Technical Report No. 70

Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities

PDA Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities Technical Report Team

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PDA would like to recognize **Glenn Wright** of the PDA Science Advisory Board for supporting this technical report as the SAB subject matter advisor.

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Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities

Technical Report No. 70

ISBN: 978-0-939459-77-3
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1.0 Introduction

While sterile product manufacturing has the most stringent application, these concepts can also be used to design a program for the manufacture of nonsterile products. To ensure a consistently controlled production environment, a comprehensive cleaning and disinfection program together with a contamination control program should be supported by the following:

- Sound facility design and maintenance
- Established documentation systems
- Validated/qualified disinfection procedures
- Reliable process controls
- Good housekeeping practices
- Effective area traffic and access controls
- Effective training, certification/qualification, and evaluation programs
- Quality assurance of materials and equipment
- Risk management mitigation

The purpose of the cleaning and disinfection program is not only to control microbial contamination, but also to serve as a corrective action for the loss of control for viable excursions contamination. While the destruction of viable cells are an integral part of the cleaning and disinfection program, the use of disinfection as a singular focus without efforts to control contamination from entering the area is without technical merit. Environmental monitoring (EM) evaluates the efficacy of controls on the manufacturing environment. It is through control of bioburden levels entering the area, along with cleaning and disinfection, that acceptable viable control of the manufacturing or appropriate testing environment is achieved. This technical report provides comprehensive information and suggested best practices as well as appropriate references to support such guidance.

For individuals wanting a historical perspective of disinfection, a summary can be found in Appendix I (**Section 17.0**).

The technical report team consisted of members who are cleaning and disinfection experts from various global pharmaceutical and biopharmaceutical companies, academia, and companies that manufacture agents used in disinfection.

1.1 Purpose

The purpose of this document is to identify systematic elements that are essential to assuring an appropriate and compliant cleaning and disinfection program for aseptic and bioburden controlled manufacturing facilities and classified environments.

1.2 Scope

The document covers cleaning and disinfection within controlled and noncontrolled environments using chemical agents that reduce or destroy microorganisms. The document provides guidance for non-product-contact surface cleaning and disinfection. This document is not intended to fully address product-contact surface cleaning from a clean-in-place (CIP) or clean-out-of-place (COP) system which is specifically addressed in PDA's *Technical Report No. 29 (Revised 2012): Points to Consider for Cleaning Validation* and *Technical Report No. 49: Points to Consider for Biotechnology Cleaning Validation* ***(1,2).***

This document should be considered as technical guidance; it is not intended to establish any mandatory or implied standard.

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2.0 Glossary of Terms

Active Pharmaceutical Ingredient (API)
Any substance or mixture of substances intended to be used in the compounding of a drug preparation, thereby becoming the active ingredient in that preparation and furnishing pharmacological activity o other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or affecting the structure and function of the body.

Adverse Trend
A series of alert-level or action-level excursions that indicates the system or areas are not in control and have the potential to affect the product quality.

Airlock
A room that controls the airflow between two rooms of different classification.

Analyte
Substance for which an analysis is being performed.

Antimicrobial Chemical Agent
Substance used to destroy or suppress the growth of microorganisms, whether bacteria, fungi, or viruses, on inanimate objects and surfaces.

Area Disinfection
Disinfection of floors, walls, ceilings, and other surfaces.

Aseptic Processing Area (APA)
A controlled environment that directly supports the aseptic processing of product consisting of several zones in which the air supply, materials, equipment, and personnel are regulated to control microbial and particulate contamination to acceptable levels.

Bioburden Load
A measure of the number of viable organisms in a given environment or material.

Change Control
A documented system for reviewing proposed or actual changes that might affect a validated system or process; change control includes the determination of any corrective action required to ensure that the system remains in a validated state.

Clean (v.)
The implementation of procedures to render an area, piece of equipment, system, or object free of adulterants and contaminants.

Clean(liness)
The measurement for the level of particulates, microbes, or other extraneous substances on an item or surface.

Cleaning Agent
The solution or solvent used in the washing step of a cleaning process. Examples of cleaning agents are: water, organic solvent, commodity chemical diluted in water, and formulated detergent diluted in water.

Colony-Forming Unit (CFU)
The visible outcome of growth of microorganisms arising from a single or multiple cells.

Contact Time
The minimum amount of time that a sanitizer, disinfectant, or sporicide must be left in complete (wet) contact with the surface to be treated in order to be effective.

Contaminant
Any adventitiously or externally introduced material (e.g., chemical, biochemical, or microbial species) not intended to be part of the process.

Coverage
The appropriate distribution of a chemical agent needed on the equipment surface to be effective.

Degradation
The breakdown (usually chemical) of material during manufacture, including during and after the cleaning process.

Depyrogenation
Removal or destruction of pyrogens.

Detergent
A synthetic wetting agent and emulsifier that can be added to a solvent to improve its cleaning efficiency.

Disinfectant
A chemical or physical agent that reduces, destroys, or eliminates vegetative forms of harmful microorganisms but not spores.

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Disinfection
The destruction of pathogenic and other kinds of microorganisms by thermal or chemical means.

Environmental Monitoring (EM)
Describes the processes and activities that need to take place to characterize and monitor the quality of the environment.

First Air
Refers to the air exiting at the face of HEPA filters. Based on the airflow through HEPA filters and its unidirectional air flow the air exiting at the filter face is for the purposed of aseptic processing free of particulate contamination (both viable and non-viable).

Heating, Ventilating, and Air-Conditioning (HVAC)
Refers to technology of indoor and automated environmental control.

High-Efficiency Particulate Air (HEPA) Filter
A type of air filter that must satisfy certain standards of efficiency such as those set by the United States Department of Energy (DOE). The air filter must remove 99.97% of all particles greater than 0.3 micrometer from the air that passes through it.

Gamma Irradiation
The process by which a material is rendered sterile by exposing the material to a radioactive source, such as Cobalt 60.

Germicide
A compound that destroys all vegetative microorganisms.

In-Use Testing (also called In-Situ Testing)
A field study that validates the effectiveness of a disinfecting agent, the trained operators, and the approved operating procedures.

Isolates
Microorganisms that are recovered from a facility.

Largest Daily Dose
Maximum daily dose of the next product to be produced in the equipment train.

LD_{50}
Median lethal dose, or median lethal concentration, of a toxin, radiation, or pathogen; the dose required to kill half the members of a tested population after a specified test duration. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity.

Log Reduction
Log reduction is defined as the first log being 90%, the second log being 9% and the third log being 0.09% of the original inoculums.

Manual Cleaning
A cleaning procedure requiring operator-performed critical steps (e.g., scrubbing with a brush or rinsing with a hose).

Metabolite
A substance that is either the result of metabolism or a requirement for a metabolic process.

Mycoplasma
Small, flexible bacteria that lack a cell wall. Mycoplasma can pass through 0.2 μm and some 0.1 μm rated filters and are unaffected by some antibiotics, such as penicillin.

Penicylinder
A small, ceramic carrier surface used to hold cultures of microorganisms. Used in antimicrobial effectiveness testing procedures.

Pesticide
Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant and any nitrogen stabilizer.

Pyrogen
A material that elicits a pyrogenic response (fever).

Sanitize
To make physically clean and to remove and destroy, to the maximum degree that is practical, agents injurious to health.

Sanitizer
A compound that will reduce the number of vegetative microorganisms to a safe level as de-

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termined by public health requirements. Normally a reduction of 10^3 in vegetative microorganisms is obtained.

Sonicate
To use sound energy to agitate particles; generally used to accomplish mixing or cleaning.

Sporicide
A compound that destroys all vegetative microorganisms and bacterial and fungal spores.

Sterile
The absence of viable microorganisms.

Sterilization
A process by which something is rendered sterile (i.e., moist heat, dry heat, chemical, irradiation); normally validated at 10^6 organism reduction.

Substrate
Primary construction material of a surface to be cleaned or disinfected.

Total Organic Carbon (TOC)
Measurement term for the total organic carbon in a sample.

Transfer Disinfection
A disinfection process conducted on materials and equipment that coats the surface for a validated wetted time to remove bioburden prior to introducing such items into classified areas.

Trend Analysis
Analysis of environmental data over time indicating a shift; adverse trends require investigation.

Vapor Phase Hydrogen Peroxide (VPHP)
A disinfection system in which 35% hydrogen peroxide is changed to a vapor phase and used for bioburden reduction of a chamber or items in a chamber.

Validation
A documented program that provides a high degree of assurance that a specific process, method, or system will consistently produce a results meeting predetermined acceptance criteria.

Visually Clean
Absence of materials that would adulterate a product when inspected with the eyes.

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3.0 Sanitizer, Disinfectant, and Sporicide Claims and Classifications

Sanitizers, disinfectants, and sporicides are chemicals agents that reduce, eliminate, or destroy microorganisms. Registration testing of these chemical agents to meet the requirements of organizations such as the U.S. Environmental Protection Agency (EPA), EU Biocidal Directive, Australian Therapeutics Goods Administration (TGA), Health Canada, and many others are performed at very high levels to address the high-bioburden environments in which they may be used. High-bioburden environments include hospitals, food processors, clinical laboratories, institution, consumer, and others. Registration and approval are required prior to sale in the marketplace and are defined by regulatory requirements in most every county. The label claims seen on product are approved in this fashion. See Appendix II for additional information on registration of sanitizers, disinfectants, and sporicides.

Testing for the use of these same chemical agents in GMP manufacturing operations also requires testing prior to use. However, this testing is significantly different, as the bioburden load within a clean room environment is much lower. As such, the testing is performed with lower bioburden levels, decreased dry (wetted) time periods, and on varying substrates. This type of testing or internal qualification (performed by the firm who intends to use the product or by a third party contract laboratory) is an expectation of drug regulatory agencies worldwide such as the U.S. FDA, the European EMEA, and health ministries throughout the world and may be reviewed as part of the inspection process.

Sanitizers can best be described as chemical agents that reduce the number of vegetative microorganisms to a safe level but do not destroy bacterial and fungal spores. Disinfectants are chemical agents that reduce, destroy, or eliminate vegetative forms of microorganisms but not spores. Sporicides are chemical agents that will destroy all vegetative microorganisms as well as bacterial and fungal spores. However, time frames to destroy high levels of vegetative microorganisms and spores may be extensive and reach far beyond the bioburden level and normal dry (wetted) times characteristic of the clean room operation.

The classifications of sanitizers, disinfectants, and sporicides include the following:

- Alcohols
- Iodine/bromine-containing compounds
- Aldehydes
- Quaternary ammonium compounds
- Phenolic
- Hydrogen peroxide
- Chlorine and sodium hypochlorite
- Peracetic acid/hydrogen peroxide
- ß-Propiolactone
- Ethylene oxide
- Ozone
- Chlorine dioxide

The term "disinfectant" is often used as a general term as well as a term referring to a specific type of chemical agent. To avoid confusion, in this document the term *antimicrobial chemical agent* will be used when referring to sanitizers, disinfectants, and sporicides in general.

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4.0 Regulatory Expectations

4.1 Regulations and Guidance

Reference to the cleaning and disinfecting of manufacturing areas can be found in regulations and guidance documents from various regulatory and standard-setting organizations. Listed below are citations from U.S. regulations and guidances, EU guidances, the PIC/s Convention, the U.S. Pharmacopiea, and the ISO.

- CFR Title 21 Part 211.42(c), (c10i), (c10v) ***(3)***:

  > *Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups during the course of…* (c)
  >
  > *Floors, walls and ceilings of smooth, hard surfaces that are easily cleanable* (c10i)
  >
  > *A system for cleaning and disinfecting the room and equipment to produce aseptic conditions* (c10v)

- U.S. FDA, *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practices,* section X. Laboratory Controls: Sanitization Efficacy ***(4)***:

  > *The suitability, efficacy, and limitations of sanitization agents and procedures should be assessed.*

- EU *Human and Veterinary Medicinal Products,* Annex 1, *Manufacture of Sterile Medicinal Products* ***(5):***

  > *In clean areas, all exposed surfaces should be smooth, impervious and unbroken in order to minimize the shedding or accumulation of particles or micro-organisms and to permit the repeated application of cleaning agents, and disinfectants where used.*

- The Pharmaceutical Inspection Convention (PIC/S) *Guide to Good Manufacturing Practices for Medicinal Products* ***(6)***:

  > *Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimize the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination, build-up of dust or dirt and, in general, any adverse effect on the quality of products.* (Part I Chapter 3, Premises and Equipment)
  >
  > *Using cleaning and decontamination procedures of known effectiveness, as ineffective cleaning of equipment is a common source of cross-contamination.* (Chapter 5, Production)

- The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Part 1 Chapter 3: Premises and Equipment ***(7):***

  > *Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimise the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination,build-up of dust or dirt and, in general, any adverse effect on the quality of products.*

- The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Part 1 Chapter 5: Production ***(8):***

  > *Cross-contamination should be prevented by attention to design of the premises and equipment as described in Chapter 3. This should be supported by attention to process design and implementation of any relevant technical or organizational measures, including effective and reproducible cleaning processes to control risk of cross-contamination.*

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- USP <1072> Disinfectants and Antiseptics ***(9):***

  > *A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of Pharmacopeial articles to prevent the microbial contamination of these articles. Sterile drug products may be contaminated via their pharmaceutical ingredients, process water, packaging components, manufacturing environment, processing equipment, and manufacturing operators*

- International Organization for Standardization (ISO) 13408-*1*, Aseptic Processing of Health Care Product*s*; a*n*d ISO 14698, Cleanrooms and Associated Controlled Environments—Biocontamination Contro*l* ***(10,11):***

  > *This part of ISO 14698 establishes the principles and basic methodology of a formal system of biocontamination control (Formal System) for assessing and controlling biocontamination when cleanroom technology is applied for that purpose. This part of ISO 14698 specifies the methods required for monitoring risk zones in a consistent way and for applying control measures appropriate to the degree of risk involved. In zones where risk is low, it can be used as a source of information.*

These documents are general in nature, providing a limited amount of information on how cleaning and disinfection are to be executed but do convey the expectation that these programs are in place. The responsibility of proving the effectiveness of the chemical agents used remains with the individual firms.

