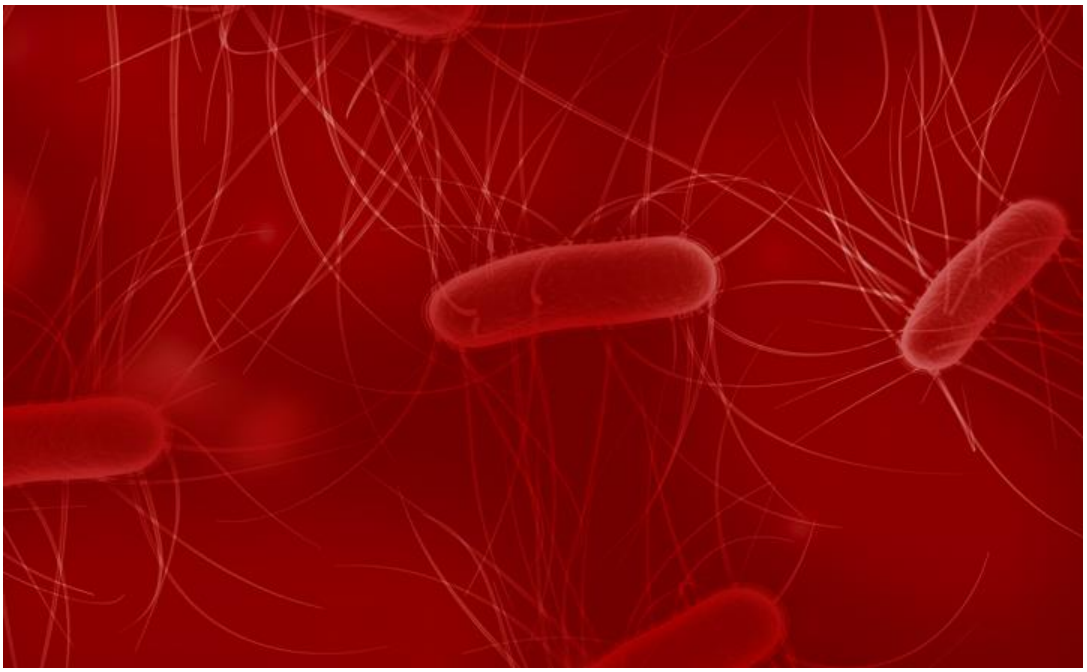
ATS LABS



DISINFECTION QUALIFICATION TESTING—CONSIDERATIONS FOR THE ASEPTIC AND CLEANROOM MANUFACTURING ENVIRONMENT

by Dave Rottjakob, M.T. (ASCP)

December, 2013

ATS LABS
EXCELLENCE IN ANTIMICROBIAL TESTING

INTRODUCTION

Obtaining the highest confidence that aseptic, cleanroom, and other critical manufacturing environments are properly cleaned, sanitized and disinfected is paramount in assuring the production of safe and effective pharmaceutical products and/or medical devices. The microbiological safety of these products is primarily determined by the quality of raw materials, the integrity of the manufacturing process, as well as the effectiveness of cleaning and disinfection procedures performed in the facility. It is for this reason that the U.S. Food and Drug Administration (FDA) requires manufacturers of pharmaceutical and other critical products to qualify and validate the disinfection procedures used in these manufacturing environments. This paper is intended to provide an overview of disinfection qualification testing and the considerations that must be addressed when designing and executing these studies.

WHAT IS DISINFECTION QUALIFICATION?

Disinfection and sanitization in the pharmaceutical and controlled manufacturing spaces refer to the killing, inactivation, removal or reduction of contaminating microorganisms to levels considered safe per industry standards and regulations. The terms "cleanroom disinfection qualification", "disinfection validation", and "cleaning validation" are often used in the pharmaceutical and aseptic manufacturing industries interchangeably. While these terms seem to define the same thing, they are actually rather different. In fact, validations build upon qualifications.

> "The FDA requires that all aseptic and sterile manufactures to qualify disinfection products and procedures with a formal disinfection qualification study.

