TECHNOTES

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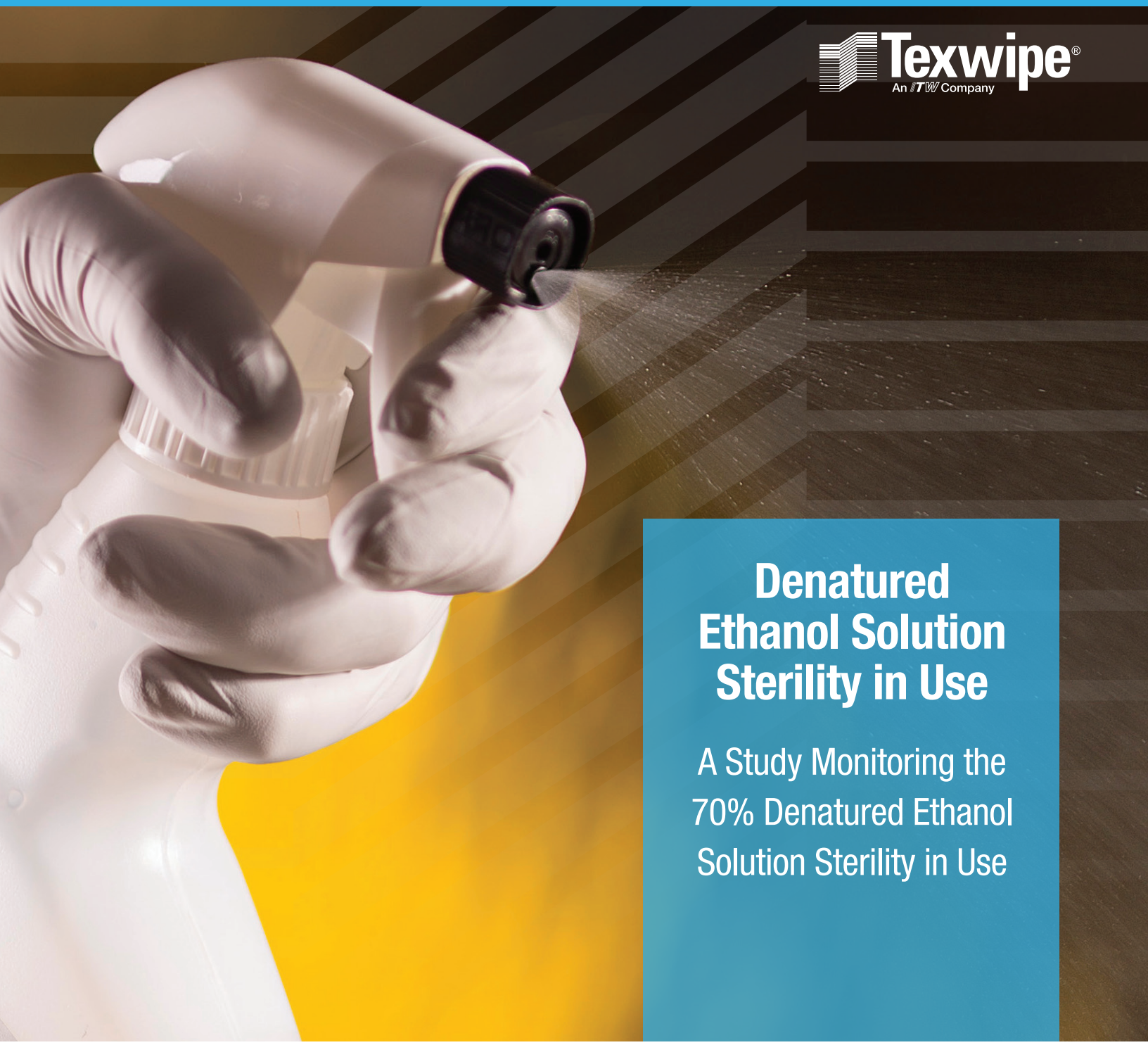
An ITW Company



Denatured Ethanol Solution Sterility in Use

A Study Monitoring the 70% Denatured Ethanol Solution Sterility in Use

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A Study Monitoring **70% Denatured Ethanol Solution** Sterility in Use

Introduction

Denatured ethanol (or ethyl alcohol) diluted to a seventy-volume percent (70%) solution is largely used throughout life science cleanrooms to sanitize work surfaces, gloves, and tools. It is common knowledge that alcohols may have the potential to kill various microbes, but not spores. The literature shows the efficacy of several alcohols tested at various concentrations against various microbes.[1] Sixty to eighty percent ethyl alcohol was found to be effective against various microbes including *Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli and Salmonella typhosa*, as well as all of the lipophilic viruses, e.g., herpes, vaccinia, and influenza virus, and many hydrophilic viruses, e.g., adenovirus, enterovirus, rhinovirus, and rotaviruses.[2]

What does denatured alcohol mean? The purpose of denaturing alcohol is to make it undrinkable. This is done by either making the solution toxic, taste bad, smell bad or nauseating. In the United States, TTB, the Alcohol and Tobacco Tax and Trade Bureau, part of the U.S. Department of Treasury, taxes ethanol unless the alcohol is denatured. The federal regulation for denaturing can be found at Title 27 Part 21 (27 CFR 21) Formulas for Denatured Alcohol and Rum.