4.2 Regulatory Inspections

Due to their importance and direct impact on manufacturing operations, the cleaning and disinfection programs have been and continue to be a focus during regulatory inspections. Key components of any cleaning and disinfection program, which are often reviewed during inspections, include the following:

- Qualification of suppliers and agents
- Cleaning and disinfection methodologies
- Decision to use ready-to-use vs. ready-to-prepare chemical agents as well as the quality of water to be used (if needed) during their preparation
- Process used for sterile filtering of antimicrobial chemical agents
- Sterilization and storage of antimicrobial chemical agents used in aseptic processing areas
- Sterilization and storage of cleaning equipment (sprayers, buckets, mop heads, and mops)
- In-use expiration dating of antimicrobial chemical agents
- Rotation of agents
- Training, qualifications, and responsibilities of personnel and supervisors
- Frequency of cleaning and disinfection
- Contact times (wetted period)
- Method for addressing residuals
- Documentation for cleaning and disinfection
- Hold times for cleaned and disinfected areas and equipment
- Hold times for soiled areas and equipment
- Cleaning and disinfection performed after a shutdown or an excursion

While the preceding list may not be complete, it serves as a basis for the program and for inspection readiness.

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5.0 Qualification of New Suppliers and Agents

New suppliers and new antimicrobial chemical agents for use in the disinfection program should be qualified prior to use following established procedures. A satisfactory audit, qualification testing, and a clearly defined Certificate of Analysis (CoA) are important aspects to be considered as part of the qualification. If changes occur in the agent's formulation, packaging, or manufacturing site, an evaluation should be performed to determine if requalification is required.

When choosing a new antimicrobial chemical agent from a supplier, evaluate the supplier's:

- Product literature/technical data
- Material compatibility
- Storage conditions
- Expiring dating
- Efficacy data
- Material safety information
- Compatibility information
- Packaging presentations
- Disposal requirements
- Sterility and sterilization information (if the product is provided sterile)

In evaluating supplier information related to the efficacy of an antimicrobial chemical agent, it is important to understand the testing methodology and standards used. These often vary depending on where the agent was registered and the claims made regarding its use. See **Appendices II–V** for more information on this topic as well as safety-related information.

Depending on the specific use of the antimicrobial chemical agent and experience with the specific supplier, an audit may need to be performed. Extra attention should be given to the following during an audit:

- Environmental control and cleaning of the manufacturing or packaging area and equipment used to manufacture the antimicrobial chemical agent.
- Control and disinfection or sterilization of the antimicrobial chemical agent packaging containers.
- Documentation and review of antimicrobial chemical agent production processing activities.
- For aseptically filled agents, the environmental monitoring (EM) program data, including alert and action levels, trending, corrective actions taken, and the use of neutralizing agents for the EM media used.
- For agents labeled as sterile, sterility testing data and qualification of the sterilization process.
- Water systems and the quality of water used in the manufacturing process.
- Package or container integrity studies.
- For double- and triple-bagged containers, disinfection of filled container and overwrapping integrity.
- For double- and triple-bagged containers where a claim of sterility is made for inner bags, qualification of the sterilization process used.
- Handling and storage of finished product containers or work in progress.
- Study results to support label claim of agent.
- Documentation related to regulatory approval of agent.
- Change control: customer notification of ingredient changes or process changes that would affect the finished product—for example, wrapping, irradiation, and sterilization.

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5.1 Qualification Testing

Qualification testing of a new antimicrobial chemical agent should include both laboratory and in-situ testing. Chemical analysis of the actives and microbial efficacy testing should be performed. Chemical analysis of the actives may be provided by the vendor or, alternatively, performed in-house or by a qualified contract laboratory using the vendor's method. Microbial efficacy testing, whether in suspension or in carrier studies, should be performed in-house or by a qualified contract testing laboratory. The antimicrobials chemical agents used for testing should be close to or beyond their stated in-use expiration date (this should take into account a ready to use and/or a use dilution prepared from a concentrate expiry). Testing should be done in replicate on multiple lots of the antimicrobial chemical agent where applicable. It should be noted that significant registration testing on multiple lots of the agent is performed by the company registering the product to ensure product consistency between lots and stability throughout the stated shelf life.

Additional qualification may be performed if changes in product formulation or packaging or site investigations deem it necessary. Information supporting the qualification includes the following seven areas:

- Description of packaging, label, and container type
- Description of ingredients and concentrations
- Lot or batch number
- Efficacy testing results
- Irradiation or other sterilization verification certification
- Safety data sheet information
- Disposal information

5.2 Efficacy Testing

The demonstration of antimicrobial chemical agents to provide their respective kills is a function of the concentration of microorganisms present, the type of microorganisms, the choice of agent, the concentration of the agent, the porosity or texture of the surface to be cleaned, the method of application, and the contact time. Routinely, the agent used should be effective against the normal microbial vegetative flora recovered from the facility. Many efficacy testing guidelines, such as the Association of Official Analytical Chemist (AOAC), suggest high microorganism inoculum levels requiring longer contact times to destroy the population of cells (see Appendix VI, **Section 22.0).** As the normal clean room bioburden level is very low, the inoculum levels for testing would ideally depict levels seen in the controlled area. As this would not be practical in a test environment a higher inoculum level should be used and should not exceed 10^5. The antimicrobial chemical agent used within the industry can be broken into three general areas: sanitizers, disinfectants, and sporicides.

- **Sanitizers**
  Sanitizers provide minimal reduction in thirty seconds to ten minutes and are often used for low levels of vegetative microorganisms. The type of sanitizer will dictate the appropriate contact time required. Alcohol is an example of a commonly used sanitizer.

- **Disinfectants**
  Disinfectants exhibit a higher level of efficacy than sanitizers, and their kill is dependent on the inoculums and the contact time. Disinfectants will typically kill vegetative microorganisms with the exception of spore-forming microorganisms. Examples include quaternary ammonium compounds and phenolics.

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- **Sporicides**
  Sporicides provide up to a total kill depending on the inoculums and the wet contact time and will kill bacterial spore formers as well as mold. Products commonly used today include bleach, hydrogen peroxide, and a mixture of hydrogen peroxide and peracetic acid.

In general, contact or dry times in qualification studies should not exceed 120 seconds for alcohols (70% isopropanol and 70% denatured ethanol) and 10 minutes for disinfectants and sporicides. Longer contact times may be required based on the specific chemical agents used.

Methods to demonstrate efficacy include in-suspension and surface carrier (coupon) studies. In general, a total of three antimicrobial chemical agents (sanitizer, disinfectant, or sporicide) are all that would be qualified within the typical biopharmaceutical or pharmaceutical facility. While historically it was thought that a wide array of disinfectants were required to minimize the buildup of facility-resistant microorganisms, this is no longer a widely held belief (see **Section 11.0).**

5.2.1 In-Suspension Studies

The in-suspension studies may be used to quickly screen various chemical agents to determine which may be the most effective. However, these types of studies should not be considered a replacement for carrier/coupon surface studies (discussed in **Section 5.2.2**) in determining antimicrobial chemical agent performance on clean room surfaces. The test may also be used, where applicable, to demonstrate an agent's efficacy in destroying suspended organisms in solutions (for tanks, holding vessels, bioreactors, etc.).

For in-suspension studies, a panel of six to ten microorganisms, including bacteria, yeast, and mold, should be used. Selection of organisms should be based on the type of environmental isolates recovered from the facility (environmental isolates are preferred); however, if facility isolates are not available ATCC cultures (or cultures from other recognized international culture collections) representing facility isolates are acceptable until facility isolates can be obtained. From the panel, the microorganisms chosen for each study should correlate with the type of antimicrobial chemical agent being evaluated (sanitizer, disinfectant, or sporicide).

One method that may be used to complete the studies is described here:

1. A fresh culture of each organism is prepared to a known CFU/ml concentration.

2. For each organism, a small volume of the culture is transferred directly into a sterile preparation of the chemical agent and mixed. (An inoculum level of 10^3 to 10^4 is suggested.)

3. The mixture is allowed to sit for a specified time to simulate the desired chemical agent contact (wetted) time.

4. Once the desired time has been reached, the entire solution is filtered and rinsed three times with an appropriate neutralizing agent. The filter is subsequently plated to suitable media such as Trypticase Soy Agar (TSA) and incubated to assess the survival level of the microorganisms. Commonly used neutralization agents are provided in **Table 5.2.1-1.**

   Alternatively, the solution can be subjected to a serial dilution, with the first dilution using the neutralizing agent and subsequent dilutions using a saline solution. Selected dilutions are then filtered and plated as described above. A pour plate or spread plate method can also be use used with this approach.

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5. A positive control to verify the inoculum concentration for each organism should be prepared as part of each test. For each positive control, the method used in the study should be followed with the exception that the chemical agent should be replaced with saline. Based on the concentration of the inoculums used, appropriate serial dilutions should be made to allow the recovery of between 10 and 300 CFU per plate.

6. A negative control should also be used to verify that appropriate aseptic technique was employed during the performance of the method. For the negative control, the method used in the study should be followed with the exception that no inoculum should be used. No CFUs should be recovered from the negative control.

7. After the completion of the study, the log reduction achieved against each organism should be determined based on the CFUs present in the inoculum (as determined in the positive controls) and the CFUs recovered from the inoculum exposed to the chemical agent. This level of reduction should be assessed against a set of pre-established criteria to determine if the chemical agent provided the level of reduction required.

Table 5.2.1-1 Commonly Used Neutralization Agents

Antimicrobial Chemical Agent	Neutralizing Agent
Alcohols	Dilution or polysorbate 80
Sodium hypochlorite	Sodium thiosulfate
Quaternary ammonium compounds	Polysorbate 80 and lecithin
Phenolic compounds	Dilution or polysorbate 80 and lecithin
Hydrogen Peroxide/Peracetic Acid and Hydrogen Peroxide	Catalase

The methods used should be validated to ensure that the neutralizing agent selected does not prevent growth of the various organisms chosen for the studies yet is effective in neutralizing the chemical agent.

1. To validate the ability of the test organisms to grow in the presence of the neutralizing agent, the test organism (typically at a concentration of <100 microorganisms) and the neutralizing agent should be plated together using a standard pour plate technique and using the same media type that will be used in the studies. The number of CFUs recovered should be comparable with a positive control to which the neutralizing agent is not added.

2. To validate the ability of the neutralizing agent to neutralize the chemical agent, the study method should be performed as written with the exception that the inoculums (typically at a concentration of <100 microorganisms) should be added after the neutralization step has occurred. In the case of a method that uses membrane filtration, the inoculums should be added to the last rinse performed on the membrane before plating. If a pour plate technique is used, the inoculums should be added to the vessel containing the neutralizing and chemical agents. The number of CFUs recovered should be compared to a positive control in which no chemical or neutralizing agent has been added.

5.2.2 Carrier Surface Studies

Carrier surface studies are performed to provide a verification of the ability of the antimicrobial chemical agent to reduce the microorganism levels that may be present on the types of material surfaces present within the facility.

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A variety of surfaces that are commonly found in the facility and represent a worst-case porosity or most difficult to clean due to their surface texture should be considered. These may include stainless steel, plastic, plastic bags, glass, vinyl curtains, polycarbonates, and various floor material, such as terrazzo, epoxy, vinyl, and laminate, and wall material, such as painted epoxy and polysubstrates. The number of facility surfaces selected should be based on the criticality of the surface and the risk of such surfaces to harbor contamination that may have an impact on the final product. An example of criticality would be stainless steel. While it is often not found to be the most difficult to clean it is often included due to its close proximity (criticality) to the manufacturing operation.

The carrier surface used for testing should be made of the material surfaces selected. If coatings, such as clean room paints and epoxy, are selected, they should be coated on non-linting or absorbent surfaces that will not adversely affect test results. It is recommended that the carrier's measurements not exceed 1.5 inches (38 mm) by 1.5 inches (38 mm) so as to avoid false positives during handling of the carrier. Carriers of this size and smaller will fit into a standard test tube without significant manipulations. However, the size of the carrier will be dependent on the specific method being employed and larger carriers may be used. Prior to use all carriers should be cleaned if needed and properly decontaminated to remove any microorganisms present. Precautions should be taken to ensure that no residual antimicrobial chemical agents are present on the carriers prior to testing. Based on variability within the test methods multiple replicates should be performed, three (3) or more replicates are recommended.

As with the in-suspension studies, a panel of six to ten microorganisms that include bacteria, yeast, and mold should be used. The organisms chosen should be based on the type of environmental isolates recovered from the facility (environmental isolates are preferred); however, if facility isolates are not available, ATCC cultures (or cultures from other recognized international culture collections) representing facility isolates are acceptable until facility isolates can be obtained.

Presented next are two methods that may be used to complete the studies.

The first method is a total kill method and the second an enumeration method. The total kill method is as follows:

1. A fresh culture of each organism is prepared to a known CFU/ml concentration.

2. For each organism, a small volume of the culture is transferred onto the surface of the selected carrier. A sufficient number of microorganisms (10^3–10^5) are placed on the carrier to demonstrate a sufficient reduction of these organisms.

3. The inoculum applied to the carrier is allowed to thoroughly air dry, after which either the antimicrobial agent is generously applied to the carrier by spraying or wiping or the carrier is submerged within the antimicrobial solution. The chemical agent is then allowed to stay in contact with the carrier for a defined period of time (e.g., five to ten minutes).

4. After being in contact with the chemical agent for the specified time, the carrier is then submerged in a vessel containing a neutralizing agent and appropriate growth medium such as Trypticase Say Broth (TSB) to neutralize the chemical agent.

5. After a set time, the carrier is placed within a second vessel containing the same growth medium without the neutralizing agent.

6. Both of the vessels are incubated at the appropriate temperature for a suitable time.

7. Using this method, a result of no growth is required in both containers to demonstrate that the required log reduction has been achieved. This level of reduction should be assessed against a set of pre-established criteria to determine if the chemical agent provided the level of reduction required.

8. A positive control to verify the inoculum concentration for each organism should be performed as part of each test. For each positive control, carriers that have been prepared with those to be exposed to the chemical agent should be submerged in a vessel containing a known concentration of saline and gently sonicated or mechanically scrubbed to remove the microorganisms from the carrier. A serial dilution should then be performed from the vessel and plated using the same media type that was used in the test to allow the recovery of between 10 and 100 CFU per plate. For example, if TSB is used in the test, TSA should be used for the control.

9. A negative control should also be used to verify that appropriate aseptic technique was conducted during the performance of the method. For the negative control, the method used in the study should be followed with the exception that a sterile carrier should be used. No CFUs should be recovered from the negative control.

10. After the completion of the study, the log reduction achieved against each organism should be determined based on the CFUs present in the inoculum (as determined in the positive controls). The level of reduction should be assessed against a set of pre-established criteria to determine if the chemical agent provided the level of reduction required.

The total kill method should be validated to ensure that the neutralizing agent selected does not prevent growth of the various organisms chosen for the studies yet is effective in neutralizing the chemical agent. The validation can consist of the following:

1. To validate the ability of the test organisms to grow in the presence of the neutralizing agent, each of the test organisms (typically at a concentration of <100 microorganisms) and the neutralizing agent should be plated together using a standard pour plate technique and using the same media type that will be used in the studies. After appropriate incubation, the number of CFUs recovered should be comparable to a positive control to which the neutralizing agent is not added.