Disinfection qualifications formally evaluate the efficacy and suitability of antimicrobial products and procedures used to eliminate contaminant microorganisms on various surface types and components within an aseptic, sterile or otherwise controlled manufacturing environment. Disinfection qualifications are critical in assuring the microbial control of a manufacturing environment by qualifying the appropriate use and effectiveness of disinfection products and procedures.

Disinfection qualifications **are not** disinfection validations. A disinfection validation assures that the sterile, aseptic and even non-sterile manufacturing environments are under microbial control as measured by a comprehensive and continuous environmental monitoring program.

Furthermore, disinfection qualifications are not cleaning validations. Cleaning validations are studies designed to measure a procedure's effectiveness at removing by-products or residual chemicals which may result during the manufacturing process.

ATS LABS
EXCELLENCE IN ANTIMICROBIAL TESTING

WHY ARE DISINFECTION QUALIFICATION STUDIES IMPORTANT?

Disinfection qualifications effectively mitigate the microbial contamination that may occur during the manufacture of a product. Mitigating such contamination ultimately helps to ensure a safe product for the end-user or patient. Control of microbial contamination is required by the FDA's CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS as defined in 21 CFR §211.113 which states:

> **Control of Microbiological Contamination**
>
> (a) Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed.
>
> (b) Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes.

These requirements are additionally clarified in FDA's 2004 Guidance for Industry – "Drug Products Produced by Processing- Current Good Manufacturing Practice." This guidance addresses disinfection qualification stating:

> **Disinfection Efficacy**
>
> 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.

While federal regulations and guidance documents mandate microbial control of sterile and non-sterile manufacturing environments, the interpretation and enforcement of these regulations can be seen through GMP inspections and the resulting FDA Form 483 warning letters. The extent of the deficiencies listed in a 483 warning letter can mean the difference between being operational or being shut down. It has been well documented that the observations citing "the failure to ensure disinfection" in 483 warning letters have been trending upward.

ATS LABS
EXCELLENCE IN ANTIMICROBIAL TESTING

RECENT FDA FORM 483 WARNING LETTER EXCERPTS:

- "Your firm has not established procedures designed to prevent microbiological contamination of drug products purporting to be sterile." Warning Letter dated February 22, 2012

- "Your disinfectant qualification for (b)(4) and (b)(4) bi-spore disinfectants documented that the log reduction criteria (Bacteria>4, Fungi>3) was not met when challenged with multiple organisms in a variety of surfaces. After disinfection, you recovered *Micrococcus luteus* on vinyl, (b)(4), stainless steel, glass and wall laminate and *Enterobacter cloacae, Rhodococcus* sp, *Burkholderia cepacia, Pseudomonas aeruginosa*, *Methylobacterium mesophilicum* and, *Acinetobacter lwoffi* on glass. However your procedures for routine cleaning of the aseptic manufacturing area continue to require the use of unqualified disinfectants during days (b)(4) through (b)(4) of your disinfection program." Warning Letter dated October 7, 2011

- "The qualification of your disinfectant (b)(4) failed to demonstrate that it is suitable and effective to remove microorganisms from different surfaces. Specifically, this disinfectant failed to meet the qualification criteria when challenged with multiple organisms." Warning Letter dated October 7, 2011.

- "We note that the cGMP violations listed in this letter include similar violations to those cited in the previous inspection in February 2008 […] 3) failure to adequately conduct disinfectant efficacy studies, and 4) inadequate quality control unit oversight." Warning Letter dated July 14, 2011.

- "The materials that were tested in the Disinfectant Efficacy study were not representative of all the surfaces present in the Aseptic Processing Area. […] The stainless steel coupon tested did not represent these damaged surfaces." Warning Letter dated May 25, 2011.

Resource: http://www.fda.gov/iceci/enforcementactions/WarningLetters/default.htm

ATS LABS
EXCELLENCE IN ANTIMICROBIAL TESTING

WHY DOES THE FDA REQUIRE DISINFECTION QUALIFICATION STUDIES?