The allowed denatured product is defined by the denaturing ingredients and their concentrations and authorized uses. The formula chosen for Texwipe's denatured ethanol products was Formula No. 3-C (27 CFR 21.37). This formulation consists of **USP-grade ethanol and USP-grade isopropanol (IPA)** as the denaturant, five gallons of IPA for each 100 gallons of ethanol. The authorized uses list the processing of items found in the **life science market**. These uses include, but are not limited by, these examples: processing crude drugs, glandular products, antibiotics and vaccines, blood and blood products. Other formulas are available, but these contain ingredients that could leave residues, e.g., nicotine, or use aggressive solvents, e.g., toluene, methyl isobutyl ketone.

The EU 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."*[3] It logically follows that the disinfectant and detergent's sterility be maintained while in use.

This guidance indicates that **it is required to validate the sterility of the bottled denatured ethanol solutions used in ISO Class 5 (Grade A and B) life science cleanrooms**. Life science users can save time and money by purchasing prepared sterile denatured ethanol solution in bottles that come with certificates of compliance, analysis and irradiation for each manufactured lot to ensure that the product being purchased meets the 10^{-6} Sterility Assurance Level found in the ANSI/AAMI/ISO 11137-2 2013 Sterilization of healthcare products – Radiation – Part 2: Establishing the sterilization dose standard.

Trigger-spray bottles perform by expelling the contained solution as the trigger is squeezed then aspirating the environmental air to re-pressurize the bottle. There is an article in **Cleanroom Technology**[4] that indicates that the typical trigger-spray bottle system can disperse biological contaminants that were previously aspirated from the cleanroom. If the aspirated air were to have spores present, the alcohol could become contaminated and dispense the aspirated spores into the air or onto a surface again.

Some common questions are: ***How long can an alcohol bottle remain uncontaminated inside a controlled environment? At what point will the solution become contaminated, 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."[8,9] It can be inferred that manufacturers are required to validate the amount of time a sanitizer bottle can be used inside the controlled environment and remain sterile. Usage can range from discarding after every shift or daily, weekly, monthly, or until the bottle runs out. As long as manufacturers have the data to support that the contents of the bottle are sterile, remain 70% denatured ethanol and the outside of the bottle is also sterile, manufacturers can operate as the data justify.

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 is not that simple. Alcohol is made from petroleum. It is also a hazardous chemical and VOC of which the US EPA tracks emissions. So, it would require more resources to dispose it. Bottles of Denatured Ethanol solution are sometimes used over several days. It is important to know how long the ethanol solution remains sterile in the bottle.

Tested Product

Texwipe's 70% denatured ethanol solution is a blend of USP-grade ethanol, USP-grade isopropanol and USP-grade purified water. Denatured ethanol is diluted with water to form the 70% denatured ethanol solution used in the TX3265 and TX3267 products. This solution is **0.2 µm filtered**, filled into cleaned bottles, and double-bagged. Then, the products are **gamma-irradiated** to a Sterility Assurance Level of 10^{-6} according to the ANSI/AAMI/ISO 11137-2:2013 standard. **Products are validated for sterility according to AAMI guidelines** at a third-party lab and are subject to periodic dose audits to comply with the sterilization standards.

Texwipe offers two sterile bottled products containing 70% denatured ethanol solution: **TX3265**, a 32 oz. trigger spray bottle, and **TX3267**, a 16 oz. trigger spray bottle. Each bottle is lot coded with an expiration date for easy recordkeeping. Bottles come fully-assembled and are ready for use. The adjustable spray nozzle offers stream or coarse spray delivery options.

The 70% Denatured Ethanol solution products are intended to be used for cleaning and residue removal:

- Cleaning general surfaces
- Pre-cleaning before disinfectant application
- Removing residues after disinfectant application
- Cleaning gloved hands
- Wiping down items for pass through to controlled and classified environments

Purpose

The purpose of this study is to show for how many days bottles of sterile 70% denatured ethanol in Texwipe's TX3265 spray bottles remain sterile after repeated use in a nonsterile environment.

