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COMMENTARY

Disinfectant Efficacy: Understanding the Expectations and How to Design Effective Studies That Include Leveraging Multi-Site Data to Drive an Efficient Program

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ABSTRACT: For manufacturers of both sterile and nonsterile pharmaceuticals, there is an expectation that the manufacturing process is performed in a manner that prevents extraneous contamination so that the products are provided in a safe, integral, pure, and unadulterated form. As part of that process, cleaning and disinfection are an absolute necessity. Although cleaning and disinfection support control of microbial contamination through preventive and corrective action, specific compendia methods do not currently exist. The intent of this paper is to provide a general guidance on how to perform disinfectant efficacy validation and implementation. This includes how to make sure the concepts are understood, how to interpret facility data and utilize it to demonstrate control awareness for your facilities, and how to leverage the data to reduce redundancies in validation or verification. This paper represents the thoughts and best practices of the authoring team and their respective companies and provides an efficient way to qualify disinfectants without impacting the quality of the study. If you choose to follow the recommendations in this paper, you must ensure that the appropriate rationale is sound and the scientific data is documented. It is the belief of the authoring team that only then will this approach meet regulatory requirements.

KEYWORDS: BioPhorum, Disinfectant efficacy, Cleaning, Cleaning requirements, Control awareness.

1. Introduction

For manufacturers of both sterile and nonsterile pharmaceuticals, there is an expectation that the manufacturing process is performed in a controlled manner by which the products are provided in a safe, integral, pure, and unadulterated form. As part of that process, cleaning and disinfection are an absolute necessity. Although cleaning and disinfection support the control of microbial contamination through preventive and corrective action, specific compendia methods do not currently exist. The following is a list of regulatory references that provide guidance for cleaning and disinfectant requirements.

1. Code of Federal Regulations (CFR) Title 21: Food and Drugs, Part 211: Current Good Manufacturing Practice for Finished Pharmaceuticals, Section 211.67 Equipment Cleaning and Maintenance (1).
    - Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals.
2. Code of Federal Regulations (CFR) Title 21: Food and Drugs, Part 211: Current Good Manufacturing Practice for Finished Pharmaceuticals, Section 211.182 Equipment Cleaning and Use Log (2).
    - Cleaning procedures must be documented appropriately, and a cleaning and use log should be established.
3. U.S. Pharmacopeia (USP) General Chapter <1072> Disinfectants and Antiseptics (3).
    - Use-dilution tests.
    - Surface challenge tests.
    - A statistical comparison of the frequency of isolation and numbers of microorganisms isolated

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prior to and after the implementation of a new disinfectant.

4. U.S. Food and Drug Administration (FDA), Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice (4).

    - A sound disinfectant program includes a sporicidal agent.

    - Microorganisms associated with adverse trends can be investigated as to their sensitivity to the disinfectants employed in the clean room in which the organisms were isolated.

5. American Society for Testing Materials (ASTM) International—used by manufacturers of disinfectants.

    - ASTM E2614-15 Standard Guide for Evaluation of Cleanroom Disinfectants (5).

    - ASTM E2197-17 Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals (6).

6. The International Organization for Standardization (ISO), ISO 14698: Cleanrooms and Associated Controlled Environments (7).

    - Surface evaluation focused on cleaning.

    - Parenteral Drug Association Inc. (PDA) Technical Report 70: Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities (8).

Without compendial methods, how do we ensure that our cleaning and disinfection activities are adequate and effective? As stated in PDA Technical Report 29: Points to Consider for Cleaning Validation (9), "[the industry and the regulatory agencies must be accountable to interpret the current Good Manufacturing Practices (cGMPs) in order] ... to create programs and policies which establish the general requirements as specific practices." As a result, regulatory agencies are demanding strong evidence through validation or verification; therefore, the industry must be diligent and thorough.

Disinfection and cleaning are two critical components of control measures for minimizing contamination during manufacturing.

Cleaning refers to the removal of foreign materials (e.g., soil, organic materials, residue, and so forth) from objects and is typically accomplished by using water with detergents (acidic, alkaline, neutral pH, and so forth). Cleaning may have microbiocidal effects but the intended purpose of cleaning is to reduce the dirty load—that is the amount of foreign materials present on the surface that may interfere with a disinfectant's ability to be effective. Thorough cleaning maximizes disinfection by enabling complete contact of the disinfectants with the surface. The removal of foreign materials helps to minimize the possibility of neutralization and the presence of residues that can lead to other issues. Residue can be left behind by disinfectant application as well. Surfactants and corrosive/oxidative compounds, if not properly removed, can cause issues like rust or safety concerns such as slippage.

Disinfection is a broad term to describe chemical and physical agents used to destroy various forms of microorganisms. Although cleaning and disinfection are vital to both aseptic and nonsterile facilities, all pharmaceutical manufacturing clean rooms are expected to be as free from microorganisms as possible, and for that reason disinfectant efficacy is a metric by which that can be assessed. Microorganisms come in various shapes and sizes ranging from viruses to spore-forming bacteria and mold and as such specific disinfecting agents are needed to combat these contaminants. The elements of disinfectant validation, product implementation, and creation of an effective program leveraged across the company will be discussed throughout this paper.

Disinfectants can be classified into four categories: sanitizer, general disinfectant, sporicide, and sterilant. Below are the definitions of each category, taken from USP <1072> Disinfectants and Antiseptics (3).