United States Pharmacopoeia (USP) <1072> "Disinfectants and Antiseptics" states that the disinfection qualification study "is considered necessary since critical process steps like disinfection of aseptic processing areas, as required by GMP regulation, need to be validated and the EPA registration requirements do not address how disinfectants are used in the pharmaceutical, biotechnological and medical device industries."

To better understand this nuance, you must first understand the regulation of disinfectant and sanitizer products themselves. Before disinfectant products can be sold in the United States, the products must be "registered" with the Environmental Protection Agency (EPA) to support the efficacy label claims. In short, standardized testing is performed using quality control strain microorganisms (e.g., *Staphylococcus aureus* and *Pseudomonas aeruginosa*) on a single representative hard surface such as stainless steel or glass to generate disinfection claims. In contrast to these claims, the manufacturing environment utilizes a wide variety of surface types and encounters a wide variety of microbes. This difference is notable. A disinfectant product that has a standard label claim for efficacy, as supported on stainless steel or glass surfaces, does not often demonstrate the same level of efficacy on other surface types against the specific environmental isolates found in the manufacturing environment. Therefore, disinfection assessment techniques must be modified to evaluate the true uses of the product in these environments. For example, it is often necessary to increase the contact time from what is listed on the product label to achieve the desired efficacy.

Table 1 displays the differences found in $\log_{10}$ reductions using the same test organism and disinfectant on multiple surface types. The table illustrates that with the particular disinfectant studied, microbial reduction from plastic based surfaces was relatively less efficient than the obtained reduction from stainless steel. The anomalies are highlighted in red.

Table 1: Example of Differences in Microorganism Reduction Found on Multiple Surface Types Using the Same Product

Active Ingredient (5 min. Exposure Time)	$\log_{10}$ Reduction of Environmental Isolate (*Staphylococcus* sp.) on the Following Routine Surfaces							
	PVC	Lexan	Vinyl	Epoxy	Stainless Steel	Poly-propylene	Glass	Poly-ethylene
Peracetic acid/ Hydrogen peroxide	>4.62	>4.45	>4.38	>4.25	>4.67	>4.21	>4.55	>4.32
Quaternary Ammonium	>4.21	>4.12	**2.78**	4.66	>4.23	**2.62**	>4.35	>4.6
Hydrogen Peroxide	**1.62**	**1.15**	**<1.21**	**1.87**	>3.79	**<0.87**	**1.72**	**1.25**
Sodium Hypochlorite	>4.03	>4.36	>4.83	>4.20	>3.85	3.94	>4.54	>4.62
Alkaline Phenolic	>4.03	>4.36	**2.57**	4.20	>3.85	**<1.02**	>4.54	**1.68**

WHEN SHOULD DISINFECTION QUALIFICATION STUDIES BE CONDUCTED?

The ideal time to conduct a disinfection qualification study is at the construction of the manufacturing facility, prior to operation, when disinfection processes and products are being considered. At a minimum, a qualification should be performed prior to starting full scale GMP manufacturing operations and prior to an FDA GMP audit. Unfortunately, disinfection qualifications are often performed reactively instead of proactively, either in response to a product contamination, an environmental monitoring excursion, or to the observations listed in the feared FDA Form 483 warning letter. Waiting to perform a qualification study in these scenarios can lead to a cease in manufacturing operations and the commissioning of a disinfection qualification study much larger than necessary.

Once the procedures have been qualified, the manufacturing environment should be continuously monitored to identify newly trending environmental isolates. This allows manufacturing facilities to successfully determine when additional disinfection qualification testing is necessary. In addition, supplemental qualifications should be performed following a change in a disinfectant product, a modification in cleaning or disinfection procedures and the incorporation of any new surfaces into the cleanroom or aseptic manufacturing area.

> **BE PROACTIVE!**
> Studies should be conducted:
>
> 1. Before full-scale manufacturing
> 2. When changing disinfectants
> 3. When changing procedures
> 4. Before FDA audits
>
> Do not wait until it's too late.

HOW SHOULD DISINFECTION QUALIFICATION STUDIES BE PERFORMED?

