Disinfectant Qualification – A Multifaceted Study

In this article, Ziva Abraham, Microrite, Inc., gives us a brief overview of the disinfectant qualification study process. An effective cleaning and disinfection program is critical to ensuring the quality of products in an aseptic manufacturing facility. A disinfectant qualification study will help you implement a sound cleaning disinfection program. This study should be thoroughly documented to comply with regulatory requirements.

Getting Started and Planning

The United States Food and Drug Administration (US FDA) Guideline for Aseptic Processing states, "The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces." Planning is an important part of the process of disinfectant qualification. When proper planning is accomplished, half the battle is won.

Factors to consider when planning a disinfectant study include:

1. Which chemical agent to use - Sanitizer? Disinfectant? Sporicide? Or all?
2. Which and how many microorganisms to use for the challenge test? Reference Cultures? Environmental Isolates? Or both?
3. Which and how many hard surfaces to test?
4. What test method to use - Suspension Test? Surface Test?

Types of Chemical Agents

Contamination prevention in an aseptic manufacturing facility begins by choosing chemical agents that are best for removing environmental (in-house) microorganisms. Three categories of chemical agents are sanitizers, disinfectants and sporicides. Sanitizers reduce the number of vegetative cells to a level accepted by public health ordinance. Disinfectants destroy or remove

vegetative forms of harmful organisms. Sporicides kill bacterial and fungal spores as well as vegetative cells.

The following are some factors which should be considered when choosing a chemical agent:

1. Operator safety
2. Cleaning ability of chemical agent
3. Compatibility with the surface to be disinfected
4. Types of in-house microorganisms in the aseptic facility
5. Residual activity of the disinfectant
6. Regulations, for example, Environmental Protection Agency (EPA) and Occupational, Safety and Health Administration (OSHA)

Basic knowledge regarding the effectiveness of different chemical agents against vegetative bacteria, fungi, and spores aids in selecting chemical agents. It will also be useful in troubleshooting and validating the data generated during the qualification study.

Challenge Organisms

This is where trending of environmental microorganisms and product contaminants come in handy. Trending data will provide a good assessment of the microorganism strains that are commonly present in your facility. Challenge microorganisms for disinfectant qualification should include both reference cultures and environmental isolates. Microbiologics provides a variety of solutions that make disinfectant challenges easy.

Reference Cultures

The type of reference cultures is determined by the test method. For example, United States Pharmacopeia (USP) lists typical challenge reference organisms in Chapter <1072>. Microbiologics offers a wide variety of ready-to-use, lyophilized microorganism preparations that are convenient, economical and reliable. Cultures for disinfectant qualification can be purchased in qualitative form such as KWIK-STIK™ and LYFO DISK®, or in quantitative form such as Epower™. Each Microbiologics Quality Control microorganism is authentic and traceable.

The Microbiologics® KWIK-STIK™ features a lyophilized microorganism pellet, reservoir of hydrating fluid and inoculating swab

Microbiologics® Epower™ microorganism preparations are available in concentrations ranging from 10^2-10^8 per pellet

Below are examples of typical microorganisms listed by USP for disinfection challenge testing.

Catalog #	Description
0681	*Escherichia coli* ATCC® 11229™*
0485	*Staphylococcus aureus* ATCC® 6538™*
0693	*Pseudomonas aeruginosa* ATCC® 15442™*
0443	*Candida albicans* ATCC® 10231™*
0896	*Candida albicans* ATCC® 2091™*
0392	*Aspergillus brasiliensis* ATCC® 16404™*

Environmental Isolates

Use of environmental isolates is important because they can be harder to kill than reference cultures. Regulatory authorities want to see evidence of the efficacy of disinfection agents against environmental isolates found on cleanroom surfaces. The most predominant environmental isolates should be used. Proper preservation of environmental isolates will ensure the strains remain stable and viable.

Maintaining environmental isolates in-house is not only time consuming and labor-intensive, it can also prove to be problematic. Microbiologics offers environmental isolate preservation services. Companies send their environmental isolates to Microbiologics where they are preserved and packaged to the customer's specifications in easy-to-use, convenient formats. They are then safely and reliably shipped to the customer's testing location(s) throughout the world. In the event a revalidation is necessary in the future, the preserved isolate can be used to compare past to present results.

Test Methods

Publications or regulatory guidance/standards such as Chapter <1072>, "Disinfectants and Antiseptics" in the United States Pharmacopeia address the issue of disinfectant testing but do not state which method to use. The US FDA expects evidence of efficacy of disinfectants. The validating data should support sanitization and disinfectant procedures.

There are three basic methods for qualifying disinfectants. They are the suspension test, the carrier test, and the surface test. The suspension test suspends organism directly into disinfectant. The carrier test submerges inoculated steel or glass penicylinders in disinfectant. The surface test directly inoculates the manufacturing surface.

Suspension tests (for example, the AOAC Use-Dilution Test) are performed in order to register disinfectants with EPA or other foreign agencies. They yield a high log reduction but do not reflect the conditions in the clean room. The outcome of suspension tests might be a poor predictor for the efficacy of a disinfectant on clean room surfaces.

Hard surface tests are used to mimic clean room conditions, including the nature of the surfaces and the application method, be it spraying or mopping. One hard surface method involves testing the disinfectant on coupons, which are approximately 2" by 2" samples of representative surfaces found in the clean room. Examples of surfaces are stainless steel, vinyl, and tile. Below is a brief summary of a method that uses coupons:

1. The coupons are inoculated with 0.1 ml inoculum of microorganism.
2. The disinfectant is applied onto the coupon.
3. The disinfectant is left on the coupon for a specified amount of time. (For example, 0, 5 and 10 minutes.)
4. The coupons are sampled for recovery.
5. The samples are neutralized and plated or filtered and transferred onto media, and incubated.
6. Colonies are enumerated.
7. Log reduction is determined for treated coupons versus untreated coupons. The use of positive controls (untreated coupons) is important. Log reduction calculations depend upon good control counts.
8. The goal for sporicidal activity is at least a 2 log CFU reduction and for bactericidal activity is at least a 3 log CFU reduction.