2. Categories of Disinfectants and How They Differ

Sanitizer—An agent for reducing, on inanimate surfaces, the number of all forms of microbial life including fungi, viruses, and bacteria. Examples include 70% ethanol and 70% isopropyl alcohol (IPA).

General disinfectant—A chemical or physical agent that destroys or removes vegetative forms of harmful

microorganisms when applied to a surface. Examples include quaternary ammonium and phenolics.

Sporicide—An agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms. Examples include sodium hypochlorite and hydrogen peroxide/peracetic acid compounds.

Sterilant—An agent that destroys all forms of microbial life including fungi and viruses and all forms of bacteria and their spores. Examples include sodium hypochlorite, vaporized hydrogen peroxide, and hydrogen peroxide/peracetic acid compounds.

These terms are often used interchangeably or are thought to be associated with specific requirements. For example, 70% IPA can be called a sanitizer or a general disinfectant. In this case, these terms are both accurate. 70% IPA is effective at reducing all forms of microbial life including fungi, viruses, and bacteria (marginally against bacterial spores) and is effective at destroying vegetative forms of microorganisms, thus meeting the definitions of both a sanitizer and a general disinfectant. The misconception comes from the idea that a sanitizer may not have to meet a specific requirement, whereas a general disinfectant does. That statement is not correct. Whether the agent is a sanitizer or a general disinfectant, to be considered "qualified" that agent must meet the acceptance criteria set by the end user.

The most important aspect to consider is that the agents identified for use meet their intended purpose. An often misunderstood concept is the rotation of disinfectants. If a general disinfectant is qualified for use it is not necessary to qualify another general disinfectant to rotate with the first. For example, if a phenolic agent is qualified for use against vegetative bacteria and fungi, it would not be necessary to qualify another phenolic or a quaternary-based product for the same purpose if the initial phenolic agent qualified meets its intended purpose. However, it is beneficial to qualify multiple general disinfectants for the purposes of having an alternative disinfectant as a backup or accommodating biocidal registration restrictions in various countries of operation. But it is not necessary to rotate back and forth to create variability, because developing resistance to chemical disinfectants is not plausible in the application of clean room disinfection owing to several factors that are discussed further in Section 5 (Challenge Organism Selection for Disinfectant Efficacy Testing).

It is however important to utilize different classes of disinfectants to ensure that the products qualified and used address all areas of concern (i.e., vegetative bacteria, fungi, bacterial spores, and viruses) as applicable. If a general disinfectant is qualified for use against vegetative bacteria and fungi, a sporicidal agent should be qualified to address bacterial spores and another agent to address viruses (if applicable). For this reason, it is important to understand the mode of action and microbial activity of the various disinfecting agents. Examples of disinfectants, classifications, microbicidal activity, and modes of action are listed in Tables I–III.

3. Regulatory expectations with Regard to Disinfectant Efficacy Testing

A compendial method for disinfectant efficacy testing has not been established, and therefore disinfectant efficacy can be demonstrated in several ways. The intent, from an end user's perspective, is to qualify the use of such disinfectants/sanitizers/sporicides in their cleaning and disinfection program on nonproduct contact surfaces in their clean room environment. Careful evaluation of the sanitizers/disinfectants/sporicides and demonstration of their effectiveness by a qualification study should enable biopharmaceutical manufacturers to achieve the specified clean room standard and the desired microbial contamination control of drug products. The effectiveness of sanitizers/disinfectants/sporicides can be demonstrated in a number of ways, and the test method should be specified in a disinfectant efficacy study (DES) protocol. It is important that the protocol be approved with a clear rationale documented, followed by an approved summary report explaining any deviations. A major point not to be overlooked is that the executed protocol must demonstrate the same practices, contact times, and so forth as the practices that will be used during production disinfection.

To meet disinfectant regulation requirements and obtain Environmental Protection Agency (EPA) registration, sanitizer/disinfectant/sporicide manufacturers base the test methodologies for their products on guidance such as:

1. European Norm Standard (EN) 13697, Quantitative Surface Test for the Evaluation of Bactericidal or Fungicidal Activity (10);

2. ASTM E1153-14, Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces (11); and

TABLE I
General Classification of Antiseptics, Disinfectants, and Sporicidal Agents[a]

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% Isopropyl alcohol, 70% alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite
Phenolics	General purpose disinfectant	500 µg per gram Chlorocresol, 500 µg per gram chloroxylenol
Ozone	Sporicidal agent	8% gas by weight
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per gram H_2O_2 vapor, 10%–25% solution, 3% solution
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 µg per gram peracetic acid
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride

[a] Table adapted from Reference 3.

3. *Official Methods of Analysis*, 21st Edition (2019), Chapter 6.2: "Hard Surface Carrier Test Methods" (12).

Sanitizer/disinfectant/sporicide manufacturers must also meet the acceptance criteria dictated by the chosen method to obtain registration. When designing disinfectant efficacy studies, these methods can be used interchangeably and acceptance criteria may be defined separately or taken from the chosen method, whichever is determined to be most appropriate by the end user.