As previously mentioned, the disinfection and sanitization of surfaces in the pharmaceutical and controlled environment manufacturing space refers to the killing, inactivation, removal and reduction of contaminating microorganisms to levels considered safe per industry standards and regulations. But how are disinfection qualification studies to be performed? Unfortunately, there is no standard method outlining step-by-step instructions of *how* disinfection qualifications studies should be conducted. However, general guidance can be found in the United States Pharmacopeia (USP) <1072> document and in the ASTM International E2614 guidance document.

Suspension-based Testing vs. Coupon-based Testing

In general, disinfectant efficacy evaluations are made using either suspension-based methods or coupon/surface-based methods. Suspension methods evaluate the reduction of a known organism population inoculated directly into a sample of the liquid disinfectant. Following inoculation and the observation of a pre-determined contact time, samples of the inoculated substance are removed, neutralized and evaluated for survivors as compared to an untreated control suspension. Since the simulation of organism films on the specific surface types used in the space is not accounted for in this method, it is recommended that suspension-based tests be used only for initial disinfectant screening purposes.

In contrast, coupon/surface-based testing is more rigorous and involves the creation of a dried organism film onto representative surface types which best simulates the contaminated environment. The surfaces are then exposed with the disinfectant utilizing a simulated-use procedure. Following a pre-determined contact time, each surface is neutralized and surviving organisms are enumerated in a quantitative fashion for comparison to untreated surfaces.

As is evidenced by FDA 483 warning letters, coupon-based testing is recommended to qualify disinfectants used in the pharmaceutical and aseptic manufacturing arena.

Scope of Qualification Testing

Each manufacturing facility is different and therefore each qualification study is different. Considering the various combinations of surfaces, organisms, disinfectant products and disinfection procedures, these studies can become rather complex. Tables 2, 3 and 4 list common considerations that contribute to the complexity of these tests and help illustrate why careful scientific rationale should be used in the design of the studies.

Table 2: Surface Types

Surface Types Commonly found in the Pharmaceutical/Aseptic Manufacturing Environment
Stainless steel (304 or 316 grade)
Polyvinyl chloride (PVC)
Glass
Lexan® (plexiglass)
Polyethylene
Tyvek
Terazzo tile
Teflon
Polypropylene
Fiberglass-reinforced Plastic
Anodized Aluminum alloys

Table 3: Challenge Microorganisms

Typical Challenge Microorganisms Used in Disinfection Qualification Studies	
Standard Reference Isolates	**Environmental Isolates**
Vegetative Bacteria	
Staphylococcus aureus	*Micrococcus luteus*
Escherichia coli	*Staphylococcus hominis*
Pseudomonas aeruginosa	*Staphylococcus epidermidis*
	Burkholderia cepacia
	Corynebacterium species
Yeast and Mold (Fungi)	
Candida albicans	*Aspergillus* species
Aspergillus brasiliensis	*Penicillium* species
Penicillium chrysogenum	
Spore-forming Bacteria	
Bacillus subtilis	*Bacillus species*
	Paenibacillus glucanolyticus

Table 4: Active Ingredients of Typical Disinfectant Products and Typical Method Applications

Active Ingredients of Typical Disinfectant Products Used in Aseptic Manufacturing	Typical Methods for Application Of Disinfectants in the Aseptic Manufacturing Environment
Peracetic acid/ Hydrogen peroxide	Mop-on
Quaternary Ammonium	Spray
Alcohol (Ethanol/Isopropyl)	Wipe
Sodium Hypochlorite	Flood
Alkaline Phenolic	With or without rinse step
Acidic Phenolic	With or without squeegee
Hydrogen Peroxide	Fogging or Submersion

In addition to the aforementioned testing considerations, the following technical elements, amongst others, must be given serious consideration while developing the scope of the study:

- Coupon/Carrier Preparation
- Organism Preparation, and Coupon Inoculation
- Drying Conditions for Optimal Organism Viability
- Disinfectant Preparation
- Determination and Testing of Expiration time for Diluted or Activated products
- Disinfectant Application and Exposure Times
- Neutralization of Coupons and Recovery of Survivors
- Disinfectant Performance Criteria (e.g., contact time and concentration)

The explanation of these technical elements falls outside of the scope of this document but will be the subject of a forthcoming white paper. The following provides an overview of the procedures commonly used in these studies.

