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Standard Guide for Evaluation of Cleanroom Disinfectants[1]

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1. Scope

1.1 This guide identifies important factors to consider when selecting a disinfectant for use in a cleanroom or similar controlled environment and recommends test methods suitable for evaluating disinfectants. The proper selection of disinfecting agent combined with qualification testing is a key element of a successful disinfection program. Regulatory guidance such as United States Pharmacopoeia Chapter <1072>, "Disinfectants and Antiseptics" and the FDA Guidance for Industry, "Sterile Drug Products Produced by Aseptic Processing–Current Good Manufacturing Practice" address the necessity of disinfectant effectiveness testing but do not clearly define acceptable test methods.

1.2 An understanding of microbiology and microbiological techniques is essential. Knowledge in the following areas is recommended: microorganisms, antimicrobial products (disinfectants, sporicides, and decontamination agents), the chemistry of disinfection, mechanism of activity of disinfectants on cells, application procedures, cleanroom surfaces, and environmental conditions within a cleanroom. This information is available in several published texts listed in the bibliography.

1.3 The theoretical basis for disinfectant activity is not addressed in this guide. An understanding of the effect of disinfectant concentration on microbial reduction (concentration exponent) and kinetics is desirable in determining the use-dilution of different disinfectants and in using dilution to neutralize a disinfectant for efficacy testing. USP chapter <1072> provides further information on this topic.

1.4 This guide is written for the cleanroom environment, although many of the principles outlined in this standard are applicable to manufacturing and processing environments outside of the cleanroom.

1.5 Evaluation of disinfectants for biofilm control is outside the scope of this document.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E2111 Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemicals

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

E2315 Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure

2.2 *BSI Standards:*[3]

BS EN 1040 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics. Test method and requirements (phase 1)

BS EN 1276 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1)

BS EN 1650 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1)

BS EN 13704 Chemical disinfectants. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1)

BS EN 13697 Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2, step 2)

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from British Standards Institute (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., http://www.bsi-global.com.

2.3 *Other Standards:*

AOAC International, Official Methods of Analysis, Chapter 6—Disinfectants, 18th Edition, 2005[4]

ISO 14644-1 Cleanrooms and associated controlled environments -- Part 1: Classification of air cleanliness[5]

United States Pharmacopeia 38, Chapter <1072> Disinfectants and Antiseptics, May 1, 2015[6]

3. Terminology

3.1 *Definitions:*

3.1.1 *antimicrobial, n*—describes an agent that kills bacteria or suppresses their growth or reproduction.

3.1.2 *bioburden, n*—the number and type of viable microorganisms that can be estimated using prescribed recovery procedures.

3.1.3 *biocide, n*—a physical or chemical agent that kills organisms.

3.1.4 *carrier, n*—a surrogate surface or matrix that facilitates the interaction of test microorganisms and treatment.

3.1.5 *cleanroom, n*—an area that establishes and maintains the level of microbial control necessary for the intended manipulations.

3.1.6 *contact time, n*—predetermined time that a test microorganism is exposed to the activity of a test material.

3.1.7 *disinfectant, n*—a physical or chemical agent or process that destroys pathogenic or potentially pathogenic microorganisms on inanimate surfaces or objects.

3.1.8 *efficacy, n*—the proven performance of a product established under defined conditions.

3.1.9 *effectiveness, n*—a measure of the performance of a product.

3.1.10 *inoculum, n*—the viable microorganisms used to contaminate a sample, device, or surface, often expressed as to number and type.

3.1.11 *neutralization, n*—the process for inactivating or quenching the activity of a microbiocide, often achieved through physical (for example, filtration or dilution) or chemical means.

3.1.12 *qualification, n*—to determine effectiveness in the context of a given process.

3.1.13 *sanitizer, n*—chemical or physical agent(s) used to reduce the number of microorganisms to a level judged to be appropriate for a defined purpose and/or claim.

3.1.14 *soil load, n*—a chemical or physical material(s) included in a test procedure to simulate conditions or use.

3.1.15 *sporicide, n*—a chemical or physical agent(s) that kill spores.

3.1.16 *substrate, n*—surface on which an organism can grow.