2. To validate the ability of the neutralizing agent to neutralize the chemical agent as used in the study, the study method should be performed as written with the exception that the inoculums (typically at a concentration of <100 microorganisms) should be added to the vessel containing the neutralizing agent and growth medium after the neutralization step has occurred. Both vessels must show growth.

The second method is enumeration, and can be completed as follows:

1. The carrier, after having been exposed to the antimicrobial chemical agent as described in method 1, is placed in a vessel containing the neutralizing solution and gently sonicated to remove any organisms.

2. The entire solution is then filtered, with the filter subsequently plated to a suitable media such as TSA and incubated to assess the survival level of the microorganisms.

Alternatively, the solution can be subjected to a serial dilution using a saline solution. Selected dilutions are then filtered and plated as described above. A pour plate or spread plate method can also be use used with this approach.

3. A positive control and negative control should be performed as described in method 1 above.

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4. After the completion of the study, the log reduction achieved against each organism should be determined based on the CFUs present in the inoculum (as determined in the positive controls) and the CFUs recovered from the inoculum exposed to the chemical agent. This level of reduction should be assessed against a set of pre-established criteria to determine if the chemical agent provided the level of reduction required. Recommended acceptance criteria are provided in **Table 5.2.2-1.**

The method should be validated to ensure that the neutralizing agent selected does not prevent growth of the various organisms chosen for the studies yet is effective in neutralizing the chemical agent. The validation should be performed as described in the first method (total kill) above.

Table 5.2.2-1 Recommended Acceptance Criteria

Antimicrobial Chemical Agent	Organism Type	Suggested Contact Time[1]	Suggested Minimum Reduction[2]
Sanitizer	Non-spore formers	max. 90 sec	>1 Log
Disinfectant/Sporicide	Non-spore formers	1–5 min	>1 Log
Disinfectant/Sporicide	Mycoplasma	1–5 min	>1 Log
Sporicide	Mold spores	1–5 min	>1 Log
Sporicide	Bacterial spores	1–5 min	>1 Log

1. Suggested contact time depends on surface dry times as well as on the room classification the agent is used in, action/alert levels, normal flora, and inoculums. Worker exposure time should also be taken into consideration.
2. Log reduction is defined as the first log being 90%, the second log being 9% and the third log being 0.09% of the original inoculums.

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6.0 In-Use Expiration Dating

Sanitizers, disinfectants, and sporicides should be assessed to ensure their performance throughout their assigned in-use period. They should be stored for use no longer than the predefined period as specified by written procedures. The expiration dating provided by the manufacturer relates to the expiration of a closed or "primary" container. Once the container (ready-to-use or concentrate) has been opened, the manufacturer's expiration date is no longer valid for active ingredient potency and sterility.

The important points surrounding in-use expiration relate to the length of time that the solution retains its ability to destroy microorganisms (evaluated in the efficacy testing performed) and, for controlled areas, how long the container and its contents maintain an appropriate bioburden level. This is determined by performing bioburden testing on samples of sanitizer, disinfectant, or sporicide taken from containers (spray bottles, squeeze bottles, etc.) used in the disinfection process at the end of their in-use period.

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7.0 Control of the Environment

An effective cleaning and disinfection system starts by limiting the introduction of contamination into the facility by controlling its entry. Stopping as much viable as well as nonviable contamination from entering controlled areas is critical to assuring that the desired environmental conditions are met. If entry of contamination is controlled, the cleaning and disinfection process becomes much less challenging as the quantity of contaminants is reduced.

On the scale of importance, the control over the introduction of contamination into the environment is the most critical concern in the entire cleaning and disinfection process. This control begins with the cleanliness of items such as components, personnel, carts, tanks, tools, and instruments that are transferred into the facility. A list should be constructed of every item that enters the controlled area, followed by the evaluation of each item, to determine whether or not it can be cleaned and disinfected (or sterilized if needed) effectively. Items that can't be appropriately cleaned and disinfected before entry should be replaced by items that can. The cleaning and disinfection procedures for these items must be formalized. Instituting strict entry controls for all items, including personnel, greatly reduces the level of contaminants entering the controlled areas and as a result reduces the probability of excursions occurring.

In addition to controlling the ingress of contamination, concern must also be focused on the proliferation of viable contaminants that are present in the controlled areas. Proliferation of certain types of microorganisms in or on product-contact surfaces such as tanks or bowls, if not cleaned appropriately, can contribute to the level of endotoxins, derived from the cell wall of gram-negative microorganisms, present within a product. Proliferating can also result in a level of bioburden that is difficult to eliminate. Molds, for example, can grow into surfaces or areas that are hard to reach, such as where equipment is attached to the facility's structure, making its elimination much more difficult. For this reason, controlling the environment includes not only limiting the entry of contaminants into the controlled areas of a facility but also limiting the proliferation of these contaminants.

There are three high-risk time periods when the entry of contamination can impact operations:

1. After cleaning and disinfection and before manufacturing begins, production personnel and the components enter the area and potentially shed particulates and microbes. This is a critical time period as setup occurs after disinfection is complete.

2. During manufacturing interventions, personnel may contaminate disinfected or sterilized surfaces through inappropriate clean room behavior and poor aseptic techniques. Shedding of particulates, microbes, and fibers onto manufacturing surfaces can cause contamination.

3. After manufacturing and before cleaning and disinfection, personnel must remain conscious of the impact that their aseptic behavior and practices may have on the cleanliness of the environment.

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Areas of concern for maintaining low levels of contamination entering manufacturing areas include but are not limited to the following:

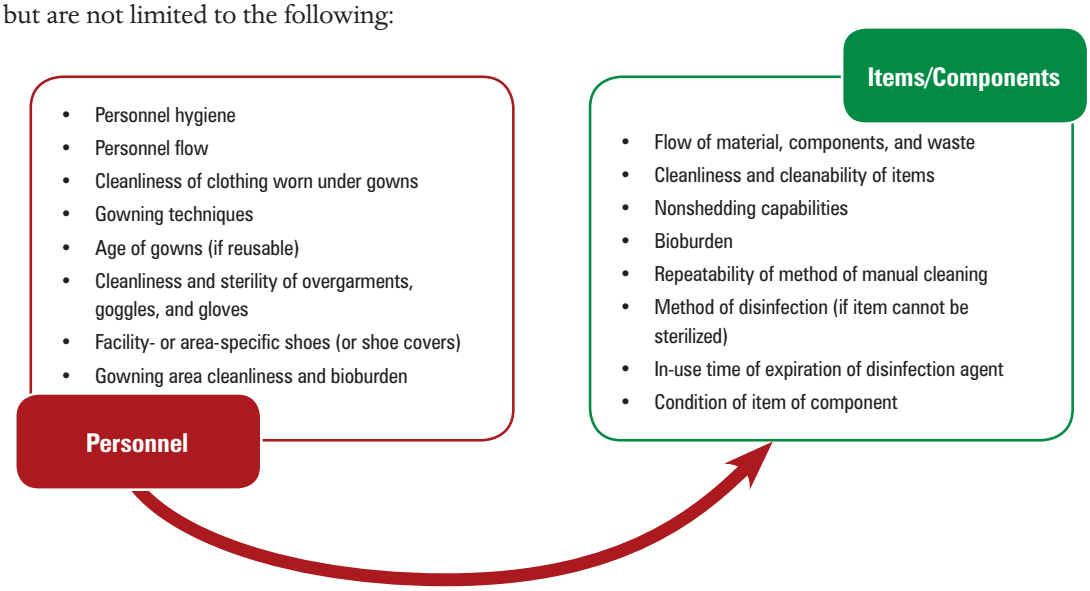

Figure 7.0-1 Considerations to Maintain Low Levels of Contamination

7.1 Introduction of Clean Room Manufacturing Supplies

The design of the facility and the procedures in place must assure the prevention of contamination from the flow of components, drug products, containers, closures, labeling, in-process materials, and products through the building or buildings.

As emphasized in the U.S. FDA *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practices*:

> *It is critical to adequately control material (e.g., in-process supplies, equipment, utensils) as it transfers from lesser to higher classified clean areas to prevent the influx of contaminants. For example, written procedures should address how materials are to be introduced into the aseptic processing room to ensure that room conditions remain uncompromised. In this regard, materials should be disinfected according to appropriate procedures or, when used in critical areas, rendered sterile by a suitable method. (4)*

Good facility design makes the process of item introduction easier and more consistent. Sterilizers, vaporized phased hydrogen peroxide (VPHP) chambers, airlocks, and pass-through ports all share a common design element, such that one side is designated "clean" and the other side is designated "dirty."

The following sections will discuss introduction of items into aseptic processing areas (APA) of the facility. Clean room manufacturing supplies include a broad range of items, such as:

- Mechanic's tools
- Carts
- Production supplies
- Handling implements
- Environmental monitoring supplies
- Wipers
- Antimicrobial chemical agents
- Filling equipment components
- Non-product-contact components
- Markers and pens
- Electronic equipment (e.g., meters and particle counters)

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Items entering the APA should be exposed to the highest level of decontamination that the material can withstand. If an item can be physically sterilized, it must be. The two most widely used methods of sterilizing items entering the APA are autoclaving and gamma irradiation. For items that are gamma irradiated, a number of wraps, barriers, or layers that can be removed when passing to a more stringent grade are normally used. This concept of using multiple outer layers can also be applied to items that are autoclaved, if the autoclave does not directly open into the APA. Items that cannot withstand the temperatures of autoclaving or rigors of irradiation should be introduced using a decontamination process (VPHP, for example) within a pass-through or a manual disinfection (i.e., spraying or wiping) using a sporicidal agent. Ultraviolet (UV) pass-through systems can also be used to reduce bioburden of items but should not be considered a sterilization method.

When using an automated decontamination chamber, each item must be validated using the chosen cycle. Additionally, care should be taken during arrangement of items to maximize the surface area exposed to the agent. If, for some reason, an item is incapable of being disinfected before entering the clean room, it should not be introduced and a suitable alternative should be found.

Personnel should not carry items through the gowning room.

7.1.1 Types of Clean Room Disinfecting Agents

As described earlier, antimicrobial chemical agents can be classified into three categories: sanitizers, disinfectants, and sporicides. Listed here are the types of agents that are commonly associated with each category.

1. Sanitizers: Alcohols (namely, isopropanol and ethanol) are chemicals agents that should be employed when disinfecting items that have been brought into the APA as they are quick to evaporate and leave minimal residue. Isopropyl alcohol (IPA) 70% should be used in favor of ethanol (EtOH) 70%, unless material interactions prohibit, because the bactericidal action of IPA is considered slightly greater than that of ethyl alcohol. While alcohols have relatively good biocidal activity on vegetative cells, their rapid rate of evaporation significantly reduces their effectiveness. Alcohols have no effect on spores.

2. Disinfectants: Phenols and quaternary ammonia compounds provide broad-spectrum kill of vegetative cells. These chemicals characteristically leave residues on surfaces. Immediately following their use, such residues should be removed, for example, via IPA wipe-down.

3. Sporicides: Sodium hypochlorite (bleach) and hydrogen peroxide/peracetic acid compounds are widely used sporicidal agents. Hydrogen peroxide can also be used (normally at 6%) to provide activity against molds and some spore-forming organisms. Peroxides are more active than alcohols and break down into water and oxygen, leaving no residue. Sporicidal chemicals should be employed when a disinfecting procedure requires the reduction of spore-forming organisms. Unfortunately, with the exception of hydrogen peroxide these chemicals leave some amount of residue.

7.1.2 Introduction of Tanks, Vessels, Carts, and Equipment into the APA

The transfer of equipment into an aseptic system can result in the introduction of contamination and, therefore, must be addressed accordingly. Ideally, this decontamination would be done via an autoclave or VPHP chamber; however, it is commonly performed manually.

During the disinfecting process, special attention should be given to cleaning and disinfecting the wheels of carts and mobile equipment. In the manual cleaning and disinfection of cart wheels increased contact time and mechanical wiping techniques should be employed. Wiping with a particulate free, non-shedding wipe to clean to wheel should be accomplished first followed by appropriate disinfectant application on the wheels that assures an appropriate and validated contact (dry) time. Where a VPHP or other type of decontamination chamber is used, the wheels should be wiped down with a cleaning agent and subsequently sprayed with an antimicrobial chemical agent prior to entering the chamber.

7.1.3 Introduction of Cleaning Supplies and Equipment into the APA

Cleaning supplies and cleaning equipment also represent potential bioburden sources to the controlled environment. Therefore, prior sterilization or disinfection of these items should be considered a standard practice.

Cleaning equipment such as buckets, mops, mop handles, mop heads, sprayers, wipes, and extensions should be thoroughly cleaned and rendered sterile prior to use in the Grade A (ISO 5) and adjacent Grade B (ISO 5/6) areas. Sterilization of cleaning equipment can be accomplished through steam sterilization or the use of sterile one-time-use disposable systems, among other methods.

Sanitizers, disinfectants, and sporicides can harbor resistant microorganisms in the solution and, therefore, must be addressed to reduce or eliminate such bioburden. The removal of bioburden from solutions prior to use in the Grade A (ISO 5), adjacent Grade B (ISO 5/6), and adjacent Grade C (ISO 7) areas are critical to assure that such contaminants are not transferred to the controlled areas. Microorganisms (normally spores) residing in sanitizers, disinfectants, and sporicides represent a breach in control during a critical time of cleaning prior to manufacturing. If a bioburden load is permitted to enter through the cleaning process, there is no mechanism in place that will remove their presence before manufacturing.

To ensure sanitizers, disinfectants, and sporicides do not represent a source of contamination, they should be sterile-filtered or sterilized before use in Grade A (ISO 5) and adjacent Grade B (ISO 5/6) areas. A firm should validate the sterilization or sterile filtration process or require appropriate proof of sterilization from outside vendors before use of the solutions in these areas. Examples of approaches that may be used to ensure that the sanitizers, disinfectants, and sporicides are not the source of contamination are listed below:

- Aseptic filtration at 0.2 μ of the final use dilution from outside the Grade A/B (ISO 5/6) area directly into presterilized holding containers or vessels located in a Grade A/B (ISO 5/6) area. If filtration into presterilized holding containers or vessels is conducted outside of the Grade A/B (ISO 5/6) area, then routine bioburden sampling should be conducted prior to its entry into the Grade A/B (ISO 5/6) area.

- Mixing of a solution in the Grade A (ISO 5) and adjacent Grade B (ISO 5/6) areas using a presterilized unit dose container where the solution has been certified as sterile. Such unit dose containers should be mixed with sterile USP Water for Injection.