OVERVIEW OF STUDY DESIGN

Once the study parameters have been established, a testing protocol is developed. The overall testing process is generally executed as follows. Each surface coupon is individually inoculated with test organism. A sufficient number of coupons must be inoculated to evaluate each disinfectant, each test organism and each disinfection procedure for each coupon replicate. The coupons are placed into an incubator to allow the test organism to dry as a film. Once dried, each coupon is treated and exposed to the disinfectant. Following careful monitoring of the exposure, each coupon is transferred to a pre-selected solution designed to neutralize the disinfectant and elute or rinse off any surviving test organisms. This solution is quantitatively evaluated to enumerate the number of survivors onto an appropriate agar plate medium. Untreated, inoculated, coupons are similarly enumerated to determine the starting level of test organism on each surface type prior to treatment. Appropriate controls should be included with the study to assess the sterility of the materials used in testing and to confirm the adequacy of the neutralization techniques used.

After incubation, the recovery plates are enumerated and the study controls are evaluated to assure study validity. Survivors found on the treated coupons are compared to survivors recovered on the untreated control coupons to determine the $\log_{10}$ reductions. The level of reduction observed can then be used to assess the success of the disinfection procedure.

CONCLUSION

Properly designed, appropriately qualified and consistently executed disinfection procedures are critical to the production of safe and effective biopharmaceuticals, medical devices and other sterile or non-sterile products. As demonstrated in various FDA Form 483 warning letters, the proper qualification of these disinfection procedures is required. The major considerations and potential variables that must be addressed when considering the design and execution of a successful disinfection qualification study have been outlined in this document. Careful review of the data collected in properly executed qualification studies will help facilities monitor potential deficiencies in their cleaning and disinfection program. As a result of the disinfection qualification studies, future trends that fall outside the pre-established disinfection program will allow facility staff to investigate and take corrective action to re-establish environmental control ultimately ensuring a safer product for the end-user or patient.

> “With a properly designed **disinfection program**, manufacturers can better control the risk of microbial contamination, ensuring safe products for patients.

REFERENCES

1. United States Pharmacopeia (USP) 34, Chapter <1072> Disinfection and Antiseptics – General Information pp.579-580, May 1, 2011.
2. American Society for Testing and Materials (ASTM). Test Method, Standard Guide for Evaluation of Cleanroom Disinfectants, E2614-08.
3. U.S. Food and Drug Administration (FDA), Guidance for Industry Sterile Drug Products Produced by Aseptic Processing- Current Good Manufacturing Practice, 2004.
4. Block, SS, *Disinfection, Sterilization, and Preservation*. Lippincott, Williams and Wilkins, Philadelphia, PA., 2001.
5. Madsen, R.E and Moldenhauer, J. *Contamination Control in Healthcare Product Manufacturing,* Volume 1., DHI Publishing, River Grove, IL. (2013).
6. Inspections, Compliance, Enforcement, and Criminal Investigations, Retrieved http://www.fda.gov/iceci/enforcementactions/WarningLetters/default.htm, 2013.
7. Current Good Manufacturing Practice for Finished Pharmaceuticals, 21 CFR Part 211.

ABOUT ATS LABS

ATS Labs is an antimicrobial testing laboratory providing the developers, manufacturers, and users of antimicrobial products with a comprehensive range of microbiology, virology and product chemistry services to meet both product development and regulatory testing needs. ATS Labs has delivered the highest quality scientific data to clients for over 20 years. The scientific, quality assurance and administrative staff have built a strong reputation for antimicrobial technical expertise, regulatory compliance knowledge, and client service.

FOR MORE INFORMATION CONTACT

651.379.5510 | www.ats-labs.com
TOLL FREE 1.877.ATS.TEST (1.877.287.8378)
FAX 651.379.5549

1285 Corporate Center Drive, Suite 110
Eagan, MN 55121