3.1.17 *surfactant, n*—an agent that reduces the surface tension of water or the tension at a water-liquid interface

3.2 *Acronyms:*

SDS = Safety Data Sheet
GMP = Good Manufacturing Practice
IPA = Isopropyl Alcohol
USP = United States Pharmacopeia

4. Summary of Guide

4.1 Selecting and qualifying the appropriate disinfection agents is an integral factor in developing a compliant cleaning and disinfection program for a cleanroom. Significant factors to consider when selecting disinfectant for use in cleanrooms and controlled environments are discussed in this guide. A summary of the most common test methods used to determine disinfectant effectiveness is also presented.

5. Significance and Use

5.1 Requirements for aseptic processing areas include readily cleanable floors, walls, and ceilings that have smooth, non-porous surfaces; particle, temperature, and humidity controls; and cleaning and disinfecting procedures to produce and maintain aseptic conditions. These controls, combined with careful and thorough evaluation of the chemical agents used for the cleaning and disinfection program, should lead to achieving the specified cleanliness standards and control of microbial contamination of products. Qualification of disinfectants in pharmaceutical, biotechnology, medical device facilities, and associated controlled environments, along with validation of the cleaning and disinfection process, is subject to scrutiny by regulatory agencies.

5.2 An effective cleaning and disinfection program in aseptic processing areas of a Good Manufacturing Practice (GMP) - regulated facility is critical to assure product quality. Manufacturers are held to a high standard when it comes to product sterility, and regulatory agencies increasingly request validation data to support sanitization and disinfection procedures. Regulatory authorities expect evidence of the effectiveness of disinfection agents against environmental microorganisms isolated from the facility. The FDA Guideline for Aseptic Processing states, “The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces.”[7]

5.3 Basic knowledge regarding the effectiveness of different chemical agents against vegetative bacteria, fungi, and spores will aid in selecting chemical agents.

5.4 An understanding of test methods used to assess disinfectant effectiveness is important. Most methods are adaptable, allowing the user to customize the methods to their specific requirements.

[4] Available from AOAC International, 481 North Frederick Ave., Suite 500, Gaithersburg, Maryland 20877-2417, http://www.aoac.org.

[5] Available from International Organization for Standardization (ISO), Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[7] FDA Guideline for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Process, September 2004. Available at http://www.fda.gov/cder/guidance/index.htm

6. Procedure

6.1 *Selection Procedure:*

6.1.1 In the pharmaceutical, biotechnology, and associated industries, the selection of one sanitizer, one or two disinfectants, and one sporicide is typical. More than one type of chemical agent may be needed to obtain the proper balance of effective microbial control and minimal surface damage because products vary in spectrum of activity and formulation. Most facilities select one or two disinfecting agents to use on a routine basis and supplement them with a sporicide which is used on a less frequent routine basis to address spores that may not be killed by the routine disinfectant. All three product types should be evaluated for effectiveness with appropriate test methods. Typical agents used in cleanrooms are:

Sanitizers:	70 % v/v Isopropyl Alcohol (IPA) 70 % v/v Ethanol
Disinfectants:	Phenolic Detergent Compounds Quaternary Ammonium Compounds Hydrogen Peroxide 3% Sodium Hypochlorite <0.10%
Sporicides:	Sodium Hypochlorite > 0.3 % Hydrogen Peroxide > 6 % Peracetic Acid Chlorine Dioxide Vaporized Hydrogen Peroxide

6.1.2 Sanitizers such as 70% solutions of IPA or ethanol are not intended for use as primary microbial control agent in cleanrooms. Their use should be limited to specific purposes such as glove decontamination, cleaning processes, and residue removal.

6.1.3 It is important to choose a disinfectant that incorporates a surfactant in its formulation to help clean surfaces. Occasionally, the choice will be made to rotate different disinfectants. These agents are designed for frequent use.

6.1.4 Sporicidal formulations typically contain highly reactive chemicals (for example, sodium hypochlorite or hydrogen peroxide) and may cause damage to surfaces, even stainless steel, if used too frequently. However, they are a necessity in the cleanroom since routine disinfectants are not effective against bacterial endospores or resistant mold spores. Typically, they are not used on a daily basis to avoid damage to substrates and reduce the potential irritation to cleaning personnel.

6.1.5 *Critical Factors in Selection of Chemical Agents:*

6.1.5.1 *Product Information*—One of the first steps in selection of a chemical agent is to collect all pertinent and available information from the potential supplier. This would include EPA registration information, technical data sheets, SDS, recommended directions for use as well as data on substrate compatibility, stability, and microbiocidal efficacy performed according to acceptable standards.