- Purchase of a ready-to-use or ready-to-mix (where two solutions are packaged together such that they can be mixed) sterile solution from an outside vendor that is delivered with appropriate documentation confirming its sterility.

- Sterilization of the mixed solution in an autoclave (if acceptable based on the composition of the solution).

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Containers that aspirate air into the container, such as squeeze bottles, trigger sprayers, and bulk containers that are opened and closed, should be used for a limited time as defined in written standard operating procedures. As an illustration of the potential problems, 70% IPA bottles that aspirate have been known to harbor mold cells that may have been introduced into the container from the clean room environment. Nonaspirating containers neither introduce contamination to the master reservoir nor allow active ingredients to escape, which would lessen their effectiveness. Nonaspirating containers may be used until the validated expiration period defined for the product.

A sanitizer, disinfectant, or sporicide solution in an open container that has been used in the Grade A (ISO 5) area can subsequently be used in the adjacent Grade B (ISO 5/6) and Grade C (ISO 7) areas, in that order. However, extensively used solutions (dirtied solutions) can compromise the cleaning operation and the antimicrobial effectiveness of the solution. Sterile solutions used in the Grade A (ISO 5) area and subsequently used in a lower classification cannot be used in a Grade A (ISO 5) area again, unless the contents of the solution are kept under pressure, so as not to return contamination to the vessel. Consideration should be given to establishing limits for the total area covered for each batch of solution. Where open bucket systems are used the contents should be discarded upon completion of the cleaning operation as stated above.

7.1.4 Introduction of Components into the APA

The term "components" refers to items that are used directly in the manufacturing process. Stoppers, plungers, vials, and cartridges are some of the most common components. Depyrogenation and sterilization are required for components that come into direct contact with the sterile product (e.g., vials and stoppers) within the aseptic processing area ***(1)***. A validated sterilization process must be used for components entering the APA, and this sterility must be maintained after components have entered the APA through integration into the final product. Depyrogenation and sterilization can be achieved with dry heat or through a validated washing and sterilization process. If components are sterilized outside of the APA, multiple outer wraps, layers, or barriers should be used to allow for appropriate disinfection before entering the Grade A (ISO 5) environment for integration into the final product.

Sterilization and depyrogenation of product-contact surfaces are of the utmost concern. The appropriate methods to accomplish this are outside of the scope of this document. For additional information see PDA *Technical Report No. 3 (Revised 2013): Validation of Dry Heat Processes Used for Depyrogenation and Sterilization* ***(12)***.

7.2 Environmental Monitoring Data Analysis

Environmental monitoring data demonstrates the effectiveness of the microbial contamination control system, which includes the cleaning and disinfection program. The actual genus and species of organisms found; the numbers; and the distribution within the facility compared to the trending history, indicate if the data are consistent with historical area performance or if there has been a shift in control. The data attain a predictable profile, and typical organisms have been isolated when the facility is considered in control. The most common isolates are typically those from people, with fewer isolates from air or soil and water or liquid sources. Data may be analyzed in a number of ways, such as by area, by product, by process, or by organism type. Formal, documented analysis of all microbial environmental data trends should be performed periodically. Evaluating the effectiveness of control, cleaning, and disinfection programs and the adequacy of the current alert and action levels should be performed at least annually. The analysis should include the types and numbers of organisms found and their locations. Following the analysis, this information should be reported to site management, then reviewed and documented by quality management. In addition to the long-term reporting, short-term analysis should also be performed to determine if the areas are in control.

Any change from the normal condition creates a signal, usually referred to as an adverse trend or excursion. An adverse trend will usually require some type of action. Actions can vary from simple notifications for heightened awareness when alert levels are reached or atypical organisms are isolated, to special cleaning and disinfection of the area, to a full investigation when action levels are exceeded on multiple occasions or at multiple monitoring sites.

Adverse data trends should be evaluated to establish the microorganism source (personnel related, from the air or soil, or related to water or liquid sources), if organisms are different from ones previously encountered or if they were found at locations where they would not be expected. Organism-specific corrective actions could include increased use of sporicides or further control mechanisms if organisms producing spores or fungi are found. The reaction to data shifts, signals, or trends should be proportional to the risks imposed by the sampling location and the potential for the contamination to spread. All aspects related to control should be considered and methodically evaluated as the possible cause of the deviation. Return to sustained acceptable data is the long-term measure of success.

For example, the recovery of vegetative organisms above action levels would signal the possible need for temporarily increased control and/or increased disinfection. However, detection of spore-forming organisms above the action level could indicate the need for an immediate response using sporicidal agents. The investigation into a data shift, signal, or trend may indicate that the disinfection program needs to be adjusted.

Additional information on environmental monitoring can be found in PDA *Technical Report No. 13 (Revised 2014): Fundamentals of an Environmental Monitoring Program* ***(13)*****.**

7.3 Attaining and Selecting Environmental Isolates

Recoveries of microorganisms from environmental monitoring samples should be identified to genus and species level when exceeding alert or action levels, and periodically when limits are not exceeded. Organism identifications should be evaluated to determine the most frequently occurring organisms. Representative organisms should be preserved and included in the panel of organisms in efficacy testing of antimicrobial chemical agents used in the facility.

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8.0 In-Situ Field Studies

The true test of the effectiveness of a cleaning and disinfection program is the monitoring data collected from the manufacturing area. Evaluation of the in-situ data being generated from a robust environmental monitoring program will verify that the program is capable of attaining and maintaining a level of cleanliness that minimizes the probability of contamination of the manufacturing process by the environment.

These in-situ data may include the following:

- Nonviable (total particulate data)
- Viable data for surfaces and ambient air
- Personnel-monitoring data
- Microbial identification of representative isolates from the environment
- Residual testing of surfaces
- Product quality (in-process bioburden and sterility testing)

Two approaches to conducting in-situ monitoring are commonly used.

8.1 Environmental Monitoring Before and After the Start-up of a Facility or Area

In this approach, several scenarios may be applicable. They include the opening of a new area of a facility, area shutdown due to adverse events, an area that has undergone significant modifications with no special constraints to personnel or material entry, or an area that has be left idle for a significant period of time with no special constraints to personnel or material entry. Facilities should strongly consider having special start-up cleaning and disinfection programs in place following shutdowns or when significant construction has been performed.

Many programs follow viable monitoring after each step of a start-up program to document the effectiveness of each stage of the cleaning and disinfection program, with this approach:

- An initial cleaning is performed. An initial cleaning entails the removal of soil using a broom or vacuum; for example, cleaning the facility after completion of construction to prepare the facility before starting the formal cleaning process.
- Increased viable monitoring of air and surfaces is performed to attain baseline data for comparison with data acquired after the cleaning and disinfection processes are performed. Nonviable air monitoring may also be performed.
- Facility cleaning and disinfection are performed.
- After the cleaning and disinfection are complete and surfaces are dry, the increased viable monitoring of surfaces should be repeated. Nonviable air monitoring should also be performed. Non-viable air monitoring provides date related to the cleaning process overall and the resulting particulate level present.

After the cleaning and disinfection program has been implemented, the monitoring results from before and after the implementation are analyzed. The expectations from a robust cleaning and disinfection program would be the reduction of the level of viable and nonviable counts and minimization of any spore-forming or mold contaminants initially found. If the results do not demonstrate an acceptable level of reduction, the cleaning and disinfection program should be reviewed and modified where appropriate.

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8.2 Environmental Monitoring Before and After Cleaning and Disinfection During Routine Operation

In this approach, a facility is in routine manufacturing operation to evaluate the effectiveness of the cleaning and disinfection program the follow approach is taken:

- Increased viable surface and air monitoring is performed after operations have occurred and just before cleaning and disinfection take place. Nonviable air monitoring should also be performed.
- Cleaning and disinfection are performed.
- Increased monitoring is performed again after cleaning and disinfection.

The data gathered before and after implementation of the cleaning and disinfection program is then analyzed. The expectations from a robust cleaning and disinfection program would be the reduction of the level of viable and nonviable counts and minimization of any spore-forming or mold contaminants initially found. If the results do not demonstrate an acceptable level of reduction, the cleaning and disinfection program should be reviewed and modified where appropriate.

Additional information on environmental monitoring can be found in PDA *Technical Report No. 13 (Revised 2014): Fundamentals of an Environmental Monitoring Program* ***(13).***

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9.0 Cleaning And Disinfection

Cleaning is a critical step in the cleaning and disinfection process because the buildup of antimicrobial chemical agent residues, product residues, particulates, and other contaminants can inhibit an antimicrobial chemical agent's efficacy. Cleaning requires a nondestructive mechanical action that loosens and removes contaminants from the area or equipment surface. Procedurally, a cleaning agent is applied via a nondestructive mechanical action method. Contaminants and residues are loosened and rinsed from the surface and removed with a squeegee or dry cloth. By lessening the level of particulates, microbes, and residues on the surface, cleaning prepares the surfaces for disinfection and the disinfection efforts become more effective because of the following:

- There are fewer organisms to destroy, as most have been removed from the area.
- Obstructions blocking the chemical agent from contacting the organism are minimized.
- Chemical interference that would reduce the stability and effectiveness of the active agents is removed.
- Lessening of residual that can interfere with future disinfection and/or can dry or flake off and release to the environment.

An antimicrobial chemical agent's efficacy requires the saturation and penetration of the organism's cell wall by the chemical agent for a set amount of time depending on the agent used. Disinfection efficacy depends on a number of factors, including the active ingredient used, air and surface temperatures, saturation and penetration of the cell wall, wetted (contact) time, surface material substrate and bioburden of the surface, existent soil load, concentration of the chemical agent, and pH. Provided the appropriate chemical agent is used, the key to disinfection in the clean room is keeping the surface wetted for a sufficient period of time for the chemical to accomplish its mode of action. Drying time is a variable that must be carefully evaluated as the movement of air in clean rooms (especially in areas where unidirectional airflow is present) tends to dry surfaces quickly.

The effect of the buildup of residues, particulates, and possibly microbes is also affected by the surface itself. Irregular or porous surfaces trap residues and other contaminants and make the surface more difficult to clean and disinfect. Development of appropriate cleaning systems is critical to successfully preparing a surface for disinfection. Cleaning operations should be performed routinely, with frequency based on area classification, usage, risk, and visible cleanliness.

A good cleaning agent is formulated to contain an effective surfactant system that will support the water in its efforts to release particles, residues, and other foreign materials. Procedurally, strict cleaning (without the use of a sanitizer, disinfectant, or sporicide) should be conducted on a routine basis as defined by written procedures.

9.1 Area Classifications and Cleaning and Disinfecting Approaches

Area classification for controlled environments based on airborne particulate levels have been in use for many years. The classifications used are based on one retired and two active industry standards (**Table 9.1-1**):

- U.S. Federal Standard 209E : Defining Classes 100, 1,000, 10,000, and 100,000 ***(14)****
- EU Annex 1: Defining Grades A, B, C, and D ***(5)***
- ISO 14644: Defining ISO Classes 5, 6, 7, and 8 ***(15)***

*Obsolete U.S. Federal Standard 209E classification added for continuity.

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Terminology and adherence to specific guidelines varies among GMP firms throughout the world. U.S. federal standard 209E was retired in 1999, paving the way for worldwide harmonization by new clean room protocols from the International Organization for Standardization (ISO). The following table provides a comparison of these standards.

Table 9.1-1 Area Classifications

Cleanroom Standards – Airborne Particulate Limits (particulates/m³) *(13)*

Particle Size	ISO 14644	U.S. FDA (Aseptic Processing Guidance)	USP <1116>	EU Annex 1 and WHO Annex 4	Japan (Aseptic Processing Guidance)	JP XVI
	ISO 5	Class 100[1,2]	ISO 5/Class 100	Grade A Grade B (at rest)	Grade A Grade B (at rest)	Grade A Grade B (at rest)
≥0.5 μm	3,520	3,520[3]	3,520	3,500	3,520	3,520
≥5 μm	29	Not specified	Not specified	20[4]	20	Not specified
	ISO 6	Class 1000	ISO 6/Class 1000	NA	NA	NA
≥0.5 μm	35,200	35,200	35,200	NA	NA	NA
≥5 μm	290	Not specified	Not specified	NA	NA	NA
	ISO 7	Class 10,000	ISO 7/Class 10,000	Grade B (in operation) Grade C (at rest)	Grade B (in operation) Grade C (at rest)	Grade B (in operation) Grade C (at rest)
≥0.5 μm	352,000	352,000	352,000	350,000	352,000	352,000
≥5 μm	2,900	Not specified	Not specified	2,900	2,900	Not specified
	ISO 8	Class 100,000	ISO 8/Class 100,000	Grade C (in operation) Grade D (at rest)[5]	Grade C (in operation) Grade D (at rest)	Grade C (in operation) Grade D (at rest)
≥0.5 μm	3,520,000	3,520,000	3,520,000	3,500,000	3,520,000	3,520,000
≥5 μm	29,000	Not specified	Not specified	29,000	29,000	Not specified

1. Class 100 and Grade A are defined as requiring unidirectional airflow by all applicable guidelines.
2. Obsolete U.S. Federal Standard 209E classification added for continuity.
3. Class titles for U.S. FDA and USP indicate equivalent particle counts per cubic foot.
4. ISO 4.8 based on reduced limit for particles ≥5 μm.
5. Grade D operational particulate counts depend on the operation and are not defined by any guideline.

In addition to standards on airborne particulates, guidance for microbial action levels for classified areas has also been established (**Table 9.1-2**).