Sanitizer/disinfectant/sporicide manufacturers will provide product information, such as use-dilution (solution preparation), efficacy using Association of Official Analytical Chemists (AOAC) methods tested against American Type Culture Collection (ATCC) microorganisms with predetermined contact time (e.g., 10 min) based on their study designs, and results to support the directions for use provided in the labeling. Although valuable, this information may not be directly useful in supporting the products' use as sanitizers/disinfectants/sporicides in a pharmaceutical manufacturing environment. An end-user company may decide, for example, to explore a different use-dilution or be concerned about a microorganism that was not tested by the sanitizer/disinfectant manufacturer. Therefore, pharmaceutical manufacturers need to demonstrate the effectiveness of sanitizers/disinfectants/sporicides in maintaining state of microbial control in terms of:

TABLE II
General Disinfectants (Bacteria, Yeasts, and Molds)[a]

Microbicidal Activity	Mode of Action
Alcohols (e.g., isopropyl and ethanol): Broad-spectrum antimicrobial against vegetative bacteria, mycobacteria, viruses and fungi	Alcohols owe their antimicrobial activity to their ability to denature cell proteins leading to a disruption of cellular function, and to a minor extent to their ability to cause membrane damage
Alkaline: Bactericidal and virucidal	Hydrolyzes and removes proteins, nucleic acids, endotoxins, and viruses
Acidic: Broad spectrum of bacteria	Attack lipid-rich cell walls

[a] Information sourced from Reference 19.

TABLE III
Sporicides (Spore-Forming Bacteria)[a]

Microbicidal Activity	Mode of Action
Accelerated Hydrogen Peroxide Technology: Bactericidal, virucidal, fungicidal, broad-spectrum sanitizing	Hydrogen Peroxide: Produces destructive hydroxyl free radicals that attack membrane lipids, DNA and other essential cell components
Peracetic acid, hydrogen peroxide, and acetic acid: Range of bacteria, molds, yeast and viruses (Registered uses: disinfectant, sterilant, sporicide, sanitizer, microbicide, virucide, fungicide, sporicidal disinfectant, antimicrobial solution, mold killing, one-step cleaner and disinfectant)	Hydrogen Peroxide: Produces destructive hydroxyl free radicals that attack membrane lipids, DNA and other essential cell components
	Peracetic Acid: Denatures proteins, disrupts cell wall permeability

[a] Information from Reference 20.

1. use concentration;
2. contact time;
3. surfaces representing construction materials in the clean room;
4. microbial isolates recovered from EM programs and/or media fills and sterility tests; and
5. application frequency.

Although disinfectant manufacturers perform various tests to show the efficacy of their products as required by the EPA and European Standards, it is important for end users of these products to focus on the testing that is most applicable to their intended use. In the case of pharmaceutical manufacturing, clean room disinfection encompassing surfaces and material ingress is best represented by surface coupon testing.

USP <1072> Disinfectants and Antiseptics (3) and the FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice (4) specify the need for disinfectant efficacy testing to be performed by drug manufacturers. However, acceptable test methods for the study design are not clearly defined. Because a compendial method does not exist, regulatory authorities will cite such guidance with the expectation that companies align with the criteria set forth. This is a very important aspect of disinfectant efficacy validation to recognize. As a result, the guidance should be viewed as almost compendial. That is why it is crucial that companies are diligent to provide a rationale supporting the testing design and criteria, especially when it differs from such guidance.

Although USP <1072> Disinfectants and Antiseptics (3) is considered a general informational chapter, it is a good resource for general guidance when designing the sanitizer/disinfectant/sporicide qualification study for selected sanitizers/disinfectants for use in a pharmaceutical manufacturing environment. It is much like USP <1231> Water for Pharmaceutical Purposes (13), which can be used when selecting a water source and validating a water system. ASTM E2614-15 Standard Guide for Evaluation of Cleanroom Disinfectants (5) also provides useful general guidance. USP <1072> Disinfectants and Antiseptics (3) discusses how to challenge disinfectant products for bactericidal, fungicidal, or sporicidal efficacy. The chapter also considers:

1. disinfectant classification;
2. microbial resistance to disinfectants;
3. selection and application of disinfectants in the sterile and aseptic pharmaceutical manufacturing area;
4. challenge organisms/surfaces; and
5. minimum acceptance criteria (at least a 2 Log_{10} reduction for bacterial spores and 3 Log_{10} for vegetative bacteria during a predetermined contact time).

This guidance provides information that can be used to help design disinfectant studies, including neutralization to support the clean room disinfection program. The challenge organisms and surfaces provided are not required or definitive; they are designated as "typical" within the chapter. Ultimately it is the responsibility of

the manufacturing sites/company to assess and decide what is sufficient and acceptable for the DES and support the use of properly qualified sanitizers/disinfectants.

The following sections provide more information to help with the design of disinfectant efficacy studies.

4. The Value of Environmental Monitoring Trending to Disinfectant Efficacy Testing

Environmental monitoring (EM) trending is an important key with respect to the overall disinfectant program within a facility. The routine review and analysis of EM data is essential to assess environmental control and the overall level of microorganisms within the classified areas of manufacturing and is considered a requirement per FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice (4). The list of microorganisms that can potentially exist in the environment can vary greatly and is extensive. For this reason, it is not practical or feasible to attempt to include every microorganism retrieved from the clean room environment in the disinfectant efficacy testing. EM data should be trended periodically (an annual review is required by FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice (4)) from all manufacturing facilities within a company to provide an accurate, comprehensive, and representative list of microorganisms. When disinfectant efficacy testing is to be performed, the data from all facilities should be compiled to establish a matrix of what the most appropriate and representative list of organisms is to test.

Trends of microorganisms isolated from viable monitoring samples are presented as the most frequently identified organisms. These microorganisms are most prevalent to the facility specific environment(s), and therefore favorable candidates to represent the worst-case microorganisms when performing disinfectant efficacy. Additionally, different elements may be considered, such as the system from which microorganisms are identified or the classification level of the clean room.

