Review Article

Guideline For Establishing the Maximum Allowable Effectiveness and Sufficient Contact Time Period for Used as Disinfectant and Sanitization Solution

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ABSTRACT

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. 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 procedures effectiveness at removing by-products or residual chemicals which may result during the manufacturing process. The FDA requires that all aseptic and sterile manufactures to qualify disinfection products and procedures with a formal disinfection qualification study. 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. In the present review article we summarized a standard guideline for establishing the effectiveness and suitability for disinfectant and sanitization solution.

Keywords: Sanitization, Disinfectant, Microorganism, Cleaning, Validation.

INTRODUCTION

Cleaning validations are conducted to show that the cleaning process and frequency, including any mechanical cleaning actions, are sufficient to maintain surfaces in a defined state free of product, cleaning chemical residues and adventitious infectious organisms. These studies demonstrate that materials can be cleaned to the desired chemical and microbiological levels.

According to Good Manufacturing Practice (GMP) regulations, FDA guidance and USP <1072> manufacturers of finished bio/pharmaceutical products must demonstrate that harmful residues or organisms are properly removed during cleaning to predetermined safety levels, thus eliminating contamination of manufacturing equipment. Disinfectant efficacy studies are performed to demonstrate that the disinfectants used on surfaces in manufacturing areas, laboratories and other facility areas are effective in inactivation or removal of microorganisms, such as bacteria, fungi (yeast and molds), bacterial spores, viruses and mycoplasma. Disinfectant studies can support cleaning studies by showing that application of the disinfectant reduces or eliminates microorganisms, but they should not be considered a substitute for establishing that the cleaning agents and physical cleaning actions are acceptable[1].

Phases for Chemistry Cleaning Validation

a) **Method Establishment and Sampling Plan Generation** - Define analyte(s) of interest, applicable surfaces and finishes, appropriate maximum contamination limit (MCL), sampling technique (swab vs. rinse) and method of detection (specific vs. non-specific).

b) **Method Development** - Develop a cleaning detection method that addresses all specific client needs.

c) **Method Feasibility** - Method is evaluated to establish performance criteria and ensure that the method will be suitable for its intended purpose (typically would evaluate swab and surface recovery, linearity, sensitivity and accuracy).

d) **Protocol Writing and Method Validation** - Create a validation protocol and execute the method validation in a controlled GMP environment.

e) **Routine Analysis** - Method is used to analyze samples as part of a routine monitoring program[2-5].

Phases for Microbiology Cleaning Validation

a) **Protocol and Sampling Plan Generation** - Establish the sites to be sampled for microorganism contamination (bioburden), the collection method to be used and how data will be handled.

b) **Risk Identification** - Define the health based exposure limits as suggested by the EMA guidelines for shared facilities.

c) **Method Qualification/Neutralization/Recovery Execution** - Establish that microorganisms can be recovered in an acceptable range from the surface using the proposed collection method. Neutralization studies of cleaning agent or disinfectant residues are incorporated into this phase of the study if needed.

d) **Sampling Plan Execution and Analysis** - Collection of samples is performed using the determined method, and samples are evaluated for microorganisms prior to and/or after the cleaning procedure are performed to show cleaning is effective. Where available, corresponding chemical analysis from adjacent areas ensures chemical residuals are not at an inhibitory range for the microorganism recovery.

e) **Routine Monitoring** - Periodically analyze collected samples after cleaning to ensure process remains in control over time.

Disinfectant Efficacy Studies

A disinfectant study confirms that disinfectant agents used are active against organisms isolated during the environmental monitoring program and/or against other relevant organisms, under experimental conditions that simulate their practical use. These studies, which are especially critical for sterile manufacturing facilities, have been performed for bacteria and fungi for many years. In the past decade, as more biopharmaceutical products enter the market, disinfectant efficacy studies are being routinely performed for products using mycoplasma and viruses.

Phases for Disinfectant Studies

- **Study Design and Protocol Generation** - Determine microorganisms, surfaces, disinfectants and treatment conditions to be tested.
- **Surface Efficacy Studies** - Determine the effectiveness of inactivation of the desiccated microorganism by disinfectants on appropriate surfaces.
- **Suspension-Efficacy Studies** - Evaluate the effectiveness of the disinfectant in suspension (optional). This test provides relevant information about the activity of the product against nondried microorganisms. Desiccated microorganisms may be stressed and may offer different challenges.
- **Use-Dilution Expiration Studies** - Verify the effectiveness of the disinfectant up to and beyond the pre-determined expiration date.