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Table 9.1-2 Environmental Monitoring Requirements/Guidance *(13)*

Monitoring Guidance	U.S. FDA (Aseptic Processing Guidance)	USP <1116>	EU Annex 1, PIC/S and WHO Annex 4	Japan (Aseptic Processing Guidance)	JP XVI
Frequency (Airborne total particulate and viable count. Surface viable count. Personnel sampling as noted)	Class 100: Each production shift. Gloves daily or each lot. Other classes not specified.	ISO 5: Each production shift. ISO 7: Each operating shift. ISO 8: Twice per week.	A: In operation, continuous particulate monitoring required for critical operations. Frequent viable sampling. B: In operation, frequent particle monitoring is required. C, D: Monitoring on risk basis. Surfaces and personnel should be monitored after critical operations.	A, B: Each operating shift for airborne micro, surfaces and personnel; continuous particulate monitoring. C, D: Airborne micro twice per week; airborne particulate once per month; personnel not required.	A: Each operating shift. B: Each operating shift. C, D (potential product/container contact): Twice per week C, D (no potential product/container contact): Once per week
Airborne viable action levels (Active air sampling)	Class 100: 1 CFU/m^3 Class 10,000: 10 CFU/m^3 Class 100,000: 100 CFU/m^3	Recommends use of incident rate (% of samples with micro contamination) rather than count levels, as follows[2]: ISO 5: <1% ISO 7: <5% ISO 6: <3% ISO 8: <10% Applies to all active air, passive air, and surface samples.	A: <1 CFU/m^3 C: 100 CFU/m^3 B: 10 CFU/m^3 D: 200 CFU/m^3	A: <1 CFU/m^3 C: 100 CFU/m^3 B: 10 CFU/m^3 D: 200 CFU/m^3	A: <1 CFU/m^3 C: 100 CFU/m^3 B: 10 CFU/m^3 D: 200 CFU/m^3 0.5 m^3 sample required for A, B 0.2 m^3 sample required for C, D
Airborne viable action levels (Passive air sampling)	Class 100: 1 CFU Class 10,000: 5 CFU Class 100,000: 50 CFU 90 mm diameter settle plate/4 hr Use of settling plates is optional.	Same sample incident rate as active air. 90 mm diameter settle plate/4 hr	A: <1 CFU/m^3 C: 50 CFU/m^3 B: 5 CFU/m^3 D: 100 CFU/m^3 90 mm diameter settle plate/4 hr	A: <1 CFU/m^3 C: 50 CFU/m^3 B: 5 CFU/m^3 D: 100 CFU/m^3 90 mm diameter settle plate/4 hr.	Not specified
Surface Viables Action Levels[3]	Not specified	Same sample incident rate as active air. Use contact plate or swab.	A: <1 C: 25 B: 5 D: 50 55 mm diameter contact plate	A: <1 C: 25 B: 5 D: 50 24–30 cm^2 contact or swab area	A: <1 C: 25 B: 5 D: 50 24–30 cm^2 (5.4–6.2 cm diameter contact or 25 cm^2 swab area)
Personnel viables action levels (gown)	Not specified. Gown sampling must be established based on job responsibility.	Same sample incident rate as active air.[4]	Not specified	Not specified	Not specified
Personnel viables action levels (gloves)	Not specified	Same sample incident rate as active air.	Glove print, 5 fingers A: <1 CFU/glove B: <5 CFU/glove	Glove print, 5 fingers A: <1 CFU/5 fingers B: <5 CFU/5 fingers	Glove print, 5 fingers A: <1 CFU/5 fingers B: <5 CFU/5 fingers

1. Guidance is condensed. Refer to the cited references for complete guidance
2. FDA guidance retains count limits rather than overall contamination rate
3. In general, surface and personnel monitoring should not interfere with the class protection and should be done after critical operations
4. Operators may not be aseptically gowned in ISO 8 support areas

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9.1.1 Cleaning and Disinfecting Grade A (ISO 5) and Grade B (ISO 5 at Rest, 6/7 in operation) Areas

Cleaning and disinfecting these areas take on three varying procedures:

- Cleaning and disinfecting conducted on an established frequency.
- Cleaning and disinfecting conducted in response to an adverse trends and/or a return from a shutdown.
- Routine disinfection conducted without a prior cleaning step

These areas traditionally incorporate 100% HEPA-filter modules in the ceilings, and, thus, the filter should not be exposed to cleaners or antimicrobial chemical agents on a routine basis. Accidental wetting of the filter matrix with a cleaner or disinfecting agent can cause the proliferation of microorganisms and degradation of the filter matrix, which can lead to the integrity of the filter system being compromised.

For cleaning and disinfecting conducted on an established frequency in the Grade A and Grade B areas the following order is commonly followed (from lowest bioburden to highest bioburden) to ensure contamination from the cleaning process itself is minimized.

- A sterile cleaning agent (high surfactant based product) is applied to ceilings (not HEPA filters), then walls, then equipment is cleaned and finally the cleaning agent is applied to the floors in a succession from the furthest point to the closest point to the room exit. Mopping is the preferred method of application for ceilings, walls and floors.
- A squeegee is used to remove the excess liquid and contaminants from the ceiling (not HEPA filters), then walls and floors again in a succession from the furthest point to the closest point to the room exit.
- The dirtied liquid should be lifted from the area via a sterile dry mop, sterile dry wipe, or HEPA-filtered wet vacuum. This prepares the surface for the disinfecting agent.
- After the surfaces have dried they should be sufficiently wetted with a sterile disinfecting agent via mop, spray or wipe following the same sequence being used for the ceiling (not HEPA filters), walls, and floors as described above.

In cases where the cleaning and disinfection is being performed in response to an adverse event or a return form a shutdown the cleaning and disinfection process may need to be repeated for several cycles to ensure the area bioburden is reduced to acceptable levels.

The frequency of the cleaning and disinfection steps may be different with disinfection occurring more frequently. In these cases where disinfection is performed without a prior cleaning the application of the disinfecting agent should follow the same sequence being used for the ceiling, walls, and floors as described above.

Grade A and B work surfaces, and equipment (production lines, dedicated carts, tanks, racks, etc) should be wiped using a sterile cleaning agent and a dry wipe. The dry wipe is used to soak up contaminants in the liquid. After assured drying the surface should be sufficiently wetted with a sterile disinfectant or sporicide. Items found in the cleanroom represent an equivalent contamination level to other surfaces in the clean room (ceilings, walls and floors) as they are also exposed to sources of contamination present within the area.

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In general the cleaning frequency for Grade A and Grade B areas as well as work surfaces and equipment should be based on the facility design, area classification, usage (process being performed), risk, and visible cleanliness. See **Section 10.0.**

9.1.2 Cleaning and Disinfecting Grade C (ISO 7 at rest / ISO 8 in operation) and Grade D (ISO 8 at rest) Areas

Cleaning and disinfecting of these areas also take on three varying procedures that are similar to those required in Grade A (ISO 5) and Grade B (ISO 5 at rest/6 /7 in operation):

- Cleaning and disinfecting conducted on an established frequency.
- Cleaning and Disinfecting conducted in response to adverse trends and/or a return from a shutdown.
- Routine disinfection conducted without a prior cleaning step

These areas traditionally incorporate partial HEPA-filter modules in the ceilings, and, thus, the filter should not be exposed to cleaning or disinfecting agents on a routine basis. Accidental wetting of the filter matrix with a cleaner or antimicrobial chemical agent can cause the proliferation of microorganisms and degradation of the filter matrix, which can lead to the integrity of the filter system being compromised.

For cleaning and disinfecting conducted on an established frequency the walls, ceilings, and floors of Grade C and Grade D areas should be cleaned in the following manner. First, a sterile or nonsterile cleaner (high surfactant based product) is applied to ceilings (not HEPA filters), then walls, then equipment is cleaned and finally the cleaner is applied to the floors in a succession from the furthest point to the closest point to the room exit. Mopping is the preferred method of application for ceilings (not HEPA filters), walls and floors.. Then a squeegee should be used to remove used to remove the excess liquid and contaminants, and finally a HEPA-filtered wet vacuum or other means of lifting the liquid from the area should be employed. After assured drying the surface should be sufficiently wetted with a sterile or nonsterile disinfecting agent via mop, spray or wipe.

In cases where the cleaning and disinfection is being performed in response to an adverse event or a return form a shutdown the cleaning and disinfection process may need to be repeated for several cycles to ensure the area bioburden is reduced to acceptable levels.

The frequency of the cleaning and disinfection steps may be different with disinfection occurring more frequently. In these cases where disinfection is performed without a prior cleaning the application of the disinfecting agent should follow the same sequence being used for the ceiling, walls, and floors as described above.

Grade C and D work surfaces and equipment (production lines, racks, tanks, dedicated carts, etc.) should be cleaned using a sterile or non-sterile cleaning agent and a dry wipe. The dry wipe is used to soak up contaminants in the liquid. After assured drying the surface should be sufficiently wetted with a sterile or non-sterile disinfecting agent. Items found in the cleanroom represent a possibly equivalent contamination level as ceilings, walls and floors as they are exposed to possibly existent contamination within the area.

In general the cleaning frequency for Grade C and Grade D areas as well as work surfaces and equipment and the use of a sterile or non-sterile cleaning and disinfecting agent should be based on the facility design, area classification, usage (process being performed), risk, and visible cleanliness. See **Section 10.0.**

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9.2 Application Methods

Four basic methods of application for a cleaning or disinfecting agent are in use today. The method selected is based in part on the design of the facility. Safety precautions should be taken when using these agents. See **Appendix V** for more information.

- **Spraying**
  This method produces the best wetting of surfaces. A spraying method that employs larger rather than smaller droplets has been found to provide better wetting results. As efficacy performance is based on saturation and penetration of the cell wall as well as contact time, this method produces very good results as long as the underlying surface has been appropriately cleaned. Spraying does not clean the surface, as it lacks mechanical action. Consistent spraying without routine use of a mechanical cleaning action will potentially result in the development of high residue levels, entrapped particulates, deteriorated surfaces, and, as the decontaminating agent will be unable to reach viable contaminants, increased bioburden levels.

- **Mopping**
  Mopping assures that a mechanical action of cleaning is employed. The use of a mopping system for either walls or floors removes residues, viable contamination, and nonviable contamination. For walls, mopping is done from the highest surface point to the lowest surface point. For floors, mopping is done from cleanest to dirtiest and from the highest grade to the lowest grade. While mopping provides the mechanical action needed, great care must be taken to ensure surfaces are wetted appropriately. In general, mopping does not provide as uniform wetting as spraying. For example, the wringing of mop heads and the inability for mop heads to hold sufficient liquid may compromise the level of surface wetting and, therefore, the contact time required. As a result, while cleaning is accomplished, disinfection may be compromised.

- **Wiping**
  Wiping with a presaturated cloth or a dry wipe that is wetted with a cleaning or disinfecting agent is a common practice in the cleaning industry. Wiping, as with mopping, cleans the surface of residues, viable contamination, and nonviable contamination with a mechanical action. Normally, wiping is associated more with cleaning than disinfection. Wiping is done on smaller surfaces that need to be cleaned, such as door handles, push plates, return vents, equipment, carts, and pass-through areas. While wiping possesses the ability to clean the surface, as with mopping, disinfection can be compromised as the surface wetting may not be sufficient to provide the required amount of disinfecting agent contact time. While wiping may remove viable contamination, great care must be taken to ensure that surfaces are adequately wetted.

- **Fogging or Gassing**
  This method can produce excellent results but does require longer periods of time to ensure adequate distribution of the agent and sufficient surface contact time. Fogging methods generate very fine droplets of the disinfecting agent, whereas gassing use a disinfecting agent in a gas form. While both are very effective, just as with spraying, they do not clean the surface. As a result, fogging or gassing without routine use of a mechanical cleaning action will potentially result in the development of high residue levels, entrapped particulates, deteriorated surfaces, and, as the decontaminating agent will be unable to reach viable contaminants, increased bioburden levels. Chemical agents that have commonly been used with this method of application are peracetic acid, hydrogen peroxide, phenol, bleach, quaternary ammonia, paraformaldehyde, and chlorine dioxide. Great care must be taken when a decision is made to use this method, as special safety considerations are required due to the potential exposure dangers and explosion hazards. See **Appendix VIII** for additional information on this method.

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As part of a cleaning and disinfection program, a combination of multiple application methods is suggested for attaining successful results.

9.3 Cleaning and Disinfecting Materials and Workstations

9.3.1 Cleaning and Disinfecting Curtains

A multitude of curtain material substrates can be used in clean room operations. The most common is vinyl. Cleaning curtains is a difficult but critical activity. Curtain materials are generally considered more difficult to clean and disinfect due to their softer surface finish which when viewed microscopically is rougher containing areas where dirt and microorganisms can be better protected from the cleaning and disinfecting agents. To ensure the disinfecting agent is effective curtains should be first cleaned to remove any dirt that may be present and then disinfected.

Cleaning should utilize a high-surfactant-based cleaning product or 70% isopropyl alcohol that is applied via a mechanical cleaning action (wiping or mopping). After cleaning, the curtains should be disinfected with a disinfectant or a sporicide that is characteristically low in residue (for example, H_2O_2 or Peracetic Acid). Curtain surfaces should be sprayed or wiped with an efficacious disinfectant or sporicide and allowed to remain wetted for the contact time validated in antimicrobial effectiveness studies. This time is normally a minimum of five minutes. Curtains should be cleaned with a greater frequency than wall surfaces, as they may come in contact with personnel more frequently. If a disinfectant or sporicide with medium to high residue is utilized, such as phenolic, quaternary ammonium, or bleach, the curtains should be subsequently wiped using 70% IPA and a dry wipe to assure a majority of residue has been removed. The transfer of residue from curtains to critical areas should be avoided. The spraying of a disinfectant or sporicide should be targeted to curtains to avoid overspray to other surfaces, including filling equipment.

9.3.2 Cleaning and Disinfecting Unidirectional Airflow Hoods, Benches, and Biosafety Cabinets

Unidirectional airflow hoods, benches, and biosafety cabinets are used by most GMP operations for a multitude of tasks. Most commonly, the workstations are used for the manipulation or transfer of cells or cell cultures, manipulations of drug products, compounding, or aseptic transfers. The cleaning and disinfecting of unidirectional air flow hoods, benches, and biosafety cabinets is commonly done before and after use.

Cleaning of the interior surfaces requires first cleaning the surface of any residual or spillage. Residing residual or spillage will adversely affect disinfection by blocking the chemical agent from contacting the microorganisms on the surface. The surface should be first cleaned using a cleaner with sufficient surfactants or, at a minimum, sterile 70% IPA. The agents should be sprayed onto the surface and wiped with a dry wipe throughout the enclosure. Wiping should occur from the top of the unit to the bottom of the unit and from the rear of the unit to the front of the unit and should include all sides and the work surfaces. During cleaning, the filter and filter grate (either vertical or horizontal) should not be wetted. Wetting of the filter with the cleaning or disinfecting agent will provide a suitable habitat for the growth of molds and can cause damage to the filter itself.

Once the cleaning step is complete, the surface should be disinfected with an appropriate disinfecting agent. The use of a disinfecting agent such as phenols or quaternary ammoniums will be less effective than using sporicidal agents and will leave residues that are more difficult to remove. For that reason, they are not the preferred chemical agents for the reduction of microorganisms in a workstation environment.

Sporicidal agents are normally applied with a wetted wipe. Sporicides may be sprayed, but vapors may be increased and care should be taken to assure safe levels are maintained. After use of the sporicidal agent, an IPA wipe (dry wipe and 70% IPA) is required if the sporicidal agent leaves a residue. Sporicidal agents such as 0.52% sodium hypochlorite or peracetic acid will leave a residue and require a wipe-down after use, while 6% hydrogen peroxide will not leave a residue and will not require a subsequent wipe-down.