Experimental

For this study, twenty-four 32-oz. (946 ml) bottles of TX3265, Sterile 70% Denatured Ethanol solution were tested in a non-classified, non-sterile laboratory environment. The bottles were sprayed two times per day, every day, during the experiment until the sample was selected as a sample for biological testing. Concurrent to the sampling days, passive biological air samples for bacteria, yeast and molds and air particle counts were taken. The study steps were as follows:

- Twenty-four sample bottles of TX3265 were numbered from 1 to 24.
- Starting on **Day 1**, all sample bottles were sprayed three times in a chemical hood, two times per day, approximately 12 hours apart. After the samples were sprayed, the bottles were returned to their storage area in the laboratory.
- Spraying continued every day, twice daily.
- On **Day 10**, after spraying all bottles, the first five bottles (Sample Nos. 1-5) were set aside. See **Figure 1** for the overall sampling regime for the entire study.
- The remaining bottles were sprayed daily until **Day 15** when another set of 5 bottles were put away (Sample Nos. 6-10). Spraying the bottles continued until the next sample day.
- Another five samples were put away on **Day 20** (Sample numbers 11-15) and **Day 25** (Sample Nos. 16-20) while continuing to spray the remaining bottles each day. The remaining 4 bottles were put away on **Day 30** (Sample Nos. 21-24).
- Measure the air particle and biological contamination levels in air on the start of the experiment and each day bottles samples are taken.

After the completion of the experiment, all 24 bottles were sent to an external microbial test laboratory for sterility testing.

Figure 1. Sampling regime for the entire study

Test start 24 bottles

- **Day 10** Samples 1-5 were placed away
- **Day 15** Samples 6-10 were placed away
- **Day 20** Samples 11-15 were put away
- **Day 25** Samples 16-20 were put away
- **Day 30** Samples 21-24 were put away

Biological and particle air sampling

Airborne particle counting was performed using a calibrated instrument, Air Particle Counter Innovation by Climet Model CI-500B-01. A ten-minute sample was taken which collected one cubic foot of air (28.3 L). Particles were measured at >0.5 µm.

The laboratory air particle test results showed particle levels expected for a normal, unfiltered air environment. The results are displayed in a chart as **Figure 2**.

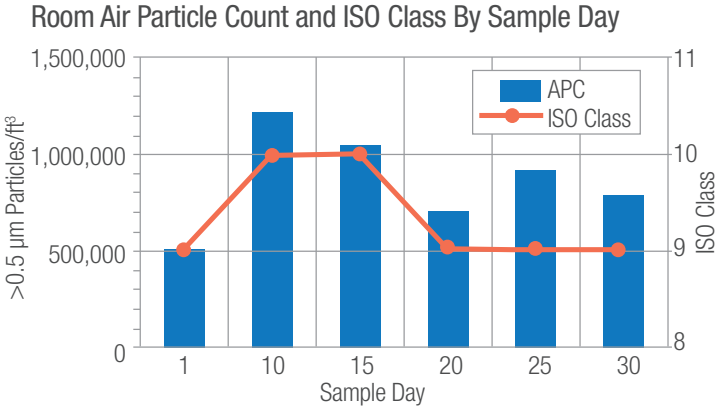

Figure 2. Air particle test results.

Microbial and particulate air sampling was performed in the laboratory on the day the experiment started and each day samples were set aside. For biological sampling, agar settle strips, Millipore HYCON® Agar Strips SDX-Y, ɣ-irradiated, catalog number 1442440040 for yeasts and molds and Millipore HYCON® Agar Strips TC-Y Total Count, ɣ-irradiated containing sodium thioglycolate, catalog number 1442260040 for bacteria, were chosen for passive air sampling. They were used as received and exposed for two hours in the laboratory near the chemical hood.

The bacteria strips were incubated at 30-35°C for 72 hours. The yeast and mold strips were incubated at 20-25°C for 72 hours. The strips were read after the incubation period, and the results were compiled and are presented as a chart in **Figure 3**. No bacteria or yeasts were observed. Bacteria are observed in the laboratory when surface samples are taken but are rarely observed through passive air sampling.

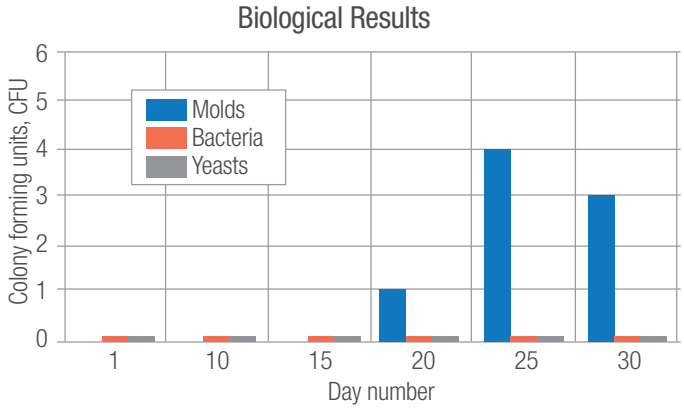

Figure 3. Mold, Bacteria and Yeasts Passive Air Sample Results

Sterility test results

All 24 Denatured Ethanol bottle samples were tested for sterility. The sterility test was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

The sterility test has shown no growth in all 24 samples. The test report is attached in the Appendix 1.

