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Disinfectant Qualification Efficacy Testing for the Pharmaceutical and Biopharmaceutical Industries

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Introduction

The COVID-19 pandemic in the last three years was a recent example manifesting the importance of biological medicinal products. For both the traditional pharmaceutical and the more recent biopharmaceutical industries, microbiological safety has been an important aspect for successful and high-quality manufacturing. Potential microbiological contaminants – from the environment or intrinsically from the source materials – must be eliminated from the final product. A multi-faceted approach which includes the selection and control of raw materials, optimization and validation of the manufacturing process, and implementation of an effective cleaning and disinfection program for the facility has been the guiding principle for mitigating the microbiological contamination risks. This concept has also been adopted by the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and other regulatory agencies worldwide.

An appropriate cleaning and disinfection procedure shall take into consideration the potential contaminants, manufacturing process, equipment, facility, and final products. For example, what types of bacteria, viruses, fungi, or spores may potentially be present? Are sanitizers and disinfectants effective in inactivating these micro-organisms? Are they compatible with the equipment and the facility surfaces? Is the shelf-life of these sanitizers and disinfectants acceptable? Will micro-organisms develop resistance to these chemicals? To address these important questions, a risk assessment shall be performed and a disinfectant qualification (DQ) or disinfectant efficacy test (DET) is highly recommended. A successful DQ (DET) will provide objective evidence of the effectiveness of the disinfection program in the (bio)pharmaceutical manufacturing environments and provide maximum assurance to stake holders of the microbiological safety of the final products.

Ensuring adequate microbial control may also be mandatory to meet regulatory requirements. For example, the FDA Guideline for Aseptic Processing[i] 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." These federal regulations and guidance documents clearly highlight microbial control of manufacturing environments. It is also an area heavily scrutinized in GMP inspections and frequently appears in the FDA Form 483 warning letters.

Overview of Disinfectant Qualification (DQ) / Disinfectant Efficacy Testing (DET)

A disinfectant qualification (DQ) or disinfectant efficacy test (DET) is performed to assess the efficacy of the desired disinfectants and sanitizers against potential microbiological contaminants that may be present in a (bio)pharmaceutical manufacturing setting, especially cleanrooms and other aseptic environment. The most used test method is surface ("carrier")-based, U.S. Pharmacopeia (USP) Chapter <1072>, Disinfectants and Antiseptics[ii]. Further guidance may be found in ASTM International E2614 Standard Guide For Evaluation Of Cleanroom Disinfectants[iii], etc., however other methods such as suspension-based and *in situ* testing have also occasionally been used. The purpose of a DQ/DET is to provide assurance that the cleaning and disinfection program intended to be used for manufacturing is appropriate and adequate to address the microbiological contamination risk.

DQ/DET may sometimes be loosely referred to as "disinfection validation" or "cleaning validation". However, it shall be noted that these terms actually have different meanings. A disinfection validation typically includes a DQ/DET study and also incorporates other aspects such as environmental monitoring. Cleaning validations measure a procedure's effectiveness at removing organic matters and other substances; it usually does not address the viability of micro-organisms. This article will focus on the requirements for the design and performance of a DQ/DET study.

Design of a DQ/DET Study

A successful DQ/DET study shall take into consideration of several aspects:

1) the raw materials and final products
2) target micro-organisms (bacteria, viruses, fungi, and spores, etc.)
3) types of disinfectants (and sanitizers and/or sterilants, if applicable) to be assessed
4) types of surfaces to be tested (for a carrier-based study); and
5) exact test parameters and conditions, such as the shelf-life, dilution ratio and diluents for the disinfectants, contact times, contact temperature, neutralization and other controls, performance criteria, and GLP/GMP compliance, etc.

Additionally, the test should match the manufacturer's Standard Operating Procedures (SOPs), if already established, to be directly applicable and relevant.