EM trending data should be leveraged to assess the efficacy of currently qualified disinfectants. Trending data indicates the degree of clean room control, identifies any areas that are trending toward the established action level, and facilitates evaluation of current alert levels. If any shifts in microorganism trends are noted, the existing data should be evaluated to determine any impact on currently qualified disinfectants.

5. Challenge Organism Selection for Disinfectant Efficacy Testing

ATCC cultures (or cultures from other recognized international culture collections) are typically challenged by the supplier of a given disinfectant or sporicide. They are not, however, considered the most representative worst-case isolates for the end user's disinfectant study. Although the development of microbial resistance to disinfectants under certain circumstances is possible, it is not feasible for it to occur in the clean room environment. Resistance is generally seen at concentrations well below the levels used in application (*Russell, Hugo & Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization*, 5th Edition (14)) and disinfectants are normally applied against low populations of microorganisms usually not actively growing, so the selective pressure for the development of resistance is low (USP <1072> Disinfectants and Antiseptics (3)). There are, however, significant differences among the different species with regard to the resistance against the lethal effects of different disinfectants. Representative microorganisms isolated from EM (in-house isolates) should therefore be challenged with the agents used in the disinfection program to confirm their susceptibility.

Trending EM data may identify reoccurring isolates present at the facility where disinfectants may not have been effective. Therefore, in-house isolates are the most appropriate for disinfectant efficacy testing. A collective panel of microbes representing each category of microorganism (gram-positive, gram-negative, gram-positive spore-former, and mold) should be chosen based on the intended use of the disinfectant(s) being tested (i.e., disinfectant versus sporicide). Once in-house isolates to be used are identified for disinfectant efficacy testing, it is best practice and strongly recommended to utilize viable microorganisms for inoculation not more than five passages removed from the original culture, which aligns with USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests (15). This will help ensure unbiased data can be achieved.

For testing the bactericidal activity, two (2) different in-house microorganism types should be used for the validation; specifically, one (1) gram-positive organism and one (1) gram-negative organism. Both gram-positive and gram-negative microorganisms present

differences in the cellular envelope that influence their resistance to disinfectants. In gram-positive bacteria, there is one membrane that is surrounded by a thick cell wall made of peptidoglycan. In gram-negative bacteria, there is an inner membrane, a much thinner peptidoglycan layer (periplasm), and an outer membrane. Gram-positive bacteria have a greater volume of peptidoglycan in their cell membranes. In other words, gram-positive bacteria have a very thick outer covering. Although mechanically strong, this wall is susceptible to the diffusion of small molecules. Gram-negative bacteria have a much thinner membrane, but this membrane can be very difficult to infiltrate as it may constitute a nonabsorbing barrier or an absorbing barrier that retains the toxic agent (14).

Some antimicrobial compounds are more effective against gram-positive organisms, some are more effective against gram-negative organisms, and some broad-spectrum antimicrobials are effective against both. It really depends on the mechanism of action of the compound. Also, the sources and occurrence of these two groups of microorganisms are very different. Gram-positive non-spore-forming bacteria are mainly inhabitants of human skin. Examples include *Micrococcus* species, *Staphylococcus* species, and *Corynebacterium* species. These microorganisms are recovered from the air and surface samples of production areas. Gram-negative species found in clean room environments are almost always rods and mainly waterborne bacteria often recovered from water samples, tanks, humid areas, and in-process bioburden samples. Examples include *Stenotrophomonas* species, *Ralstonia* species, and *Pseudomonas* species.

For testing fungicidal activity, a minimum of one (1) in-house mold isolate should be used for the validation. *Aspergillus* or *Penicillium* species are typically considered worst-case molds because they can sporulate and are very common environmental isolates; utilizing a fungal spore suspension for efficacy testing further supports this approach. When using spore suspensions, the acceptance criteria applied may mirror that of bacterial spores as these structures share similar physical qualities. The recovery of mold in production areas is typically more common than the recovery of yeast, and mold is of higher contamination risk in production areas because of its high dissemination capability. The structural characteristics of yeast cells make them more sensitive to disinfectant agents than mold spores and hyphae, providing ample justification not to test yeast. A case where yeast may be included during disinfectant efficacy testing would be if a disinfectant is specifically identified for use against yeast and not mold.

For testing sporicidal activity, a minimum of one (1) in-house gram-positive spore-forming rod (e.g., *Bacillus* species) isolate should be used. Bacterial spores are considered the most resilient form of microorganisms when it comes to disinfectant resistance. *Bacillus cereus* is often selected because of its highly resistant nature compared to those of other species within the spore-forming genera of bacteria.

If a particular microorganism group (e.g., gram-negative) cannot be represented by a facility isolate, it is then that ATCC cultures (or culture from other recognized international culture collection)—ideally relevant microbes of concern (e.g., *Bacillus cereus*, *Escherichia coli*, and so forth)—may be used to supplement the testing. These additional isolates may be added to strengthen the study even if the microorganism category is represented by a facility isolate. For example, if *Bacillus subtilis* is determined to be the most prevalent gram-positive spore-former, *Bacillus cereus* may be added to the study because of its higher resistance. If gram-negative isolates are not recovered routinely from manufacturing, an isolate like *Pseudomonas aeruginosa* may be added to represent the microorganism group because of its relevance as a microbe of concern and recognition as a hardy gram-negative organism (14).