These studies include the following controls:

- **Recovery Controls** - Evaluate the ability to recover the organisms applied to the surfaces (conducted prior to or simultaneously with efficacy studies).
- **Disinfectant Neutralization/Toxicity/Interference Controls** - Establish that disinfectants and sample matrix do not impact the assay for the organisms being tested.

Routine Chemical & Microbial Monitoring

Ongoing environmental monitoring and chemical monitoring (e.g., TOC swabs) confirm that the surfaces and equipment are free of microorganisms and inhibitory residues that might interfere with the microorganism recovery and establish that the cleaning frequency maintains the desired condition of the equipment between cleanings[6-10].

Six Steps to Qualifying Disinfectants

Sterile pharmaceutical and medical device manufacturing environments require an effective cleaning and disinfection program to maintain aseptic conditions and prevent the microbial contamination of the product. The qualification of the chemical disinfectants used in these environments is extremely important, yet it is often overlooked. Disinfectant qualifications require more planning, time and resources than many companies realize. Considering the potential issues and difficulties that could occur while performing these qualifications, contracting an outside lab experienced in disinfectant qualifications may be the most efficient and least painful way to perform this work.

The following six steps provide a framework to assist companies in qualifying the disinfectants used in their environmental cleaning processes. Whether performed internally or by an outside testing lab, they must be addressed.

Step1: Determine the Test Method There is a number of methods for qualifying a disinfectant published by the Association of Official Analytical Chemists (AOAC), yet these are for qualifying the disinfectant itself. They are not appropriate for demonstrating the efficacy of a disinfectant within the pharmaceutical, biotechnology, and medical device industries. Two of the most common methods suggested for disinfection qualification in these environments are:

- Tube method: This method evaluates disinfectants by inoculating dilutions of the disinfectant and determining the microbial reduction. It would most commonly be used as a simple screening to determine the type of disinfectant most effective against a specific set of organisms before performing a comprehensive disinfectant qualification.
- Coupon method: This method is more comprehensive and uses coupons made from actual facility surfaces. The surfaces are

inoculated and exposed to the disinfectant. The inoculum is then removed from the surfaces and the $\log_{10}$ reduction determined.

Step 2: Determine the Challenge Organisms

Typically, standard American Type Culture Collection (ATCC) test organisms representing the basic classes of microorganisms (Gram negative, Gram positive, spore-former, fungus) along with actual environmental isolates from the clients facility should be used in the qualification.

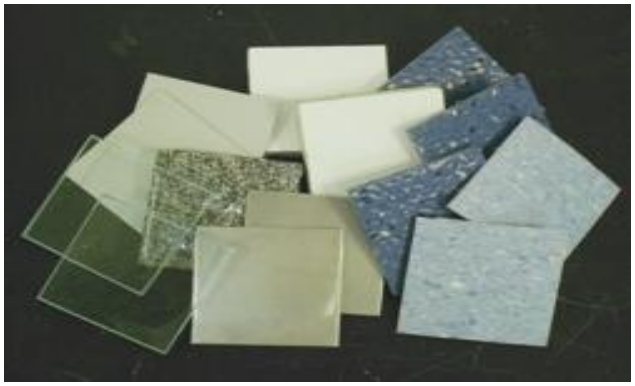

Figure 1: Examples of surface sample coupons (polypropylene, vinyl, stainless steel, epoxy coated stainless steel)

Step 3: Determine the Surface Types to be tested each of the construction materials used in the clean room and/or other controlled areas should be tested separately. Examples of common materials are stainless steel, glass, plastic, and Plexiglas Normally 2-inch by 2-inch square coupons are used for the qualification.

All coupons must be sterilized or disinfected before use in the qualification. Depending on the material, sterilization may be accomplished through steam, ethylene oxide (EO), or chemical methods.

Step 4: Determine Expiration of Disinfectants

The qualification should replicate the same disinfectant concentration and contact exposure time used in the facility. It also should be performed using the worst-case expiration date for each disinfectant. If a container has a 30-day expiration once opened, and a dilution may be prepared and put into a spray bottle with an expiration of seven days, the efficacy testing should reflect this.