6.1.5.2 *Cleanroom Bioburden*—A review of environmental monitoring data is necessary to determine the number and types of microorganisms to be controlled. Not all chemical agents are effective against all microorganisms and not all are sporicidal. Knowing the types of microbial flora in the area to be disinfected aids in selecting an appropriate disinfectant with the desired spectrum of activity. From this information, a facility can determine what efficacy is needed and identify potential product candidates.

6.1.5.3 *Contact Time*—For a biocide to be effective, a surface must remain in contact with the agent for a defined period of time. The contact time may vary depending on the target microbial contamination, air temperature, as well as the type of surface being treated.

6.1.5.4 *Preparation*—Products can be purchased in concentrate form or a ready-to-use form. If a concentrated product is selected, dilution will be necessary. Accurate preparation of a use-dilution is critical for optimal performance. Regulatory standards require that disinfectants used in ISO Class 5 environments are sterile; therefore, it may be necessary to purchase the agent sterile or to sterilize it in-house. The quality of water used for dilution is also important. Water hardness or softness can interfere with the action of some disinfectants. It is recommended that purified water or better be used to dilute the concentrate.

6.1.5.5 *Safety and Toxicity*—The product SDS should be reviewed to determine special storage and handling requirements and to identify any potential hazards to personnel working with the agent. Appropriate regulations should be reviewed regarding disposal.

6.1.5.6 *Compatibility*—Some disinfectants are incompatible with others and can cause problematic residues. Performance of disinfectants can be decreased if they are incompatible with cleaning agents used in the area. Additionally, compatibility with surface types in the cleanroom should be evaluated.

7. Disinfectant Challenge Testing

7.1 Once the chemical agents to be used in the cleanroom have been selected, they must be qualified for use through efficacy testing. This testing provides a means of assessing that a disinfectant will render a surface microbiologically safe for the intended purpose. A combination of several test methods is necessary to qualify a disinfectant for use.

7.1.1 Data from *in vitro* testing contributes to the overall picture of how a disinfectant will potentially perform in practice. *In vitro* testing includes suspension tests and carrier tests.

7.1.2 *In situ* testing demonstrates that the disinfectant, when used according to site-specific procedures, will kill the target organisms.

7.1.3 Trend analysis of environmental monitoring data provides an on-going analysis on the effectiveness of the disinfectant.

7.2 Laboratory tests for the evaluation of disinfectants must be scientifically based and ensure that the agent is safe and effective for its intended use.

7.3 Following the same basic methods when evaluating several possible disinfectants for use is a good idea as it allows direct comparison between products.

7.4 Test protocols can be based on ASTM standards, AOAC International methods, or the European standards (EN) for disinfectant efficacy testing. Often, some modification of these methods is necessary when qualifying disinfectants for cleanrooms to accurately reflect the intended use and application of

the disinfectant on the applicable cleanroom surfaces. Modifications to standard methods often include the inclusion of frequently isolated, site-specific environmental organisms and variations in the contact time, soil load, carrier material, application and exposure conditions and disinfectant preparation.

7.4.1 *Use of environmental organisms.* Regulatory authorities have been clear and consistent regarding microorganisms used for disinfectant qualification and expect frequently isolated environmental isolates from the cleanroom environment to be included in the testing. Regulatory guidelines state that "Routinely used disinfectants should be effective against the normal microbial vegetative flora recovered from the facility" which substantiates including environmental isolates in addition to reference strains.[8] It is not necessary to include all environmental isolates in disinfectant efficacy testing but it is necessary to have a valid rationale for the selection of the organisms that are included in the studies. Since these are the microorganisms most likely to present contamination risks to the product, it is critical to prove that the disinfectants used in a microbial control program can control them. Reference strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger* (or *Aspergillus brasiliensis*) can be included in the testing for microbiocides to be used in cleanrooms.

7.4.2 *Contact Time*—The contact time used for the test should be realistic. Regulatory authorities have cited facilities that failed to qualify disinfectants in the manner in which they are used, including disinfectant exposure time.[9]

7.4.3 *Soil Load*—Some published methods test the disinfectant under "clean" and "dirty" conditions. The dirty condition represents an area known to contain levels of organic and inorganic materials. Generally, testing under clean conditions only is sufficient as most sterile manufacturing areas would have little, if any, organic or inorganic contamination.

7.4.4 *Preparation*—The disinfectant used for testing must be prepared the same way it will be used in practice. While some of the published AOAC and EN methods use hard water, this would not be appropriate for disinfectants used in cleanrooms.