Guidance found USP Chapter <1072> and ASTM E2614 provide some framework and basic information on how disinfectant qualifications should be performed. Each test is a custom design

based on the manufacturing facility's SOPs and can vary greatly depending on the following combination of factors: surface types, microorganisms, disinfectant products, and application procedures. However, each test must demonstrate the efficacy of the sporicidal, disinfectant, and/or sanitizer agents (as applicable) when used to decontaminate applicable surface types.

Selection of antimicrobial products:

The sterilants, disinfectants and sanitizers utilized in manufacturing facilities are first evaluated using alternative standard test methodologies (e.g., AOAC International Use Dilution Methods[iv], ASTM E1053 Virucidal Effectiveness Method[v] and EN methods) to obtain registration with the U.S. Environmental Protection Agency (EPA) and other regulatory bodies. The guidance for disinfectant and sanitizer registrations in the U.S. is also outlined in the EPA 810.2000 series guidelines[vi].

However, the methodologies used for EPA registration do not necessarily account for how the disinfectants will be applied in the manufacturing setting. The following provides a sample comparison for EPA registration requirements compared to overall conditions for disinfectant validation testing for vegetative bacteria.

Parameters for EPA Registration	Parameters for Disinfectant Validation Tests
High surface challenge (>6-Log_{10})	Lower surface challenge (3-4 Log_{10})
Organic soil challenge	Typically no soil
Preparation using hard water	Prepared using purified or tap water
One carrier type (stainless steel and/or glass)	Various carrier types
Limited contact time	Contact time based on the facility SOPs
Qualitative efficacy criteria	Quantitative efficacy criteria

Although a disinfectant may achieve the required criteria for EPA registration, it may not always achieve the same level of efficacy on other surface types or against the specific facility isolates. Often, more than one type of chemical agent is required to achieve effective microbial control while maintaining minimal surface damage. Therefore, most facilities will select one or two disinfectants along with a sanitizer to use on a routine basis and supplement with sporicides on a less frequent basis. The application method of the disinfectant to the coupon surface can vary greatly depending on the disinfectant and the surface type it is being used to decontaminate. Application methods may include spray (with or without wiping), wipe, or mop-on.

A study design matrix should include additional considerations depending on the SOPs of the facility and may include:

- Best-case conditions: testing ready-to-use products on the day of opening (without aging) or freshly diluted concentrates of test products.

- Worse-case conditions: use of expired products or extended shelf-life products. Additional factors to consider include: the use of AOAC Hard Water or non-sterile water for the dilution of concentrates; reduced contact times that are not in alignment with the product's EPA label; or altered application methods (e.g., using a spray application rather than a wiping application which reduces mechanical action)

Selection of surface types:

Clean rooms and other controlled environments within manufacturing facilities are constructed using a wide variety of materials, in addition to common surfaces and equipment utilized for manufacturing procedures that may be subject to contamination. Each material should be independently evaluated for each combination of microorganism and disinfectant to validate efficacy. Surface types can include:

- Aluminum
- Countertops
- Epoxy
- Equipment Painted Surface
- Fiberglass
- Glass
- Neoprene gloves
- Nitrile gloves
- Plastic
- Plexiglass / Lexan
- Polyamide
- Polyethylene
- Polyisoprene gloves
- Polycarbonate
- Polyester
- Polyethylene
- Polyoxymethylene
- Polypropylene
- Polyvinyl chloride (PVC)
- Powder Coated Metal
- Rubber
- Sheet metal
- Tile flooring
- Tyvek
- Sheet metal
- Stainless steel (304 and 316)
- Vinyl
- Wall/Ceiling

Selection of microorganisms:

Regulatory guidelines state, "routinely used disinfectant should be effective against normal microbial vegetative flora recovered from the facility". Therefore, regulatory authorities require frequently isolated microorganisms from the cleanroom environment to be included in testing since these microorganisms are most likely to present contamination risks. Therefore, it is crucial to provide validation that the sanitization and disinfection procedures are effective against the microorganisms isolated from the facility. However, it is not necessary to include all environmental isolated in disinfectant efficacy testing if valid rationale for the selection of microorganisms is provided; in addition, per USP <1072>, reference strains obtained from ATCC may be included.