Figure 2: Examples of surface disinfectants

Step 5: Method Validation Method validation is a critical step to verify that the testing method allows adequate recovery of the challenge organisms in the presence of the disinfectants. Regardless of the method being used, the test system must be inoculated with a low level of challenge organism, with and without (control) exposure to the disinfectant for the designated contact time.

Typically, the recovery of the challenge organisms should be within a factor of two of the positive controls for that organism. If the recovery is not satisfactory, the testing method should be repeated using a different neutralization system and/or additional dilutions.

Step 6: Efficacy Testing

Efficacy testing is the actual testing of the disinfectant. Per the USP General Chapter <1072> Disinfectants, the test system is inoculated with sufficient inoculum to demonstrate at least a two $\log_{10}$ reduction for bacterial spores and a three $\log_{10}$ reduction for vegetative bacteria and allowed to dry. The inoculated system is then exposed to the desired concentration of the disinfectant for the desired contact time.

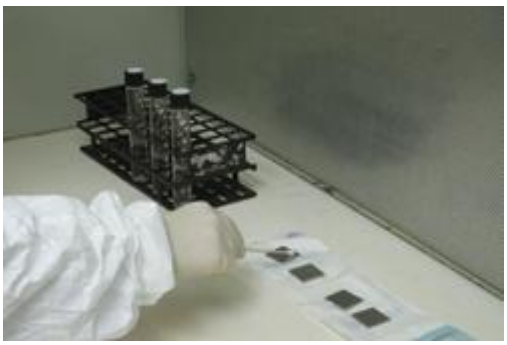

Figure 3: Swabbing the inoculated coupons

The surviving population in the test system is determined and the $\log_{10}$ reduction calculated. The log reduction data should be used to establish a scientifically supported disinfection program for the clients facility.

Beyond the Six Steps Any time a new disinfectant is introduced into the cleaning process within the facility, a qualification should be performed. From start to finish, a disinfectant qualification can require from two to 12 months to complete. The timeline will depend on a number of variables, including the number of disinfectants, contact time, and challenge organisms being tested as well as the number of surfaces (for the coupon method) being evaluated [11-15].

Process Description

Test procedure

- Selection of media.
- Requirements.
- Preparation of Media.
- Sterilization.

➢ Test Procedure.

Acceptance Criteria

There should be effective subsequent log reduction in the number of microorganisms from the initial population after the application of the sanitizing agent.

Test Procedure

Following test functions shall be checked in this section.

Selection of media

Carry out the validation study on Soyabean casein digest agar which is routinely used in microbiological testing by pour plate method.

Requirement:

Glassware & apparatus: Test tubes, Petri plates, conical flasks, sterile pipette and Stainless steel Plates.

Preparation of Media: Prepare Soyabean casein Digest Agar & Sabouraud Dextrose Agar as per SOP

Sterilization: Sterilized the media in the Autoclave at 121^0C for 15 minutes.

Test Procedure

Preparation of Disinfectant Solution

- Prepare fresh disinfectant solution using purified water from the stock.
- Prepare three dilutions- a) Dilution less than Use dilution. b) Use dilution. c) Dilution higher than Use dilution.
- Disinfectants used for sanitization :

a) Dettol 0.5%,⟶1.0%, 1.5%

b) Bacillocid 0.5%, ⟶1.0%, 1.5%

c) Triple 0.5%, ⟶1.0%, 1.5%

d) Mikrobac forte 0.5%;⟶ 1.0%, 1.5%

e) Gramicid 4.0%,⟶ 5.0%, 6.0%

f) 70% IPA 65.0%,⟶ 70.0%, 75.0%

- Prepare 500 ml of the disinfectant solution as per SOP No. ALP/QC/SOP009 for IPA preparation Filter the IPA solutions through the sterilized 0.2 µm filter collect in sterilized closed in an airtight container / Spray bottle.

Table 1: Disinfectant Preparation Record:

Sr No.	Prepared on	Name of Disinfectant	Concentration	Qty of Disinfectant	Qty of purified water	Volume Prepared	Done By/Date
01							
02							
03							

Preparation of Challenge Inoculum

✓ Prepare Soyabean casein digest agar as per below mention procedure.

✓ Transfer from stock, the following microbial cultures onto the Soyabean casein digest Agar (SCDA) & Sabouraud Dextrose Agar slant and incubate at 32-35°C & 20-25°C for 48 hours.