Discussion and Conclusion

The TX3265 Denatured Ethanol solution samples showed no microbial growth on Day 10, 15, 20, 25 and 30.

The study showed that the content of the bottles remained sterile for 30 days under in-use non-sterile conditions, i.e., repetitive sprays each day of the study duration.

The study results lead to a conclusion that, when aspirated through the product bottle's spray nozzle, microbes were exposed to the ethanol solution for an extended period of time, leading to their death. So, even the sanitization possibilities of ethanol solution are low, the long-term exposure of the microbes to the solution leads to their death.

Texwipe's 70% Denatured Ethanol solutions may be used for at least ***30 days without concern for its sterility change.***

Appendix 1.

Nelson Labs™
A Sotera Health company

ITW Texwipe
1210 S. Park Dr.
Kernersville, NC 27284

Product Sterility Final Report

Test Article:	TX3265 Lot: 180104 Exp. 01/20
Purchase Order:	TM18517LTJP
Study Number:	1059250-S01
Study Received Date:	08 Jun 2018
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0077 Rev 21 Customer Specification Sheet (CSS) Number: 201803927 Rev 01
Deviation(s):	None

Summary: The sterility test results are only valid with an acceptable method suitability test on this product (a separate study). All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

ILAC-MRA ANAB ACCREDITED TESTING LABORATORY

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Study Director Camille Coffey, B.S.

17 JUL 2018
Study Completion Date

1059250-S01

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nrg FRT0077-0001 Rev 18
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These results relate only to the test article listed in this report. Reports may not be reproduced except in their entirety. Subject to NL terms and conditions at www.nelsonlabs.com.

Results:

Test Article	Unit Number	SCDB
LTR0116-1	1	No Growth
LTR0116-2	2	No Growth
LTR0116-3	3	No Growth
LTR0116-4	4	No Growth
LTR0116-5	5	No Growth
LTR0116-6	6	No Growth
LTR0116-7	7	No Growth
LTR0116-8	8	No Growth
LTR0116-9	9	No Growth
LTR0116-10	10	No Growth
LTR0116-11	11	No Growth
LTR0116-12	12	No Growth
LTR0116-13	13	No Growth
LTR0116-14	14	No Growth
LTR0116-15	15	No Growth
LTR0116-16	16	No Growth
LTR0116-17	17	No Growth
LTR0116-18	18	No Growth
LTR0116-19	19	No Growth
LTR0116-20	20	No Growth
LTR0116-21	21	No Growth
LTR0116-22	22	No Growth
LTR0116-23	23	No Growth
LTR0116-24	24	No Growth

SCDB = soybean casein digest broth

Procedure:

Type of Test:	Membrane Filtration
Minimum Incubation Time:	14 days
Incubation Temperature:	28-32°C SCDB
Positive Control(s):	Passed growth promotion
Negative Control(s):	Negative
Approximate Media Volume:	180 mL
Rinse Medium:	Peptone Water (3 rinses, 100 mL ea)
Amount of Each Unit Tested:	Entire Test Article

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Reference

1 "Alcohols." Disinfection, Sterilization, and Preservation. Ed. Seymour S. Block, Ph.D., (5th ed.), Philadelphia: Lippincott Williams & Wilkins, 2001. 229-254." Chapter 12 presents the information for alcohols starting on page 229.

2 CDC Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

3 EudraLex, volume 4 "Good manufacturing practices", annex 1, point 62.

4 Tucker, J., Simpson, N., and Moss S., (2011) *Trigger happy*. Cleanroom Technology. Accessed on August 17, 2018 at CleanroomTechnology.com/news/article_page/Trigger_happy/60726.

8 Denny EF, Kopis EM, and Marsik, FJ. Elements for a Successful Disinfection Program in the Pharmaceutical Environment, PDA J. Pharm. Sci. Tech, 1999.

9 FDA (2004) Pharmaceutical CGMPs, *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice*, U.S. Department of Health and Human Services Food and Drug Administration, September 2004, p 34. Accessed on August 27, 2018 at FDA.gov/downloads/Drugs/Guidances/ucm070342.pdf.

For additional information, please contact Texwipe Customer Service at the number listed below.

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