VEGETATIVE BACTERIA

Reference strains:

- *Staphylococcus aureus*, ATCC 6538
- *Pseudomonas aeruginosa*, ATCC 15442
- *Escherichia coli*, ATCC 11229

Facility isolates:

- *Burkholderia cepacia*
- *Staphylococcus epidermidis*
- *Micrococcus luteus*

YEAST AND FILAMENTOUS FUNGI

Reference strains:

- *Candida albicans*, ATCC 10231
- *Aspergillus brasiliensis*, ATCC 16404
- *Penicillium rubens*, ATCC 11709

Facility isolates:

- *Penicillium spp*
- *Aspergillus spp*

SPORE-FORMING BACTERIA

Reference strain:

- *Bacillus subtilis*, ATCC 19659

Facility isolates:

- *Bacillus sphaericus*
- *Bacillus thuringiensis*

VIRUSES

Enveloped viruses:

- Baculovirus
- Bovine Viral Diarrhea Virus
- Pseudorabies Virus
- Xenotropic Murine Leukemia Virus

Non-enveloped viruses:

- Adenovirus
- Canine Parvovirus
- Murine Minute Virus
- Norovirus / Vesivirus
- Porcine Parvovirus

"Worst-case" conditions:

The manufacturers of disinfectants typically state a certain shelf-life for the products and the cleanroom SOPs may also allow a range of conditions such as the temperature of use, etc. When performing a DQ/DET study, "worst-case" conditions (i.e., those conditions that are considered to potentially give a lower performance by the disinfectants) shall be used. These include, but are not limited to, the following:

- End of shelf-life of disinfectants
- Diluent (maximum hardness, as applicable)
- Dilution ratio (as applicable)
- Disinfectant delivery
- Short contact time
- Contact temperature
- Presence of moderate/high organic load
- Wiping vs. No-wiping
- Porous surface
- In-situ testing (as applicable)

Performance of a DQ/DET Study

Once the design has been determined, each combination of surface type, microorganism, and disinfectant are tested individually. The test surfaces (coupons), ideally cut into approximately 2" x 2" pieces, are inoculated with a volume of the challenge microorganism sufficient to demonstrate the required log reduction. The inoculated coupons are dried and then treated with the selected disinfectant as applicable for actual use. The treated coupons are held for the determined contact time and then recovered with a neutralizing solution. The solution is diluted as required and quantitatively evaluated using an appropriate plating method to enumerate the number of survivors.

Inoculated, non-treated coupons are sampled and enumerated in the same manner as the treated coupons to establish the minimum baseline of the challenge microorganism. These results are used to calculate the log reduction of each challenge microorganism.

Additional controls may be included to validate the sampling procedures (Recovery Efficiency Evaluation) and the ability of the neutralizer to inactivate the active ingredient(s) of the disinfectant (Neutralizer Effectiveness Evaluation).

Per USP <1072> guidelines, the disinfectant meets the effectiveness criteria if the test results exhibit a minimum reduction over the corresponding untreated control coupons, per surface, based on the following:

- At least a 3-Log_{10} reduction for vegetative bacteria and yeast
- At least a 2-Log_{10} reduction for spore-forming bacteria and filamentous fungi

The viral performance criteria are not defined in ASTM E1053; however, per the U.S. EPA and Health Canada's criteria for a virucidal claim of a disinfectant and the European EN14476 method, a 3-4 Log_{10} reduction has widely been used for viruses.

As demonstrated by the results outlined in Table 1, many factors contribute to the efficacy of a disinfectant including surface type, organic materials present on the surface, preparation of the disinfectant (type/hardness of the diluent and concentration of the prepared disinfectant) and challenge microorganism. Therefore, it is critical to test each component independently to select the optimal course of cleaning to prevent contamination.