- Staphylococcus aureus.
- Escherichia coli.
- Salmonella abony.
- Pseudomonas aeruginosa.
- Bacillus subtilis.
- Candida albicans.
- Aspergillus Niger.
- Environmental Isolate.

❖ Prepare the Culture Suspension as per standard test procedure.

✓ Check the microbial count of the suspension by diluting the above mentioned culture suspension serially and testing the dilutions for no. of C.F.U. by Pour plate method.

✓ Records the observations in Table -2.

✓ Take the working culture suspension which contains the population between 10^5 to 10^7 microorganisms/ml.

***In Vitro* Test**

a) Dilute the disinfectant to be tested as per the recommendations by the manufacturer, i.e.at use dilution and one lower to the use dilution.

b) Filter the disinfectant solutions through the sterilized 0.22 µ filter and collect in sterilized closed container.

c) Dispense 10 ml of the disinfectant solution prepared in the sterile test tube prepare 2 sets of 2 tubes for each dilution to be tested. Mark the tube with tube number (1 & 2), name of inoculum, disinfectant name and time of contact (5 or 10 minute).

Table 2: Observation Table

Microorganism	Required Count	Count found	Dilution	Done By
S aureus	10 to 100 Cfu/ml			
E coli	10 to 100 Cfu/ml			
Salmonella abony	10 to 100 Cfu/ml			
P aeruginosa	10 to 100 Cfu/ml			
Bacillus subtilis	10 to 100 Cfu/ml			
Candida albicans	10 to 100 Cfu/ml			
Aspergillus niger	10 to 100 Cfu/ml			

Challenge Test

a) Take both the sets of dilutions. Add to each of the four tubes, 0.1 ml of one of the challenge inoculum.

b) Note the time of inoculation. Allow a contact time of 5 minute for one set (2 tubes) and 10 minute for second set (2 tubes) between the microorganisms and the disinfectant.

c) Take 1 ml of the contents from the both tubes at the intervals of 5 minutes and transfer it aseptically to the sterilized plate.

d) Pour sterilized, cooled Soyabean casein digest agar media. Rotate the plate gently to mix the content and allow solidifying. For C.albicans & A.niger use SDA in place of SCDA.

e) For negative control take 2 ml of uninoculated filtered disinfectant solution in sterile petri plate and treat similarly.

f) Perform similarly for tubes allowed to stand for 10 minutes.(Set 2)

g) Perform similarly for all other organisms.

h) Incubate the plates along with negative control.

For Bacteria: At 30-35ºC for 5 days.

For Fungi: At 20-25ºC for 5 days

At the end of the incubation count the no. of colonies on each plate and note down the results in applicable format.

***In Vivo* Test**

Validation can be conducted on the following surfaces:

a) The Stainless steel plates.

b) Glass

c) Epoxy surface

d) Partition wall

a) Double wrap the stainless steel in the butter paper piece and autoclave the surfaces at 121°C for 30 minutes.

b) Bring the test surfaces to the L.A.F. bench after sterilization, unwrap taking all precautions not to contaminate the exterior of test surfaces. Allow to cool and dry under the L.A.F. bench.

c) Select 2 areas for each of the surface for one organism. Spread to each surface1.0 ml of the challenge inoculums in an area equivalent to 5x5 cm^2 and allow it to dry under LAF bench.

d) Apply the disinfectant solution on the marked surfaces by spraying or spreading and Leave the surfaces for drying. Allow the contact time of 05 & 10 minutes between disinfectant and the test organism.

e) After 5 minutes take swab samples of the challenged surface by using sterile cotton swabs and inoculate the swab in the test tube containing 10 ml of the sterile normal saline (0.9%). Vortex the tube for 1 minute and take out 1ml of the solution from the tube and transfer aseptically to the empty sterile plate in duplicate.

f) Perform similarly as above for another set after 10 minutes of contact time between disinfectant and organism.

g) Pour 20ml sterile Soyabean casein Digest Agar and Sabouraud Dextrose Agar cooled to 45°C in each plate and allow it solidify.

h) Incubate the plates along with negative control.

For Bacteria: At 30-35ºC for 5 days.

For Fungi: At 20-25ºC for 5 days.

i) At the end of the incubation count the no. of colonies on each plate and record in prescribed format.

j) Test Perform for All Disinfectant which were used for Cleaning and Sanitization in Microbiology Section and Production Area.

Some 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 [16-17].

Conclusion and Recommendation

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.

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