When selecting a panel of challenge microorganisms for a multifacility DES, it is not necessary to test all or any of the most prevalent in-house microorganisms from each facility/area. In this case, the more resistant microbe within the same genus may be selected to represent the worst-case scenario. For example, if a company has three facilities and a *Bacillus* species is identified as most prevalent at all three facilities, but one of the *Bacillus* isolates is identified as *Bacillus cereus*, that isolate should be used to represent the others as it is one of the most resistant spore-formers in the hierarchy of microbial resistance to disinfectants. Refer to Table IV for the hierarchy of microbial resistance to disinfectants.

6. Challenge Surface Selection

Disinfectant efficacy studies typically involve application of surface disinfection procedures at a small scale to verify the removal of spiked infectious agents. Small surface coupons (that is, approximately 5 cm by 5 cm) that are representative of surfaces used in the manufacturing

TABLE IV
Hierarchy of Microbial Resistance to Disinfectants

Resistance to Disinfectants[a]			
More Resistant	Microorganism	Examples	More Resistant
↑	Bacterial Spores	*Bacillus cereus/Bacillus sphaericus*	↑
		Bacillus subtilis	
		Clostridium spp.	
	Mold Spores	*Aspergillus, Penicillium*	
	Gram-negative bacteria	*Pseudomonas, Providencia, Escherichia*	
	Vegetative Mold and Yeast	*Aspergillus, Trichophyton, Candida*	
Less Resistant	Gram-positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*	Less Resistant

[a] Adapted from Reference 19.

facilities can be used in the studies. In order to perform disinfectant efficacy studies that can be applied to multiple manufacturing facilities, surface coupons used in the studies must represent the worst-case condition of the surfaces in all facilities in the scope of the study. Characteristics of surfaces in all facilities should be collected. Based on an understanding of all the surfaces from the multiple facilities in the scope of the study, surface coupons can then be determined that represent the worst-case conditions of all surfaces. Considerations for determination of worst-case surface coupons include the type of surface, the surface soils (as applicable), and the surface condition owing to use.

6.1. Surface Type

Typical surfaces representing pharmaceutical manufacturing facilities include but are not limited to:

1. stainless steel;
2. glass;
3. vinyl;
4. epoxy-coated wallboard/flooring;
5. fiberglass;
6. acrylic (plexiglass);
7. Tyvek;
8. terrazzo tiles; and
9. polycarbonate (Lexan).

6.2. Surface Soils

Within some manufacturing facilities, residue removal can be part of the disinfection procedure for certain processing equipment and surfaces. In these cases, a representative "soil" should be incorporated into the challenge spike before drying the spike onto the coupon. If separate procedures are in place to clean the soils before application of disinfectants, then the surface soils do not need to be considered in the scope of the disinfectant efficacy studies.

6.3. Surface Condition Because of Use

The condition of the surface because of use can impact the efficacy of the surface disinfection. For example, coupons made with new stainless steel are a suitable disinfection challenge that is representative of all stainless-steel surfaces as it will provide a consistent surface to provide objective data. Damaged surfaces should not be used in disinfectant efficacy studies. Instead, procedures should be in place to repair or replace damaged surfaces in manufacturing facilities.

A matrix approach can be used to select specific materials for surface testing. Materials can be grouped based on similarity in composition (i.e., metal, plastic, rubber, and so forth). A representative material from each grouping can then be selected based on various properties and characteristics. For example, 304 L or 316 L stainless steel may be used to represent stainless-steel surface testing, provided that both surfaces are present in the facility. 304 L may be selected based on it being more susceptible to corrosion and widely used throughout the facility, or 316 L may be selected based

TABLE V
Risk Assessment Scoring for Challenge Surface Selection

Risk Level	Description	Score
High	**Surface characteristics**: The surface has high surface roughness, susceptibility to surface degradation and/or water absorption, and presence of surface soil.	3
	Prevalence: The surface covers an important proportion of the facility surfaces and/or is often represented in different production areas (e.g., bulk or finishing).	
	Operator interventions: During the process interventions, operators are in regular contact with the surface.	
Medium	**Surface characteristics:** The surface properties do not facilitate microorganism development (low surface roughness, low level of degradation, low or no surface soil, etc.).	2
	Prevalence: The surface covers a moderate proportion of the facility surfaces or is limited to a specific area (e.g., bulk or finishing).	
	Operator interventions: During the process interventions, operators are infrequently in contact with the surface.	
Low	**Surface characteristics:** The surface properties prevent any microorganism development (bacteriostatic or bactericidal properties, very low roughness, very resistant and inert, typically clean surface prior to disinfection, etc.).	1
	Prevalence: The surface covers a small surface and/or is represented in only a few production rooms.	
	Operator interventions: No process interventions with the surface.	

on its higher prevalence, operator interaction in the facility, and the procedures in place (e.g., residue removal, surface replacement) to prevent and address damage to surfaces. Similarly, polypropylene, polycarbonate, vinyl, poly(vinyl chloride), epoxy, and so forth may be selected as a representative surface to bracket plastics because they are all hard, nonporous plastic surfaces with low surface tension. Latex (glove material) may be selected as the representative surface for all natural and synthetic rubbers because of the extensive use of latex gloves in the facility. Unique materials that are not easily represented by a comparable surface should be tested individually.