7.5 *Test Methods:*

7.5.1 *Suspension Tests*—These tests are used to screen disinfectants for their efficacy at various concentrations and contact times against a wide range of environmental isolates and reference strains. They can be used for preliminary screening of several disinfectant products to determine which products should be tested further. The procedure involves pipetting a known volume of an organism directly into a test tube containing the sanitizer, disinfectant, or sporicide. After the required contact time, serial dilutions are performed in neutralization medium for enumeration. Subsequent plating of the positive and negative controls together with the neutralization confirmation and the test sample are completed. The following published methods describe methodology for suspension testing:

7.5.1.1 ASTM Guide E2315,

7.5.1.2 European Standards including BS EN 1040, BS EN 1276, BS EN 1650, and BS EN 13704.

7.5.2 *Surface Challenge Tests*—These tests provide information about the performance of the chemical agent under laboratory conditions representative of practical use. Test organisms are dried on coupons made of representative substrates from the cleanroom such as stainless steel, aluminum, epoxy, vinyl, plastic, terrazzo, and others. Once the inoculum has dried, the test product is applied to the coupon using an appropriate application method, for example, spray, surface flood, or immersion. After the specified contact time, the coupon is placed in the appropriate neutralization broth and serial dilutions are plated to determine the log reduction of the microorganism. Surface testing presents a more stringent challenge to disinfectant products and is a closer representation of practical in-use conditions. USP <1072> states that sufficient organisms need to be inoculated on the surface being decontaminated to demonstrate at least a 2 log reduction for bacterial spores and 3 log reduction for vegetative bacteria. The following published methods describe methodology for surface challenge testing:

7.5.2.1 ASTM Standard E2111,

7.5.2.2 ASTM Standard E2197,

7.5.2.3 BS EN 13697,

7.5.2.4 AOAC International, Hard Surface Carrier Methods 991.47, 991.48, and 991.49, and

7.5.2.5 AOAC International, Germicidal Spray Products as Disinfectants Official Method 961.02.

7.5.3 *In Situ Tests—In situ* testing is an important phase of the qualification process but not easily carried out since it involves shutdown of the cleanroom. *In situ* evaluations are intended to provide data on the performance of the disinfectant under actual use conditions and in a way that clearly demonstrates performance. Regulatory authorities have cited firms for failure to perform evaluation under actual use conditions.[9] The cleanroom should never be contaminated intentionally for *in situ* testing. Conducting the *in situ* evaluation under worst-case conditions is the ideal situation. As an example, *in situ* qualification can be scheduled around a preventative maintenance shutdown of the cleanroom. Surface and air sampling can be done during the shutdown prior to disinfectant application, and again after the cleanroom is back in operation and disinfected according to procedure. Data review is conducted to determine if the disinfectant effectively reduced contamination in the sampled areas. A statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of the new disinfectant is performed.

7.5.4 *Trend Analysis of Environmental Monitoring Data*—Environmental monitoring data should be trended to determine whether or not a disinfectant is effective. Frequent review of environmental monitoring data is necessary in order to assess

[8] FDA Guideline for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Process, September 2004. Available at http://www.fda.gov/cder/guidance/index.htm.

[9] GMP Trends, Issue No. 666, October 15, 2004.

the effectiveness of a selected disinfectant against the cleanroom bioburden. Environmental monitoring data over an entire year should be used for the comparison to allow for seasonal fluctuations.

7.6 *Requalification:*

7.6.1 Generally, requalification of a disinfectant would be required if one or more of the following were to occur:

7.6.1.1 A change in the disinfectant formulation or vendor,

7.6.1.2 A significant change in the number or types of organisms isolated from the environment, or both,

7.6.1.3 A modification of the surfaces in the cleanroom, and

7.6.1.4 A change in the disinfectant preparation or use procedures.

8. Summary

8.1 Disinfectant qualification is expensive and time consuming but expected by regulatory authorities. However, the choice of disinfectants for contamination control is an important part of assuring the effectiveness of a cleaning and disinfection program in critical areas and multiple factors must be considered when selecting and testing disinfectants. Companies should consider all the relevant data and make a reasonable decision to support their individual disinfection program and product choice.

9. Keywords

9.1 carrier tests; cleanroom; disinfectant; disinfectant qualification; efficacy; *in situ* testing; suspension tests

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(3) Jiminez, L., ed., *Microbial Contamination Control in the Pharmaceutical Industry*, Marcel Dekker, Inc., New York, NY., 2004.

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