitizer.”⁵ It kills vegetative organisms by breaking down cell walls. IPA is effective against common skin flora and some viruses like “vaccina, herpes simplex, and influenza.”⁶ IPA is not a panacea; it is ineffective against spores from both molds and bacteria like *bacillus* spp.

The EC Guide to Good Manufacturing Practice Revision to Annex 1 — *Manufacture of Sterile Medicinal Products*, states that, “Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilized. Sanitizers, disinfectants and detergents used in Grades A and B areas should be sterile prior to use.”⁷ This means that it is required to validate the sterility of IPA bottles used in ISO Class 5 life science cleanrooms. A way to save time and money here is to purchase prepared sterile IPA that comes with certificates of compliance, analysis and irradiation per manufactured lot to ensure that the product you are purchasing meets USP sterility requirements.

Trigger-spray bottles perform by expelling IPA then aspirating the environmental air to re-pressurize the bottle. If that air should have spores present, it is likely that the alcohol could become contaminated. How long can an alcohol bottle remain inside a controlled environment? At what point will it become or could it become contaminated? “To prevent introduction of contamination, disinfectants should be sterile, appropriately handled in suitable (e.g., sterile) containers and used for no longer than the predefined period specified by written procedures.”⁸ It can be extrapolated that firms are required to validate the amount of time a sanitizer bottle can be used inside the controlled environment. Usage can range from discarding after every shift or daily, weekly, monthly, or until the bottle runs out. As long as they have the data to support that the inside of the bottle is sterile and still 70% IPA and the outside of the bottle is also clean, they can do as they wish.

Regulatory bodies like to see that there is control and validation. The easiest way would be to discard bottles after every shift, with no chance for cross-contamination and no need for further validation.

It's not that simple.

Alcohol is made from petroleum. It is also a hazardous chemical and a VOC of which the EPA tracks emissions. The intent of this study is to determine if the environment contaminates the 70% sterile IPA inside Texwipe's TX3270 spray bottles. The potential for contamination should be inversely proportional to the cleanliness of the environment in which the product is used. To determine if the potential for contamination is significant, bottles of TX3270 Sterile 70% Isopropanol were operated in three environments of varying cleanliness over a 30-day period.

Purpose

For this study, forty-five bottles of **Texwipe TX3270 Sterile 70% Isopropanol** were distributed to three environments of different cleanliness. Fifteen bottles were brought to the ISO Class 5 Production Cleanroom (Photo A). Fifteen bottles were kept in an unclassified controlled area used for Industrial Filling, “FlexLine Area” (Photo B). Lastly, fifteen bottles were maintained in an unregulated warehouse (Photo C).

Each group of bottles was clearly labeled “CR” for Cleanroom, “FL” for FlexLine Area” and “WH” for Warehouse. The trigger sprays were depressed twice each working day for a period of 30 days. The nozzles were left open for the duration of the study. After fourteen days, five of the bottles from each area were collected and four were sent for USP sterility testing at an independent qualified laboratory. The four bottles were combined for a composite sample. After 21 days, five more bottles of each group were then collected and four tested. At the end of the 30 days the remaining five bottles were collected and four sent for final sterility testing.

The current USP <71> Sterility Test method was used to test the alcohol. Each of the four test bottles was composited and tested by vacuum filtration through a 0.45 µm filter. Three rinses were made with 100 mL of USP Fluid D each. Fluid D was chosen to neutralize the antimicrobial nature of the IPA. After filtration, half of the filters were applied to FTM (Fluid Thioglycollate Medium) and half to SCDM (Soybean Casein Digest Medium). The samples were incubated for 14 days, the FTM at 30 - 35°C and the SCDM at 20 - 25°C. A USP bacteriostasis/fungistasis (B/F) test was performed to ensure that the IPA did not inhibit growth, which confirmed the validity of the sterility tests.

Group #	1	2	3	
Location	Cleanroom	FlexLine	Warehouse	Sample Day
Bottle Label	CR-1	FL-1	WH-1	Day 14
	CR-2	FL-2	WH-2	
	CR-3	FL-3	WH-3	
	CR-4	FL-4	WH-4	
	CR-15*	FL-15*	WH-15*	
	CR-5	FL-5	WH-5	Day 21
	CR-6	FL-6	WH-6	
	CR-7	FL-7	WH-7	
	CR-8	FL-8	WH-8	
	CR-14*	FL-14*	WH-14*	
	CR-9	FL-9	WH-9	Day 30
	CR-10	FL-10	WH-10	
	CR-11	FL-11	WH-11	
	CR-12	FL-12	WH-12	
	CR-13*	FL-13*	WH-13*	

*These samples were retained.