Within each grouping, a risk-assessment approach may be used to document the justification for selection of the representative or worst-case material with respect to risk of microbial contamination. The risk assessment should, at a minimum, consider the following elements for each surface:

1. Surface characteristics—this risk evaluates the surface type and the characteristics of the surface that could contribute to microbial growth or protection—for example roughness, the risk and level of degradation, and the presence of surface soils.
2. Prevalence—this risk considers the overall prevalence of the surface at the facility.
3. Operator interventions—this risk considers operator interventions.

Table V provides the elements of risk assessment and guidance on how to score surfaces based on the surface characteristics and properties.

The overall risk score for the surface is calculated by multiplying the three scores. The surfaces within each group with high risk scores should be included in the surface efficacy testing. Table VI provides an example of overall risk scoring with the low risk/high risk delineation set at (≥6). This threshold may be adjusted as seen fit by the end user to ensure that the desired surfaces to be tested score appropriately. Surfaces that do not score above the "high risk" cutoff may be tested in addition if an adequate rationale is provided.

7. Leveraging Disinfectant Efficacy Data for Multiple Facilities

Disinfectant efficacy testing is an important part of developing a facilities cleaning program to ensure microbial control of clean room environments. The

TABLE VI
Overall risk score = (Surface characteristics score) × (Prevalence) × (Operator interventions)

Risk Priority Number	Risk Level
<6	Low Risk
≥6	High Risk

performance of this efficacy testing per EN 13697: Quantitative Surface Test for the Evaluation of Bactericidal or Fungicidal Activity (10); ASTM E1153-14 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces (11); and USP <1072> Disinfectants and Antiseptics (3) provides evidence that a disinfectant is effective for its intended use based on the isolates evaluated. Although efficacy data demonstrate a disinfectant's ability to control microbial contamination of clean room environments, performance of such activities need not be site specific.

EM trending provides a list of potential isolates that may be used as part of an efficacy test. A bracketing approach based on the isolates' genre can help reduce the number of isolates evaluated but still demonstrate the solution's effectiveness. As stated previously in Section 4 (The Value of Environmental Monitoring Trending to Disinfectant Efficacy Testing) it is not practical to include all of the microorganisms recovered from clean room environments. In addition, the use of a worst-case microorganism based on the guidance provided in Table IV can strengthen one's study and further reduce the isolates required for evaluation. Therefore, representative isolate selection is encouraged. Efficacy studies historically have been conducted at multiple sites across a company's network. Using the following guidance, disinfectant efficacy testing data (concentrations, contact time, disinfection application, and so forth) from one facility may be leveraged for multiple sites based on appropriate rationale and justification supporting a disinfectant/sporicidal agent's intended use.

1. The environmental isolates used in the disinfectant efficacy evaluation have been recovered by the facility leveraging the data.
    - If such environmental isolates have not been recovered, the use of a worst-case organism may be justified based on the hierarchy specified in Table IV.
2. The challenge organism selection criteria for a solution's activity (bactericidal, fungicidal, or sporicidal activity) have been met. Refer to Table VII for typical organisms recommended by USP <1072> Disinfectants and Antiseptics (3).
    - The use of ATCC and specified isolates per Table VII is not required. Environmental isolates (in-house isolates) of the same microbial category (e.g., gram-positive cocci) should be used to qualify a solution's intended use as they are considered the most representative for efficacy testing.
    - The documented rationale, including trending data, should be utilized effectively to support such selection.
3. The surfaces evaluated are consistent or have been deemed representative and justified with an appropriate rationale.
    - For example, 304L and 316L are common types of stainless steel that are evaluated for disinfectant efficacy. 304L may be selected based on it being more susceptible to corrosion and wide use throughout the facility, or 316L may be selected based on it having a higher prevalence and operator interaction in the facility.
4. Appropriate parameters have been established:
    - Minimum contact time (the duration the disinfectant is in contact with potential microbes on the applied surface—during this time, the material should remain undisturbed and reapplication is not required) (16). If the contact time is defined as "wet", consider the possible requirement to re-wet the surfaces if they dry prior to meeting the wet contact time. (This increases labor and product costs, so shorter contact times are most desirable.)
    - Expiry testing, that is, maximum hold time (expiration after preparation or opening of a ready-to-use (RTU) solution).
    - Temperature conditions used are representative of the manufacturing area (i.e. ambient, refrigerated).

TABLE VII
Typical Disinfectant Efficacy Challenge Organisms

Typical Disinfectant Efficacy Challenge Organisms[a]		
Intended Use	**AOAC Challenge Organisms**	**Typical Environmental Isolates**
Bactericide	*E. coli:* ATCC 11229	*M. luteus*
	S. aureus: ATCC 6538	*S. epidermidis*
	P. aeruginosa: ATCC 15442	*Corynebacterium jeikeium*
		P. vesicularis
Fungicide	*C. albicans:* ATCC 10231 or 2091	*P. chrysogenum*
	Penicillium chrysogenum: ATCC 11709	*A. brasiliensis*
	A. brasiliensis: ATCC 16404	
Sporicide	*B. subtilis:* ATCC 19659	*B. sphaericus*
		B. thuringiensis

[a] Adapted from Reference 3.

5. Log reduction criteria have been met for the solution's intended use. Guidance documents including USP <1072> Disinfectants and Antiseptics (3); EN 13697: Quantitative Surface Test for the Evaluation of Bactericidal or Fungicidal Activity (10); or PDA Technical Report No. 70: Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities (8) may be used to determine adequate acceptance criteria for a facility's disinfection program.