Table 1
Example Data - Efficacy against a Filamentous Fungus

Aspergillus brasiliensis												
Surface	**Acidic Phenolic**				**Alkaline Phenolic**				**Sodium Hypochlorite**			
	Purified Water		**Hard Water**		**Purified Water**		**Hard Water**		**Purified Water**		**Hard Water**	
	1:256	1:512	1:256	1:512	1:128	1:256	1:128	1:256	28 mL: gal	14 mL: gal	28 mL: gal	14 mL: gal
304 Steel	Pass	Pass	Fail	Fail	Pass	Pass	Pass	Fail	Pass	Pass	Pass	Pass
316 Steel	Pass	Pass	Fail	Fail	Pass	Pass	Pass	Fail	Pass	Pass	Pass	Pass
Plastic	Pass	Pass	Fail	Fail	Pass	Pass	Pass	Fail	Pass	Pass	Pass	Pass
Sheet Metal	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Fail	Pass	Pass	Pass	Pass
Cast Alum.	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Fail	N/A	N/A	N/A	N/A
Aluminum	Pass	Pass	Fail	Fail	Pass	Pass	Pass	Fail	N/A	N/A	N/A	N/A
Vinyl Floor	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass
Painted Equipment	Pass	Pass	Fail	Fail	Pass	Pass	Pass	Fail	N/A	N/A	N/A	N/A
Wall/ Ceiling	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Fail	Pass	Pass	Pass	Pass
Glass	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Pass	Pass	Pass	Pass

Hard Water = 400 ppm AOAC Hard Water
N/A = Not applicable
Pass = > 2 Log_{10} Reduction (per USP <1072>)
Fail = < 2 Log_{10} Reduction (per USP <1072>)

This example data for a filamentous fungus shows that acidic and alkaline phenolics may not pass under certain conditions (type of water, material and/or concentration), whereas sodium hypochlorite achieved passing data under all conditions evaluated.

Table 2 provides data of the different Log_{10} reductions using multiple chemistries and surfaces when challenged with a non-enveloped virus.

Table 2

Example Data - Efficacy against a Non-enveloped Virus

Surface	Log_{10} reduction - Canine Parvovirus						
	PAA / H_2O_2	Sodium Hypochlorite	Alkaline Phenolic	Acidic Phenolic	70% IPA	H_2O_2	Surfactant
304 Steel	> 4.4	> 4.5	0.1	0.0	0.0	2.3	0.0
Epoxy Floor	> 4.8	> 4.8	0.8	0.8	0.8	1.6	0.2
Epoxy Wall	4.2	> 5.1	0.1	0.7	0.0	2.5	0.0
Glass	3.8	> 4.5	0.0	0.5	0.2	1.1	0.0
Plastic	3.6	> 4.5	0.5	0.8	1.2	1.4	0.5
Cart	> 4.5	> 4.5	1.0	0.9	0.7	1.7	0.0
Fiberglass	3.6	3.6	1.0	0.0	0.8	1.5	0.3

Summary

A cleanroom or similarly controlled environment must devise an effective cleaning and disinfection program to ensure the safe and effective production of medical devices, pharmaceuticals, and other drug products. To conduct these validation assays, you must consider the experience, performance, quality, and credibility of the contract laboratory.

Microbac Laboratories, Inc. is the ideal choice as test performance, data integrity and quality assurance are the foundation of all tests performed at our laboratories. Microbac's reputation was firmly rooted more than 50 years ago because of our resolute commitment to exceptional laboratory analysis and outstanding customer service. Today our company continues to be universally recognized within the disinfectant/sanitizer, sterilant, and bioprocess industry, a distinction derived, and nurtured, by providing the best services possible to all our clients.

Contact us today and a customized proposal will be generated based on your testing requirements. Our labs are fully compliant with GLP and cGMP for FDA submissions and we are also accredited with ISO 17025. We look forward to working with you.

References

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4. ASTM International E2614-15(2020)e1 Standard Guide For Evaluation Of Cleanroom Disinfectants
5. AOAC International, Chapter 2 Hard Surface Carrier Test Methods, January 2023
6. ASTM E1053-20 Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces
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