In order to validate the disinfectant is effectiveness; manufacturers will often use a combination of test methods. Types of disinfection methods include the following:

1. AOAC Use-dilution method
2. AOAC Hard surface carrier test method
3. AOAC Germicidal Spray Products
4. ASTM Standards E2111, Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Microbicides
5. ASTM Standards E2197 Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides
6. ASTM Standards E2315 Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure
7. ASTM BS EN13697 Chemical Disinfectants and Antiseptics. Quantitative Non-Porous Surface Test for the Evaluation of Bactericidal and/or Fungicidal Activity of Chemical Disinfectants used in Food, Industrial, Domestic and Institutional Areas

Efficacy Study Using Swab Recovery Method (Courtesy of Microtest Laboratories, Agawam, MA, USA)

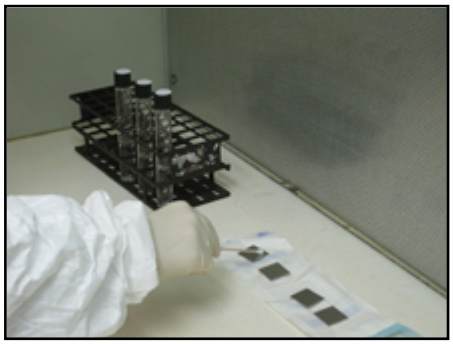

Image 1: Swab recovery method is utilized to recover microorganisms from stainless steel coupons after the inoculum is challenged with the disinfectant

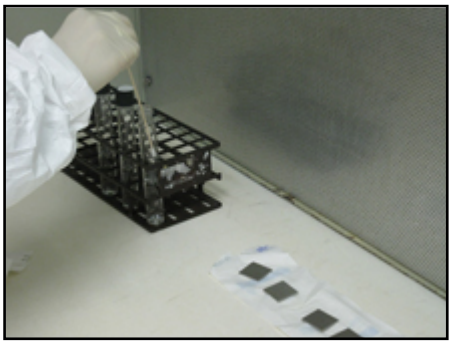

Image 2: Recovered microorganisms are delivered into buffer which is then vortexed and filtered to get count of surviving microorganisms

Conclusion

Understanding disinfectant qualification methods and the translation of qualification study data to cleaning procedures, is the key to avoiding contamination and its pitfalls such as failed media fills or sterility tests. Qualification of sanitizing agents and disinfectants is a major undertaking that requires detailed planning, careful execution and scrutiny of data generated. To learn in greater details about qualifying disinfectants for microbes from aseptic manufacturing environment and biofilms, visit www.microrite.com for the upcoming training in your area.

References

1. US Environmental Protection Agency (EPA) "Antimicrobial Pesticide Products" http://www.epa.gov/pesticides/factsheets/antimic.htm
2. US Pharmacopeia. 2010. <1072>, Disinfectants and Antiseptics, Reissue USP33-NF28.
3. B.Van Klingeren, W. Koller, S.F Bloomfield, R. Bohm, A. Cremieux, J. Holah, G. Reybrouck and H.J. Rodger, Assessment of the efficacy of disinfectants on surfaces, International Biodeterioration and Biodegradation, Volume 41, Issue 3-4, 1998, Pages 289-296, Hygiene and Disinfection.
4. Seymor S. Block, Disinfection, Sterilization, and Preservation, Fifth Edition, Lippincott, Williams and Wilkins.
5. Denny, V.F and F.J. Marsik, 1997, Current practices in the Use of Disinfectants within the Pharmaceutical Industry, PDA J. Sci. Technol, 1997, 51: 227-228.
6. Elaine Kopis, Disinfectants Program, Microbiology in Pharmaceutical Manufacturing, 2001, PDA, DHI Publication.
7. Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice www.fda.gov/downloads/Drugs/.../Guidances/ucm070342.pdf.
8. Lucia Clontz, Microbial Limit and Bioburden Tests: Validation Approaches and Global Requirements, Second Edition, 2008, CRC Press.

About the Author

Ziva Abraham, Microrite, Inc., has over 25 years of academic, research, clinical and industrial experience in Microbiology, and Quality Assurance. She has trained personnel from various industries in microbiology techniques and methods. Ziva has received her Master's Degree in Microbiology and has conducted research on developing Microbial Insecticides. She has established clinical laboratory systems in Israel, Microrite, Inc., a consulting company based in San Jose, CA and Microrite Training Center in Santa Clara, CA. Microrite helps Pharmaceutical, Medical Device, Biotechnology and Combination Product Companies in the areas of Quality Assurance, Validation, Process Development and Microbiological Quality Control. Ziva has also developed "BACTISPELL" a microbiology spellchecker to spell check genus and species names of microbes and other microbiology related terms. She is a member of PDA, ISPE, AAMI, and PMF and is an active mentor for graduate students at Stanford University working through the American Woman in Science Organization (AWIS). She is involved in Expanding Your Horizons, a program through the Math and Scientific Network to educate young girls about careers in science. Ziva serves on the editorial board of Pharmaceutical Microbiology Forum (PMF) Newsletter.

Acknowledgements

ATCC Licensed Derivative®

*The ATCC Licensed Derivative Emblem, the ATCC Licensed Derivative word mark and the ATCC catalog marks are trademarks of ATCC. Microbiologics, Inc. is licensed to use these trademarks and to sell products derived from ATCC® cultures.