This preceding guidance ensures adequate efficacy testing data has been generated for a disinfectant/sporicidal agent's intended use and eliminates the need to conduct site-specific testing.

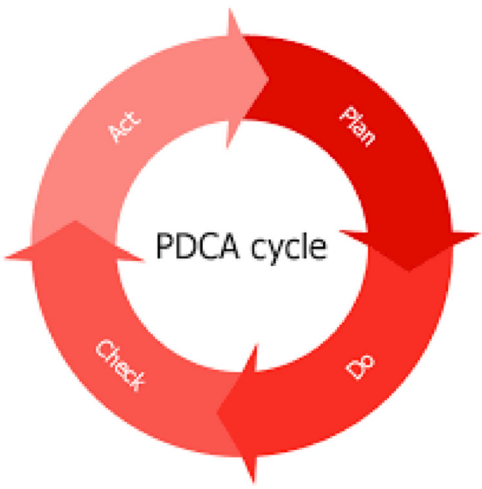

Figure 1

PDCA cycle for a successful disinfection program

8. Critical Parameters and the Holistic Management of a Disinfectant Efficacy Study Validation Program

Each facility designs its space to meet a specific area classification (i.e., ISO 5 through ISO 8, represent clean room classifications). The intent of a disinfection program is to demonstrate that it can reduce the level of microorganisms from the current load (EM trending) to below the area classification that a facility is designed to meet. In order to develop and implement a DES validation program, it is important to start by designing an appropriate EM program. A good EM program will help identify the necessary components to achieve a successful study and qualify disinfectants. The program does not stop there. Once the disinfectants are qualified, it is vital to monitor the program and watch for changes and/or gaps, and then address any gaps as required. This will ensure a confident state of compliance. The Plan, Do, Check, Act (PDCA) cycle that follows provides a high-level overview for a successful disinfection program (Figure 1).

Plan

1. Design and implement an EM program.
    - Identify sample sites and construction materials representing your facility/facilities.
    - Leverage USP, AOAC, and European Standards for testing.

2. Design a study to demonstrate the effectiveness of the disinfectant against the EM isolates obtained from and representing your facility/facilities.

3. Design effective training for proper application of the disinfectants.

Do

1. Use the EM program to identify isolates for the disinfection study.

2. Execute the disinfection study (demonstrate log reduction).

3. Implement and monitor appropriate and sufficient training for disinfectant application and usage.

Check

1. Periodic Evaluation—assessment of the disinfectant program after a defined time period (e.g., annual, biennial, etc.).

    - Review EM trending data for shifts or changes within the microbial flora.

    - Assess new surfaces being brought into the facilities. (Are they represented by current validation?)

    - Monitor for formulation changes and product availability of validated disinfectants.

    - Consider new disinfectants that can improve the program (e.g., reduced contact time, less residue, lower health risk, etc.).

Act

1. Document the evaluation.

2. Adjust the disinfection program based on the findings of periodic evaluation as needed.

9. How to Bring a New Material into the Program

9.1. Managing a New Material Introduction

When introducing a new surface to the controlled areas or disinfectant/detergent to the sanitization program, consider if a controlled process should be initiated or is required. This process should incorporate subject matter experts from departments such as purchasing, supply chain, regulatory, quality assurance, quality control, environmental health and safety (EHS), manufacturing operations, and cleaning operations. The process should ensure that the change is introduced as per regulations and that all factors are sufficiently considered by all departments affected by the change.

9.2. Choosing a New Surface/Disinfectant

Prior to initiating this change control process, the quality assurance department must ensure that sufficient testing for both efficacy and suitability of the surface/disinfectant has been carried out to ensure suitability for the manufacturing process and the sanitizing regime in place.

New surfaces should be selected based upon the following criteria:

1. be planate, continuous, smooth, nonporous, and nonabsorbent;

2. be robust, e.g., resistant to chipping, flaking, and water; and

3. be compatible with the cleaning and sanitization agents.

New disinfectants should be selected based on the following criteria:

1. cost, storage requirements, shelf life, disposal;

2. safety—personal protective equipment (PPE) requirements, consideration of evacuation during application;

3. presentation—concentrate, trigger spray, RTU, preimpregnated;

4. efficacy against selected organisms—refer to Section 4: "Challenge Organism Selection for Disinfection Efficacy Testing";

5. compatibility with the facility surfaces/representative surfaces (usually consider vendor data supplied for compatibility studies);

6. compatibility with the other disinfectants, detergents, and sanitizers used as part of the cleaning and disinfection program;

7. compatibility with the intended method of application (e.g., spraying, mopping, gassing/fogging, wiping) and ease of preparation and use;

8. contact time—check that the contact time required to be effective to the level expected is achievable around production schedules. Ensure that the validated contact time is consistent with routine use [i.e., the contact time performed during protocol execution is the same as the contact time specified in the standard operating procedure (SOP) used by facility]. If the contact time is defined as "wet", consider the possible requirement to re-wet surfaces if they dry prior to meeting the wet contact time. (This increases labor and product costs, so shorter contact times are most desirable.); and

9. requirement to rinse or use a detergent—are there inherent residue accumulation issues?

9.3. In-Situ Field Trials of the Proposed New Disinfectant

Field trials of the proposed disinfectant before implementation are beneficial to assess factors such as practicality of use and contact times, interaction with other chemicals in the regime, odors, operator comfort, and so forth This tests the feasibility of the proposed product and is most beneficial at the very beginning of the process before outlay of costs on efficacy testing.