Photo A – ISO Class 5 Production Cleanroom



Photo B – Fifteen bottles in an unclassified controlled “FlexLine” Area



Photo C – Fifteen bottles in an unregulated warehouse

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Product

The product selected for this trial was the **Texwipe TX3270 Sterile 70% Isopropanol / 30% USP water**, in a trigger spray bottle. Sixteen fluid ounces are filled into a 24 ounce bottle. In production, each pre-cleaned bottle is filled with the isopropyl alcohol-water solution that is pre-filtered through a 0.2 μm filter. The filled bottle is fitted with a trigger sprayer and double-bagged. The double-bag permits sanitizer wipedown of the exterior bag before introduction of the inner bag into aseptic areas. The bagged bottles are boxed in a case quantity of 12 units. The boxing consists of 12 double-bagged units in an inner protective case liner inside a corrugated case. The packaged product is then gamma irradiated to a 10^{-6} sterility level according to AAMI guidelines.

Test Areas

Three areas of the manufacturing facility were chosen for the exposure sites. The Group 1 bottles were placed in an ISO Class 5 (Class 100) cleanroom environment. The Group 2 bottles were placed in the FlexLine area, approximately an ISO Class 7 (Class 10,000) environment. Group 3 bottles were placed in the Warehouse area. This area had no air filtration and was open to routine warehouse traffic and operations.

Air Sampling

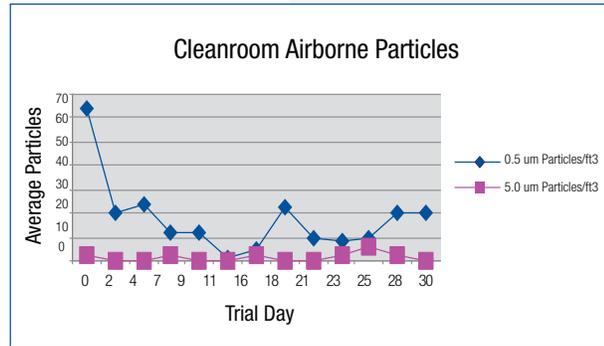
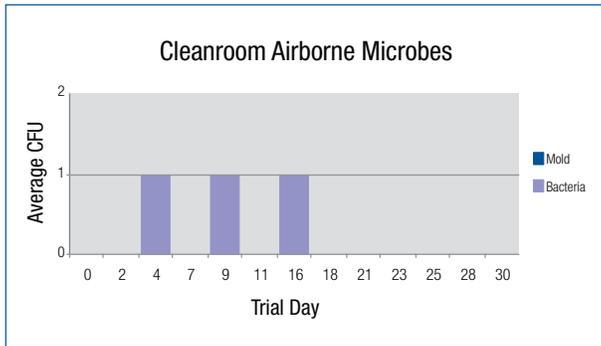
Microbial and particulate air sampling was performed for the duration of the study at each location. Settle plates were chosen for passive air sampling. The plates were prepared, sterile, standard 100 mm in diameter and exposed for two hours. The media chosen were Tryptic Soy Agar (TSA) and Rose Bengal Agar (RBA) with Antimicrobial Supplement C. Five plates of TSA and five plates of RBA were exposed on each of the trial days as indicated on the graphs. The TSA was incubated at 28 - 32°C for 48 hours. The RBA was incubated at 20 - 22°C for 96 to 120 hours.

The plates were enumerated for bacteria on the TSA and mold on the RBA. The data were compiled, averaged according to bacteria and mold and depicted graphically on the bar charts below. The averaged results were rounded up for the reason that a fraction of an organism is not viably possible.

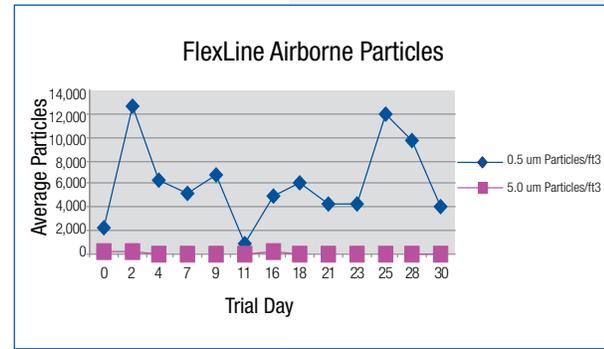
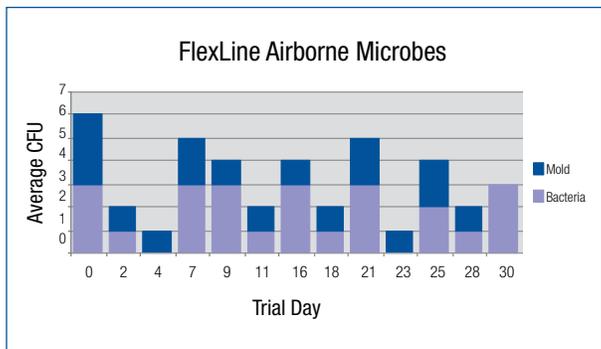
Airborne particle counting was performed using the Biotest APC Plus. A ten-minute sample was taken which collected 1 cubic foot of air (28.3 L). Particles were measured at 0.5 and 5.0 micrometers (μm).