9.4 Cleaning and Disinfecting Equipment Surfaces

9.4.1 Non-product-contact Equipment Surfaces

Non-product-contact equipment surfaces can be found in areas that are close to product-contact surfaces. Due to their critical location, caution should be taken to assure that cleaning chemicals, sanitizers, disinfectants, sporicides, wipers, and other items used on the surface do not leave a residue that may be transferred inadvertently to a product-contact surface. Residues from chemical agents, fibers from wipers, and released wiper binders and wiper size can be sources of possible contamination. Equipment should be precleaned for any past product spills, broken glass (from vials, syringes, ampules, etc.), torn stoppers, damaged caps, and other foreign matter before attempting disinfection. Once precleaning of the equipment is performed, the surface should be sprayed or wiped with an efficacious disinfectant or sporicide and allowed to remain wetted for the specified contact time. After the specified time, all surfaces should receive a wipe-down using 70% IPA if a sporicide or disinfectant with residual properties is used.

9.4.2 Work Surfaces

Work surfaces, such as work tables, carts, and setup areas, may also be near product or near components that come in contact with product. Precleaning of these surfaces should be done routinely in addition to disinfection. Routine cleaning before disinfection provides a higher level of disinfection efficacy. After cleaning, surfaces should be disinfected using either a disinfectant or a sporicide. The determination for the type of product to be used will depend on the defined risk to product or product components. After the use of a disinfectant or sporicide, a 70% IPA spray-down followed by a dry wipe may be required if the disinfectant or sporicide used is determined to leave a residue. Frequency of cleaning is normally daily but should be based on usage.

9.4.3 Nonstructural Clean Room and Hard–to-Clean Surfaces

Structural surfaces such as walls, ceilings, and floors, along with any filling equipment, should be routinely cleaned and disinfected. The frequency should be based on environmental monitoring results and/or a risk-based analysis. Equal consideration should be given to the routine cleaning and disinfection of nonstructural surfaces that exist in each classification, as these surfaces may contaminate the environmental conditions in the area. Routine scheduling for the cleaning and disinfection of surfaces and any items that may reside on them is a critical function. Surfaces can be divided into two categories: routine nonstructural surfaces and hard-to-clean surfaces. Examples of such surfaces may include those shown in **Table 9.4.3-1.**

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Table 9.4.3-1 Examples of Surfaces

Routine Nonstructural Surfaces	Hard-to-Clean Surfaces
Tanks	Tops of doors
Carts	Tracks
Countertops	Conveyers
Racks	Phones
Packaged supplies on racks	Equipment feet and legs
Storage bins	Underside of tanks, carts, and equipment
Stairs	Wheels
Exterior of tubing or pipes	Incubators, refrigerators, and cold rooms
Work surfaces	
Non-product-contact surfaces	
Non-product-equipment	
Monitors, samplers, gauges	
Tools (sterilization may be required)	

Cleaning should be done on all equipment to assure the surface is visibly free from particulate and residue. Disinfection of the surfaces should assure the removal of microbial content to below acceptable surface-monitoring levels. All equipment should be wiped after disinfection by spraying 70% IPA, 70% EtOH, or a high surfactant-based cleaner with little residue that is subsequently wiped with a dry clean room wiper. Cleaning and disinfection frequency of such equipment will depend on the room classification as well as how well contamination is being controlled in the environment and on the equipment.

9.5 Cleaning and Disinfecting Tools

Tools used in the various room classifications require varying cleaning and disinfection disciplines. A tool is any implement, usually handheld, used for performing and facilitating mechanical operations or adjustments in a classified environment. Examples include forceps, screwdrivers, wrenches, and pliers.

The cleaning, disinfection, or sterilization of a tool is based on the classification of the area in which the tool will be used. A main concern is whether or not the tool is capable of being cleaned, disinfected, or sterilized. Certain tools may incorporate electronics, construction materials, or gasket material that may be adversely affected by such decontamination processes. Another concern is whether the tool will reside in a specific area classification or will be continuously transferred from one area classification to another. The following is not a transfer procedure but rather a suggested practice for the level of cleanliness and disinfection or sterilization state for tools used in varying classifications.

- For a tool used in Grade D (ISO 8): Tools should be routinely cleaned via a wiping operation that uses a cleaning agent, 70% IPA or 70% EtOH, and a dry wipe or a saturated wiper. This should be done on a routine basis or more frequently based on use of the tool.
- For a tool used in Grade C (ISO 7): Tools should be routinely cleaned via a wiping operation that uses a cleaning agent, 70% IPA or 70% EtOH, and a dry wipe or a saturated wiper. A subsequent disinfection step may be performed as needed. This should be done on a routine basis or more frequently based on use of the tool.

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- For a tool used in Grade B (ISO 5/6): Tools should be routinely cleaned via a wiping operation that uses a cleaning agent, 70% IPA or 70% EtOH, and a dry wipe or a saturated wiper. A subsequent sterilization should be performed if feasible. If sterilization is not possible, then a disinfection step (via a sporicidal agent, if possible) should be employed prior to introduction to a Grade B (ISO 5/6) area. This should be done on a routine basis or more frequently based on use of the tool.
- For a tool used in Grade A (ISO 5): Tools should be routinely cleaned via a wiping operation that uses a cleaning agent, 70% IPA or 70% EtOH, and a dry wipe or a saturated wiper. A subsequent sterilization of the tool should be performed if feasible. If sterilization is not possible, a disinfection step (via a sporicidal agent, if possible) should be employed prior to introduction to a Grade A (ISO 5) area. This should be done on a routine basis or more frequently based upon use of the tool.

9.6 Cleaning and Disinfecting Water Points of Use

Routine cleaning and disinfection of water points of use are recommended due to the amount of handling by personnel. The scope for cleaning and disinfection includes the exit points of use for purified water and Water for Injection (WFI) systems. The frequency and methodology for cleaning and disinfection should be based on the risk level for particulate and bioburden at the site adversely affecting the product to be manufactured. Two commonly used methods are as follows:

- Use of a thorough rinse of the dispensing head with the water from the water system at routine intervals as defined by approved standard operating procedures.
- Spraying or wiping down the dispensing head with a sanitizer, disinfectant, or sporicide that has low carbon characteristics to prevent adversely affecting total organic carbon (TOC) testing. Examples of high-carbon products would be alcohol-based products. Low-carbon products would include hydrogen peroxide solutions without stabilizers. If a sanitizer, disinfectant, or sporicide is used that leaves a residue care must be taken to ensure that the residual is removed after decontamination.

For both chemical and bioburden testing, if the dispensing head to be sampled is a use point for manufacturing operations, it should be tested as it is used in the manufacturing process. That is, if a flush is required prior to use in operations, a flush should also be performed prior to sampling.

9.7 Disinfecting Drains

Drains should be limited to Grade C and Grade D areas. Drains should be capped, if possible, then opened for use and subsequently capped again. Routine disinfection of drains would provide very little success, as all surfaces of the drain's interior cannot be assured to be wetted by the antimicrobial chemical agent. Conversely, drains will most probably incorporate a biofilm on the inside of the drain that would prevent penetration of the disinfecting agent through the biofilm and from contacting the drain surface. Disinfecting the exterior of the drain's visible surface with sodium hypochlorite or peracetic acid and hydrogen peroxide may reduce bioburden, but such bioburden is expected to return within a short time period.

Firms should review local and municipal regulations regarding the use of or disposal of certain chemical agents through the sewer system.

Monitoring of drains itself may result in continually high results with no proactive corrective action that would be suitable. Monitoring directly on the drain or inside the drain should not be done. Monitoring points around the drain may provide information that is valuable to discern any possible adverse effect of bioburden that may be spread through the controlled area. However, setting of alert and action levels for such locations may prove to be without scientific value.

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9.8 Reducing Corrosion and Deterioration of Surfaces

The deterioration of surfaces that are routinely exposed to cleaners, sanitizers, disinfectants, and sporicides is a concern. Deterioration occurs for many reasons. Most notable are either a chemical reaction between the chemical agent and the surface substrate or the continual buildup of residues on the surface that deteriorate surfaces over time. Deterioration takes on several visible forms:

- **Corrosion:** Corrosion is normally associated with metal surfaces and can take the form of rust or pitting. This deterioration is an attack of the impurities in the metal by the chemical agent (normal impurities in metals relate to carbon levels and purity of the metal grade, such as 304L stainless versus 316L stainless). This is normally seen with products containing chlorine.
- **Chemical incompatibility with the surface:** Chemical incompatibility with the surface normally occurs when the chemical agent reacts with the surface substrate and can deteriorate the surface via melting, softening, or immediate discoloration. Such applications should be avoided. Incompatibility of the chemical agent with a substrate can be seen with peracetic acid and hydrogen peroxide compounds when used on softer and lower-grade metals as well as porous and nonporous substrates.
- **Drying:** Drying of the surface substrate may occur with porous and nonporous soft substrates such as vinyl, Plexiglas, Kydex, Mipolam, and epoxy. This type of deterioration occurs as the chemical agent enters the pores or slight imperfections and over dries the surface. This is most notable with peracetic acid–hydrogen peroxide compounds, hydrogen peroxide compounds, or alcohols.
- **Discoloring or staining:** Discoloring or staining of the surface is normally due to dye in the cleaning agent that stains the surface. Staining or discoloring is normally seen with the use of phenols or iodine.

Surface deterioration is an avoidable occurrence and, with appropriate cleaning steps, can be reduced to minimum levels. Several precautionary steps can be taken to reduce the possibility of corrosion and subsequent deterioration. They are as follows:

- Careful evaluation of the chemical agent's active and inactive ingredients for compatibility with the surface substrates
- Routine removal of residual buildup that may cause deterioration to the surface
- Careful evaluation for the mixing of agents or mixing of residuals on the surface
- Prevention of overexposure of the surface to chemical agents

9.9 Cleaning and Disinfection of Nonclassified Areas

Cleaning is not confined to environmentally classified areas. All buildings used in the manufacture, processing, packing, or holding of a drug product should be maintained in a clean and sanitary condition. Areas must be kept tidy and free of debris, and the introduction of materials into building areas that could impact classified areas should be evaluated to limit the introduction of bioburden, e.g. introduction of mold through wood pallets and corrugated cardboard. The building should be free of pests, and waste material should be held and disposed of in a timely and sanitary manner. The design of the areas within the building should enable thorough cleaning, allowing all areas to be clean and orderly. There should be site policies to define the environmental classifications of all the areas and describe how they are maintained. Floors represent the highest level of contamination to the controlled environment and should be cleaned routinely with an efficacious nonsterile disinfecting agent.

10.0 Frequency For Cleaning And Disinfection

The selection of an appropriate cleaning and disinfection frequency for manufacturing facility surfaces (i.e., walls, ceilings, doors, non-product-contact equipment, surfaces, and floors) is essential for maintaining effective contamination control. The pharmaceutical and biotechnology industries have developed several approaches that have used one or more of the following criteria for selecting a frequency:

- **Area Classification**
  Cleaning and disinfection frequencies based on area classification employ the most stringent cleaning and disinfection frequency for the most stringent area classification with a reduction in the cleaning and disinfection frequency as a function of reduced area classification. Based on this approach, a Grade A (ISO 5) location, for example, could be cleaned and disinfected daily, while Grade C (ISO 7) and Grade D (ISO 8) locations could be cleaned and disinfected weekly and monthly, respectively. This approach is useful, but it does not take into account the risk of product contamination that may be associated with each manufacturing area or the type of manufacturing being conducted.

- **Environmental Monitoring (EM) Data**
  The establishment of a cleaning and disinfection frequency based solely on EM data can result in a program that continually changes over time. This is due to potential fluctuations in the levels and types of bioburden recovered as revealed by daily or periodic data trending and review. This approach tends to be more reactive and retrospective in nature and has more typically been used to reduce established cleaning frequencies based on sustained satisfactory area performance.

- **Risk-Based Model**
  This approach employs elements of the preceding two approaches but also takes into account the risk of product exposure to the environment and personnel and the type of manufacturing conducted in the classified area.

Several principles can be used to help define a risk-based cleaning and disinfection frequency for classified areas:

- The cleaning and disinfection frequency of classified areas should be commensurate with the associated risks of product contamination or cross contamination. Therefore, the open versus closed nature of the process, potential exposure to personnel, and the stage of the process with respect to the final sterilization step (where applicable) should be the primary criteria for selecting a cleaning and disinfection frequency. To minimize the risk of product contamination whenever possible operations should be closed.
- Those classified areas within an aseptic manufacturing boundary (for example, Grade D to Grade C) should be cleaned and disinfected more frequently than those areas outside the boundary.
- ISO 8 manufacturing areas that are immediately adjacent and contiguous (via airlocks) with open aseptic processing areas (for example, Grade C and adjacent Grade A) may require more frequent cleaning and disinfection than those Grade D areas not immediately adjacent and contiguous with such areas (such as a Grade D area adjacent to a Grade C area versus an independent and separate Grade D manufacturing area or suite supporting closed-system processing).
- The cleaning and disinfection of manufacturing areas should be conducted for each product changeover per established procedures to reduce the risk of cross contamination.
- Areas and surfaces that can serve as a vehicle for microbial ingress into the classified area or that may support microbial growth may require cleaning and disinfection at a greater frequency than other areas or surface locations. Ingress areas include gowning entry airlocks, doors, and floors. Areas that support microbial growth include locations for charging of powdered media and/or ingredients to vessels.

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- Selected facility design issues, such as the age of the building or a difficult-to-clean layout, may warrant an increased cleaning and disinfection frequency.
- Selected events may warrant additional cleaning and disinfection beyond the routine frequency. Examples may include microbial air or surface action level excursions, power and/or HEPA filter failures, or periodic facility shutdowns.
- The cleaning and disinfection frequency selected for any classified area must be supported by ongoing satisfactory EM data. Frequent review of the environmental data should be conducted to evaluate the cleaning and disinfection efficacy. Based on the review, the cleaning and disinfection frequency for the area may warrant modification to ensure an area can meet and maintain established monitoring levels.

An example of a risk-based approach for selecting a routine cleaning and disinfection frequency is provided in **Figure 10.0-1.** Example risk levels for varying types of manufacturing processes and area classifications are provided. Based on the risk level and the manufacturing type, example cleaning and disinfection frequencies are listed. The figure illustrates the risk-based approach in that different manufacturing areas with the same area classification may have different cleaning and disinfection frequencies due to the risk of product contamination from the environment, human exposure, and the type of manufacturing performed in the area. (Note that additional controls may apply for the examples presented in the figure.).

In summary, multiple approaches have been used successfully within the industry to select an appropriate cleaning and disinfection frequency for classified areas that result in contamination control, as evidenced by satisfactory EM data. However, an approach based on the risk of product contamination due to its exposure to the environment, personnel, and the manufacturing process itself provides the greatest flexibility and the opportunity to tailor the contamination control and disinfection program to the particular facility design, area use, and manufacturing risks.