It is necessary to procure a manufacturing area for the trials (possibly during manufacturing shut down) with representative surfaces from the facility/industry standard surfaces. This area should be a size representative of the "worst case" areas during normal use. A large manufacturing room, where two or more cleaning personnel may have multiple sanitization systems of the disinfectant to make up and apply simultaneously, would best represent the "worst case". Enlist cleaning operatives, EHS, and manufacturing operations representatives to be present for the duration of the trial. Liaise with the vendor in advance of the trial to ensure that the product is being used as per the manufacturer's intent according to their SOP. If there are any concerns at the outset around safe occupational exposure levels, industrial hygienist monitoring should be included for the duration of the activity. If additional PPE, such as respirators, are required, this should be in place and personnel should be trained and qualified in their use. The trial should be performed by cleaning operatives and manufacturing technicians who are trained in the sanitization procedures and who will be using the disinfectant when it is introduced. Evaluate the ease of use, odor, and residue during this trial.

9.4. Assessment against Existing Products

1. If the trial disinfectant is replacing an existing one, ensure that the surfaces are free of any residue from the existing disinfectant through water for injection (WFI) or detergent (followed by WFI if necessary) rinse, to ensure a clean surface and thereby counteract any possibility of mixing chemistries and creating a reaction.

2. If the trial disinfectant is to be used in conjunction with other disinfectant(s)/detergent(s), use the multiple products as intended in daily operations. Assess applications during and after the trial itself, particularly if there is no rinsing step between or after the disinfectant applications.

3. If multiple sequences of application must be assessed, segregate blocks of the area where possible (which should be easily achieved for larger surface areas, such as walls, floors, and ceilings). Label each area with a test number and carry out the applications in the sequence for that test.

9.5. Residue Removal

As a precaution, the removal of residual disinfectants (through detergent or WFI applications) should be monitored for effectiveness. This includes cases where the manufacturers claim low residue. Failure to remove residual disinfectant or detergent could result in accumulation of residue thereby inhibiting disinfectant effectiveness, resulting in safety issues (slipping) or rouging.

Residue buildup can lead to mixing chemistries of disinfectants thus interfering with the expected efficacy or altering the external layer of the material so that the initial efficacy studies are no longer an accurate representation of the disinfectant's action on that surface/material.

Recommendations are often if not always provided by vendors when applicable. If such information is not available, and the surface is critical, this parameter can

be checked through a study designed around ultraviolet (UV) particle spiking of the disinfectant or through studies like cleaning validation swab or rinse samples that can then be chemically analyzed through various tests. Specific test methods include high-performance liquid chromatography, ion-selective electrodes, flame photometry, derivative UV spectroscopy, enzymatic detection, and titration. Alternatively, nonspecific methods, such as total organic carbon, pH levels, and conductivity test for the presence of a blend of ingredients may be performed for less critical surfaces.

9.6. Surface Bench Top Trials—Suspension and Surface Efficacy Testing and Ongoing Monitoring of Efficacy

Although they are not required by regulations, suspension/submersion tests can be good initial indicators for efficacy, before costs associated with coupons studies are incurred.

As a minimum, efficacy tests should consist of surface coupon efficacy tests on samples of the specified surface(s). This is according to USP <1072> Disinfectants and Antiseptics (3) and is outlined in Section 6: "Challenge Surface Selection" of this paper.

Both suspension and surface efficacy tests can be leveraged across facilities based on the worst-case isolate within the genre as discussed in Section 7: "Leveraging Disinfectant Efficacy Data for Multiple Facilities."

The EM program can be used to monitor efficacy through appropriate placement and sufficient quantity of EM surface sampling sites on floors and walls/door panels, as previously discussed.

9.7. Neutralizer Evaluations

EM is performed to assess environmental control and the overall level of microorganisms within the classified areas of the manufacturing environment. EM media contain neutralizers to ensure disinfectants and/or sporicidal agents do not impact the results. Studies must be performed to verify the effectiveness of the media's neutralizers. This is according to Eudralex—The Rules Governing Medicinal Product in the European Union, Volume 4: Good Manufacturing Practices for Medicinal Products for Human and Veterinary Use, Annex 15: Qualification and Validation (17).

The bioburden test method may be used to evaluate disinfectants for contamination. The suitability of the method must be determined if antimicrobial activity has been satisfactorily eliminated under the conditions of the test. USP <1227> Validation of Microbial Recovery from Pharmacopeial Articles (18) may be used as guidance.

10. Conclusion

Ensuring an appropriate disinfectant efficacy program is implemented requires adequate time and resources, which can be substantial. At its core, disinfectant efficacy testing provides baseline data on the effectiveness of disinfectants and EM and its trending provides actual data on the disinfection applications and practices in the manufacturing area. Currently, disinfectant efficacy has no regulatory requirements. There are, however, several guidance documents that are used by the industry to create, implement, and monitor such programs. Companies and the industry would benefit from an industry best practice document, which contains a review of the current guidance documents and practices.

This document was created to provide the end user with an industry-proposed best practice disinfectant program, which includes how to create an effective program and leverage it across the company so that there is no redundant testing. If followed, the resulting program could be more robust than any individual program developed within any one site. It could also provide time and cost savings.

The global benefits of such a disinfectant program are that it is harmonized throughout the entire organization, aligned with the pharmaceutical industry, and can withstand regulatory scrutiny.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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