As expected, the cleanroom results were low in particles, less than 100 particles per cubic foot, and had minimal airborne bacterial contamination. One organism was recovered only on three occasions. No mold was recovered. The FlexLine was more contaminated. The highest bacteria count recovered was 8 cfu, lowest <1 and on average 2 cfu. For mold, the high count was 10, low was <1 and on average 1 cfu. Particles averaged 6,000 particles per cubic foot. The Warehouse was notably contaminated. The highest bacteria count recovered was 8 cfu, lowest <1 and on average 2 cfu. For mold the high count was 51, low was 2 and on average 17 cfu. Particles averaged 256,000 particles per cubic foot.

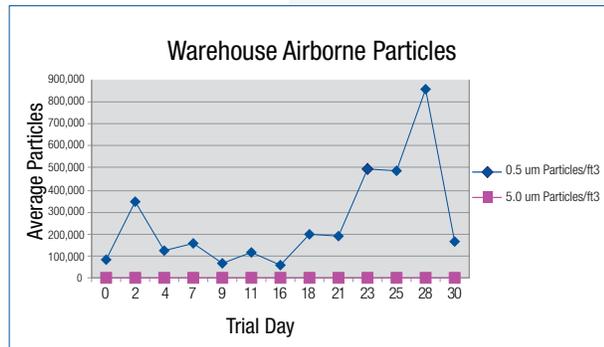
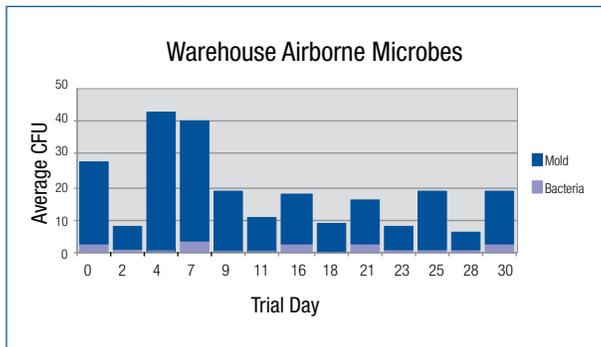
Cleanroom Results:



FlexLine Results:



Warehouse Results:



Results

Each of the tested Groups of 70% Isopropanol trigger-spray bottles was shown to be sterile.



Discussion and Conclusion

It is interesting that all results were shown to be sterile.

Microbiologists and FDA inspectors alike would be surprised of these results. Historically, there have been significant suspicions that a mold-ridden environment would assure that spores were aspirated into the bottle. Pharmaceutical and biotech companies performing aseptic manipulations are often fearful of contaminating the IPA with spores such that they throw out used bottles after every shift. It appears that thankfully this is no longer necessary. It may be interesting to fill the trigger-spray bottles with nutrient liquid media to see if organisms are indeed drawn back into the bottle. This study may be of interest to a pharmaceutical training and research institute to finally answer this question.

In conclusion, of the final 12 bottles tested for the entire 30 days all were shown to be sterile. The use of **Texwipe's TX3270 Sterile 70% Isopropanol** trigger-spray bottles in confirmed contaminated environments does not promote or sustain bacterial or mold growth within the bottle.

Reference

- ¹ Lara Soltis, BS, MBA, Regional Sales Manager
- ² Wendy Hollands, BS, MBA, Product Manager, Sterile Products
- ³ Allen Spivey, BS, Laboratory Manager
- ^{4,8} Denny EF, Kopis EM, and Marsik, FJ. Elements for a Successful Disinfection Program in the Pharmaceutical Environment, PDA J. Pharm. Sci. Tech, 1999
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- ⁶ <https://www.cda-adc.ca/jcda/vol-66/issue-10/546.html> Rotter, ML. Hand washing and hand disinfection. In: Mayhall CG, ed. Hospital epidemiology and infection control, 2nd edn Philadelphia: Lippincott, Williams, & Wilkins, 1999:1339-55
- ⁷ EC GUIDE TO GOOD MANUFACTURING PRACTICE REVISION TO ANNEX 1 – Title: Manufacture of Sterile Medicinal Products Brussels, 30 May 2003

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