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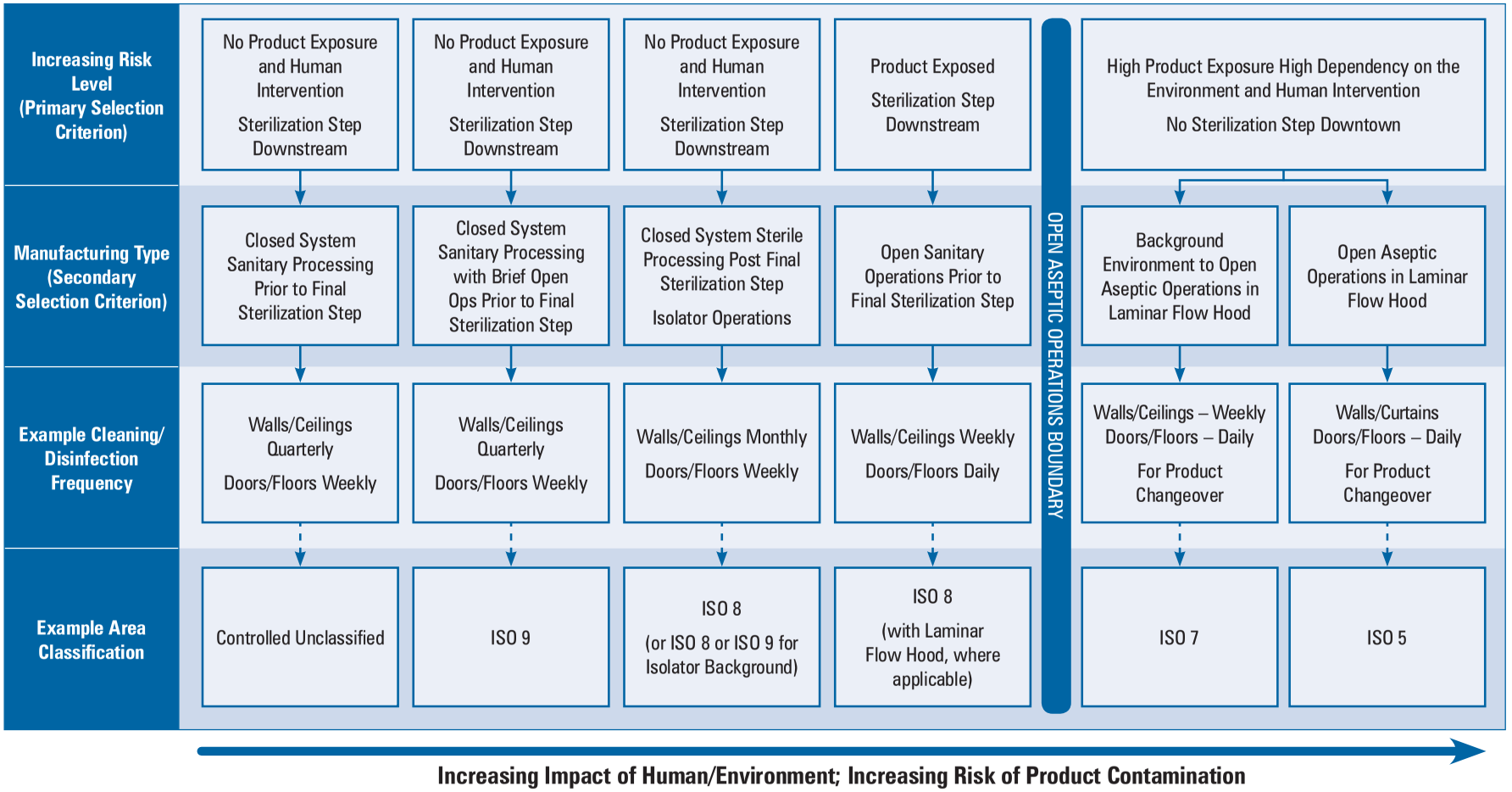

Figure 10.0-1 Example Risk-Based Approach for Selection of Routine Cleaning and Disinfection Frequencies for Classified Manufacturing Areas

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11.0 Resistance and Rotation

For many years there has been a great debate on the subject of the possible development of resistance of microorganisms to sanitizers, disinfectants, and sporicides. Concerns for the possible resistance of organisms to these products are based on a theoretical relationship to resistance found with antibiotics. To date, there is no conclusive published test data proving such development of resistance by organisms to these agents. Resistance to antibiotics is usually acquired through modification of a single gene (or acquisition of a single gene) that blocks the very specific action of the antibiotic. The antimicrobial agents typically employed in clean rooms continue to be effective because they have numerous effects on a number of aspects of cellular physiology. This means that multiple mutations would be required in a short period of time (e.g., five-minute contact time) with exposure to low numbers of cells typically found in a clean room to overcome their detrimental effects. As such, resistance of a cell to agents used in the disinfection process would be highly unlikely given the environmental conditions and low cell numbers.

This is also supported by the current USP <1072> Disinfectants and Antiseptics ***(9):***

> *The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance is less likely, as disinfectants are more powerful biocidal agents than antibiotics and are applied in high concentrations against low populations of microorganisms, so the selective pressure for the development of resistance is less profound.*

Based on this, the pharmaceutical and biotechnology industries have moved away from the rotation of two disinfecting agents. This formerly common practice led to high residue levels and subordinate efficacy performance. Today, most firms use a system whereby a disinfectant is rotated with a sporicide to more effectively reduce the bioburden levels. The rotation of a disinfectant with a sporicide is superior to the rotation of multiple disinfectants. If desired, the sole use of a sporicidal product that has proven efficacy can be implemented without a rotation. If used on a routine basis, the sporicide should destroy the level of contamination necessary to assure acceptable environmental conditions. However, the use of sporicidal agents alone is discouraged due to their inherent corrosive nature.

All rotation systems should be evaluated via the use of area classification, environmental monitoring data, and/or risk assessment.

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12.0 Return From a Shutdown

A shutdown is a planned or required stoppage of operations that is likely to compromise the environmental conditions in the classified area. A shutdown can occur because of regularly scheduled activities such as preventive maintenance and construction activities or because of unscheduled activities such as unexpected power outages. The extent and duration of the shutdown can result in varying levels of viable and nonviable contamination being introduced to the area or facility. Actions must be taken following a planned or unplanned shutdown to bring the area or facility back to a state of control in accordance with the area's environmental classification.

Criteria for returning the facility to a state of control after a shutdown should include verification that all control systems, such as air handlers, are functioning properly. Prior to disinfection, cleaning with detergent or water should be performed first to remove dirt left from construction or other shutdown activities. After cleaning, disinfection should be performed in accordance with established procedures to reduce the bioburden to acceptable levels. All surfaces, including walls, floors, and equipment surfaces, should be included in the disinfection process. When possible, the cleaning and disinfection process should be supported with in-situ data to demonstrate that the multistep cleaning and disinfection regimen employed and the types of antimicrobial chemical agents used are qualified to bring the facility back to a state of control.

After cleaning and disinfection is complete, and before normal operations resume, the area should be monitored for viable and nonviable particulates. If possible, the monitoring data should be evaluated to verify the area is back within a state of control before it is returned to use.

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13.0 Hold Times For Cleaned Areas, Non-Product-Contact Equipment, and Utensils

After an area or facility has been cleaned and disinfected, studies should be performed to establish a maximum time period between cleaning and disinfection in the absence of production activities. Studies should be based on viable and nonviable sampling performed after cleaning and disinfection and at or beyond the maximum time allowed between cleaning and sanitization. Entrance into the area after cleaning should be limited. The study should include the normal level of nonproduction activities that would occur in the area.

Hold times for non-product-contact equipment and utensils should be established and validated. Those materials should be stored in a manner that ensures integrity is maintained for the established hold times. If hold times are exceeded for areas, non-product-contact equipment, or utensils, they need to be cleaned and disinfected again.

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14.0 Training

Personnel need to be properly trained on the standard operating procedures that govern the program as well as the specific cleaning and disinfection techniques used. This training should be documented and readministered periodically. It is important that both the individuals performing the cleaning and disinfection activities and their supervisors be trained.

As many of the cleaning and disinfection processes are manual in nature and their outcome (contamination reduction) dependent on execution, the level of training and general understanding of the process becomes a critical factor in implementing a successful cleaning and disinfection program. While it is clear that training on the SOPs that govern the cleaning and disinfection program is required, additional general training should also be developed to ensure the appropriate level of underlying knowledge is present.

The scope of the training should encompass but not necessarily be limited to the following aspects, which will be discussed in more detail:

- Basic microbiology
- Contamination sources and risks
- Facility design and airflow
- Gowning
- Clean room behavior and personal hygiene
- Basic environmental monitoring
- Aspects of a cleaning program
- Aspects of a disinfection program
- Relevant SOPs
- Assessment of understanding

For personnel to be able to do the best job in cleaning and disinfection, they should have a basic understanding of the total framework of where they need to carry out their work. Once the framework in which they have to operate is better understood and they are supplied with the right tools, they should be able to perform their jobs at the required level of proficiency.

14.1 Basic Microbiology

The aim of disinfection is to destroy viable microorganisms in the clean room. It is therefore important that operators and cleaning staff understand what organisms may be present, how they are multiplying, how they are recovered, and the mechanism by which they are destroyed. Topics that staff needs to understand at the end of the training include but are not limited to:

- The type of viable microorganisms that exist in clean rooms
- How microorganisms multiply and what is needed for multiplication
- The difference between vegetative organisms and spores
- The mechanism by which vegetative organisms and spores are destroyed
- Methods used to detect their presence
- The definition of a colony-forming unit (CFU)
- How microorganisms can compromise final product (safety, purity, and potency) if not minimized
- Endotoxin and methods used for its reduction

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14.2 Contamination Sources and Risks

For operators or cleaning staff to perform their cleaning and disinfection tasks appropriately, they need to understand where contamination might come from, how it enters the clean room, and what the risks are if the contaminants are not addressed properly. At the end of the training they need to be able to identify the following as possible sources of contamination:

- People
- Mobile equipment
- Fixed equipment
- The process being performed
- Air
- Water
- Cleaning supplies

14.3 Facility Design and Airflow

Principles of "first air" and aseptic techniques should, of course, be well understood by operators and the cleaning staff. They should understand how filtration (specifically, HEPA filtration) and airflow are used to remove contaminants. Personnel also need to identify high-risk zones. Topics they need to understand at the end of the training include but are not limited to:

- Basic HVAC design and HEPA filtration principles, including the purpose of unidirectional airflow
- The role of airflow in contamination containment and how smoke studies are used to visualize it
- Facility surfaces and how to clean them appropriately
- High-risk zones in regards to introduction of contamination

14.4 Gowning

The cleaning staff will contribute to the level of bioburden in a clean room. As an added risk, physical labor may result in increased perspiration, which may increase contamination emitted by personnel and may compromise the gowning barrier efficiency. Topics cleaning staff need to understand at the end of the training include but are not limited to:

- The importance of gowning in the clean room environment
- How the gown barrier optimally functions
- Basic principles of gowning (gown, head cover, beard cover, glasses, gloves) to prevent contamination
- Human factors that influence the ability of the gown to provide the level of protection needed
- Liquids and their impact on gowning materials

14.5 Clean Room Behavior and Personal Hygiene

All people entering a clean room should understand what is expected of them with regards to personal hygiene. Topics trainees need to understand at the end of the training include but are not limited to:

- The importance and components of appropriate personal hygiene
- The impact of dry skin on shedding
- Proper washing and sanitization of the hands
- Appropriate movement within a clean room
- Appropriate handling of materials, cleaning supplies, surfaces, and equipment

14.6 Basic Environmental Monitoring

Individuals involved with cleaning and disinfection should understand why environmental monitoring samples are taken and how the data is used. Topics they need to understand at the end of the training include but are not limited to:

- What an EM sample is and how it is taken
- How EM data are used to evaluate the performance of the cleaning and disinfection program
- How EM can help cleaning staff or operators improve their job performance
- What the limitations of EM reporting are
- How an evaluation of the EM data is used in understanding whether the product was manufactured under the appropriate environment

For detailed information on environmental monitoring see PDA Technical Report 13: *Fundamentals of an Environmental Monitoring Program* ***(13)*****.**

14.7 Aspects of a Cleaning Program

Personnel need to understand that cleaning is a separate operation from disinfection and that dirtied surfaces can sometimes complicate disinfection. Topics they need to understand at the end of the training include but are not limited to:

- What the term *dirty surface* means in our industry
- What the typical types of dirt are in our industry (product residue, antimicrobial chemical agent residue, organic or inorganic material, etc.)
- What the difference is between cleaning and disinfecting
- What the general approaches used for cleaning are
- How cleaning agents are prepared
- How cleaning tools are used
- How to handle the cleaning agents from a safety point of view
- How to approach the cleaning of:
  - Walls
  - Ceilings [outside of Grade A (ISO 5) areas]
  - Floors
  - Carts and furniture
  - Equipment and machinery
  - Curtains and barriers

14.8 Aspects of a Disinfection Program

Although the people involved in the cleaning and disinfection of clean rooms are normally not involved in validation, they should understand why certain antimicrobial chemical agents are chosen for specific surfaces and how validation or qualification is performed. The training should also focus on how to apply the agents correctly and how to remove residues on critical surfaces. Topics they need to understand at the end of the training include but are not limited to:

- Which antimicrobial chemical agents are used for disinfection
- How antimicrobial chemicals work
- How antimicrobial chemical agents used in disinfection are chosen and qualified
- What a residue is and how it is removed

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- How to prepare the antimicrobial chemical agent and how to make the correct dilutions
- What the goal is with each disinfection step
- What surfaces should be disinfected
- What the limitations of disinfection are
- How to correctly apply and remove antimicrobial chemical agents
- The how and why of "back to front" mopping techniques
- How to disinfect, using what tools where
- Room hold time before entering the clean room
- How to clean the disinfecting tools
- How to handle the antimicrobial chemical agent from a safety point of view
- Room hold time after disinfection and before entering the clean room again for disinfection
- Contact time for disinfection
- How EM results help identify areas where the disinfection program may need to be modified

14.9 Assessment of Understanding and Qualification

Effective training evaluates what trainees understand before training begins and again after training has completed to assess what was retained. Assessing prior knowledge may be useful in the development of the appropriate course material. Post-evaluations indicate the effectiveness of the training. Post-assessments are critical as the trainee is now performing procedures from training on a daily basis. Assessments should be rendered by supervisory personnel of the individual performing such capacities. At the end of the training, all aspects of that training should be assessed, measuring the level of understanding with respect to what has been discussed. Based on the outcome, further training may be deemed necessary.

15.0 Conducting Investigations Related To Cleaning And Disinfection

Cleaning and disinfection programs and practices that are not followed can result in unacceptable microbial levels in areas or on equipment within the facility. Investigation related to negative shifts, excursions, or trends in the EM data should include a review of the cleaning and disinfection program.

For investigations related to viable contamination, the type of organism can be an important factor in understanding the possible source. The following list offers three organism types and common sources:

- **Gram-positive cocci and small non-spore-forming gram-positive rods**
  The most prevalent contamination source is personnel.
- **Gram-positive rods and fungi**
  The most prevalent contamination source is the external environment (air and soil), which can include the facility's interstitial spaces.
- **Gram-negative rods**
  The most prevalent contamination source is water or liquid related.

When reviewing the cleaning and disinfection program as part of an investigation, areas that should be considered based on the data available include but are not limited to:

- Antimicrobial chemical agent residue buildup and soil that have not been adequately removed by cleaning, thus preventing adequate disinfection
- Inappropriate application of antimicrobial chemical agents
- Insufficient contact times on surfaces
- Inappropriate decontamination of components or bagging before transfer to the controlled area
- Use of inadequate clean room– tools
- High bioburden or shedding from inappropriate cleaning apparatus
- Expired solution
- Incorrect selection of agents in relation to the organisms found
- Incorrectly prepared solutions
- Lack of adherence to established cleaning and disinfection procedures

The following questions may be helpful to ask during an investigation based on the area of focus:

Equipment Related

- For area disinfection (floors, walls, countertops), is the disinfection equipment clean and dry before each use? If it is a Grade A/B (ISO 5/6) area, is the equipment, including tanks and tubing, sterilized before each use?
- Is the equipment stored properly in a clean area and covered until use?
- For transfer disinfection, are spray containers either single use or properly cleaned before reuse?
- Are carts used for transport properly disinfected, including the wheels?

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Materials and Solutions Related

- Is cleaning and disinfection performed starting from the cleanest area and progressing to the dirtiest area?
- Are disinfection efficacy studies available at the site to include methods and expiry dating, as well as any predominant site isolates?
- Are antimicrobial chemical agents adequately rotated with the use of a sporicide to maintain low levels of spore-forming organisms without causing erosion of surfaces from overuse?
- Is the antimicrobial chemical agent applied such that the surfaces remain wet for the required or validated contact time, yet are not overly wet so as to cause puddles to remain, which may allow non-fermenting gram-negative organisms to proliferate?
- Are the site procedures clear on how to apply antimicrobial chemical agents and designated contact times for each agent?
- Are in-situ studies available that demonstrate effectiveness of the site's restart disinfection program for activities such as after construction, after a power outage, or after prolonged shutdown of an area?
- Do the certificates of analysis on the antimicrobial chemical agents used show any changes?
- If the antimicrobial chemical agent is not purchased sterile and is sterile filtered in house, do the records from the sterile filtration process as well as bioburden data available reveal any issues?

Personnel/Training

- Are accurate and complete SOPs in place and available?
- Are operators trained and qualified on how to apply the antimicrobial chemical agent, including contact time and removal of residuals where applicable?
- Have the antimicrobial chemical agents been prepared properly?
- Are personnel instructed not to enter areas that have been disinfected until after the contact time has been exceeded?
- Are areas of construction properly segregated from in-services areas to prevent cross contamination? Are there additional precautions and disinfection and cleaning activities for personnel in the transition areas?
- Are clean room staff trained in and exhibiting consistently good aseptic technique?

Additional information on investigating environmental monitoring excursions can be found in PDA *Technical Report No. 13 (Revised 2014): Fundamentals of an Environmental Monitoring Program* ***(13)*****.**

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16.0 Conclusion

A robust contamination control system starts with controlling contamination from entering classified areas. Such a system stages items from the exterior environment to be cleaned, disinfected and sterilized prior to entry. Without a system for controlling contamination from entering the loss of control for the environmental conditions is very likely. Cleaning compliments the control system as the blockage of all contamination from entering is extremely difficult. Cleaning with a non-abrasive type action and subsequent lifting of dirtied soils, liquids, particulates and microbes prepares the surface to be characteristically lower in soil/residue and bioburden making disinfection of what remains a simpler and more successful process. The disinfection of areas utilizing a validated agent is done correct the errors that have occurred during the control and cleaning process. Cleaning and disinfection of classified areas is not a preventative measure but rather a corrective action procedure done to equalize the failures of the control system. Many pertinent details defined in this technical report combine together to help provide the opportunity for success which is measured as consistent acceptable environmental conditions. Consistent control is the ultimate goal. This technical report is not intended to replace any existing requirements or standards, it is a best practice reference documents regarding the fundamentals of cleaning and disinfection.

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17.0 Appendix I: History Of Disinfection

A disinfectant is a substance that kills microorganisms, also known as bacteria, viruses, and other pathogenic microorganisms, on inanimate objects. People were routinely killing microorganisms long before they even knew of their existence. The ancient Egyptians, Persians, and Chinese used whatever substances had been observed to be effective at keeping "pestilence" at bay. Their random observations of the ability of certain substances to prevent food from spoiling, or people from getting sick, led to the discovery of what can be considered the first disinfectants. These early disinfectants included wine, pine pitch, copper, silver, and even mercury. All of these early disinfectants were, of course, poisonous to humans as well at higher concentrations, but they proved useful at lower dosages for preventing rotting and spoilage. Centuries passed before they were purified and the exact mechanisms of their actions (the killing off of the microorganisms that can cause disease) were finally understood.

17.1 Disinfecting Technologies of the Past

The earliest intentional use of a specific chemical, sulfur dioxide, as a disinfectant was reported as far back as 800 BC by the Greek poet Homer. Fumigation and disinfecting vapors were used in AD 500 by Hindu physician Sushruta Samhita. Venetian cargo ships were reportedly fumigated in attempts to control diseases. During the plagues of the Middle Ages, sulfur dioxide was again used to disinfect contaminated items or areas, although fire was also often used in response to this extreme threat.

When Anton van Leeuwenhoek perfected his microscope in the mid-1600s, he became the first person to view bacteria. A "fellow of insatiable curiosity," Leeuwenhoek also discovered that pepper, vinegar, and other common chemicals killed what he dubbed the "animalcules" (little animals) that he saw with his microscope. Thus, he became the first person to disinfect, or knowingly kill bacteria, with a chemical substance.

Around the same time, Sir Francis Bacon was experimenting with different substances or methods for preventing putrefaction, which he likened to gangrene and other medical conditions. Bacon noted that the process could be prevented by astringents, acids, salt, sugar, or lack of oxygen.

17.2 Disinfecting Technologies in the Age of Chemistry

The science of sterilization via chemical methods (that is, using disinfectants) progressed with Sir John Pringle's experiments with various septic and antiseptic solutions in the mid-1700s. His work led to recommendations for using salts, astringents, vegetable gums, and fermented liquors to prevent spoilage and disease. Using salt as the standard, in 1750 he developed a table of coefficients to help compare the effects of these substances to each other and was possibly the first to ever call these chemicals "antiseptics."

Another chemical disinfecting agent, chlorine, was discovered around the same time by Carl Wilhelm Scheele to prevent putrefaction and accompanying noxious odors. This led to the use of calcium hypochlorite in hospitals, sewers, stables, and other areas. Chlorine was used mostly as a deodorant until its germicidal properties were discovered. During World War I, a 0.5% sodium hypochlorite and alkali solution was used to disinfect wounds. Widespread use of chloride salts continues today, especially in the treatment of water.

Creosote, a mixture of phenols distilled from the tar of beech trees, was discovered by Carl (Karl) Ludwig von Reichenbach in 1832 and also was used first as a deodorant to remove noxious odors. The word creosote is derived from two Greek words that mean "I preserve flesh," and it was used widely in medicine to prevent wounds from becoming infected. Later, a mixture of alkylphenols distilled from coal tar creosote was found to be a more effective wound disinfectant. This distillate was emulsified with soap and marketed as Lysol.

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Tincture of iodine was introduced to the United States Pharmacopeia (USP) in 1830 and was used to treat wounds during the Civil War. Other chemists began to discover and isolate many more disinfectants, including copper sulfate, sodium permanganate, and various alcohols, sulfurs, acids, and alkalis. In fact, most of today's most common disinfectants have been used since the nineteenth century.

17.3 Discovering Microorganisms as a Basis of Disease

Theodor Schwan, who was a codiscoverer of yeast cells, used sterile media and heat to demonstrate that microorganisms in the air produce putrefaction. His experiments were later confirmed and expanded upon by Louis Pasteur. Pasteur was a genius who played a major role in the development of the field of microbiology, as well as in advances in chemistry, medicine, and bacteriology. He was one of the first to advocate the use of heat in medical settings to destroy the microorganisms that cause disease but are invisible to the naked eye. After reading Pasteur's idea that microbes in the air caused putrefaction, Joseph Lister began to experiment with various antiseptics to kill the microorganisms causing wound infection. He found that a phenol called carbolic acid effectively prevented infection of open wounds in his patients. Phenol proved to be a potent germicide that can even kill spores, but it was toxic to the body tissue at full strength. One of his talks in the United States inspired a physician from Missouri named Joseph Lawrence to develop Listerine in 1879, thus immortalizing Lister's name. A pharmacist from New York named Robert Johnson was also inspired by Lister's talk, selecting phenols for use on wound dressings as the first product of his surgical products company Johnson & Johnson. Later, the search for other effective phenols would lead to the development of Lysol from coal tar.

Robert Koch later conclusively demonstrated that bacteria cause disease in live tissues and wrote the report "On Disinfection" in 1881 ***(16).*** This report compared the ability of various chemical agents to kill bacteria and their spores. Kronig and Paul later expanded on this, noting that bacteria are killed at a faster rate with increasing temperature and/or chemical concentrations. Rideal and Walker later developed the very practical "phenol coefficient method of testing disinfections," modifications of which are still used today ***(17).***

In 1776, Spallanzani found that microorganisms could be destroyed by heat. Some microbes proved to be more resistant and required boiling for about an hour for a surface to be totally sterile (free of microbes). Appert later used this method of heating with boiling water to preserve food during canning. Koch later defined hot air and steam as sterilizing agents. Tyndallization, a process of sterilizing through discontinuous heat, was then developed by John Tyndall to reduce the activity of any sporulation bacteria left after boiling. Louis Pasteur discovered the benefits of using superheated steam in sterilization that killed bacteria as well as spores, which eventually led to the development of the modern autoclave in the mid-1800s.

17.4 Microbiological Contamination Control Today

Sterilization is the process of totally destroying all microbes using either physical or chemical methods. Once all microorganisms are destroyed, the resulting product or environment is said to be "sterile," or germ-free.

Physical methods of sterilization include the processes of dry heat and steam sterilization that were refined in the late 1800s through the work of William Henry along with Pasteur, Koch, and Wolffhugel ***(18).*** Much later, gas vapors such as ethylene oxide, formaldehyde vapor, and plasma gas were used, although subsequent research proved some of these gases to be too toxic or, in the case of formaldehyde, even carcinogenic. Steam and dry heat sterilization continues to be the method of choice in many settings, including biopharmaceutical and medical device manufacturing. Decontamination of isolators uses a chemical decontamination agent, for example, hydrogen peroxide.

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Filtration had been used to purify water for centuries before air was filtered through cotton by Schröder and von Dusch in 1854. Devaine then demonstrated that bacteria in the air could be retained in porcelain filters, but it was John Tyndall, in 1877, who clearly demonstrated that a decrease in visible air particulates with their accompanying microorganisms helped maintain sterility in liquids open to this filtered air. Later, the Pasteur-Chamberland filter was devised to filter out bacteria in fluids as well ***(19)***.

Ultraviolet (UV) radiation was also found to be an effective method of sterilization, first by Rieder in 1898 and then by Gates in 1928. However, the need for direct exposure and the chance that some microbes can "hide" in cracks and crevices have been major limitations of this method. Still, UV radiation is used today for certain situations, assuming procedures are such that all critical surfaces receive maximum exposure for the time needed to kill all of the microorganisms present.

The advancement of organic chemistry in the twentieth century brought a wide variety of disinfectants. Although many common disinfectants have been used for centuries, we now have a much better understanding not only of their mechanism of action but also of the possible harm that these chemicals can cause to humans. In addition, because we can now easily grow and isolate microbes, we are better able to test specific disinfectants and antiseptics to help determine the appropriate level of use for all of the various types. Therefore, disinfection research of today is focused mostly on finding a balance between the two variables of effectiveness and safety. The goal is to design procedures that allow for the application of enough disinfectant to effectively remove all of the microbes, but minimize the risk of harm to the product, the workers who have to use these chemicals in their workplace, and the health of the public at large.

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18.0 Appendix II: Registration Of Sanitizers, Disinfectants And Sporicides

Understanding what legislative body is in charge of registrations of sanitizers, disinfectants, and sporicides throughout the world is imperative to understanding their regulation. At the same time, understanding the variable worldwide types of antimicrobial effectiveness claims and test methods for products that will be used for hard-surface disinfection is critically important. In each country the legislative authority is different. In the United States, the Environmental Protection Agency (EPA) governs hard-surface disinfection and related claims. In the European Union (EU), the EU Biocide Regulation as determined and written by the member states replaces the past segregated individual country governing bodies as the new all-encompassing EU legislature. Many who follow Good Manufacturing Practices (GMPs) assume that the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA) must approve sanitizers, disinfectants, and sporicides for use in pharmaceutical, biotechnology, and health-care settings. This assumption would be incorrect, as most medicinal governmental registration authorities become involved only if the chemical agent comes in contact with the human body, comes in contact with a medical device that is implanted or inserted into the human body, or is taken internally into the human body.

As an example, the U.S. FDA registers products (under a 510K registration) that will be used to clean or sterilize medical device products that will come in contact with the human body. This does not include products that will be used for hard-surface disinfection within a controlled environment. Nor does it include pharmaceutical or biotechnology product-contact surfaces where products may be used. Understanding registration authorities and registration claims is imperative in understanding the claims made on products. In the following sections, the U.S. EPA and the EU Biocide Regulation requirements and framework are discussed. These are examples of the types of registration that are required within their legislative regions. In other regions of the world, legislative authorities, antimicrobial effectiveness claims, and associated requirements vary per region. This complex, worldwide multiregistration system is without harmonization and confuses the average end user, who should confer with local, state, and country requirements prior to use of chemical agents for disinfection purposes.

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19.0 Appendix III: Overview Of The U.S. Environmental Protection Agency

The United States Environmental Protection Agency (EPA) regulates antimicrobial products under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). FIFRA requires U.S. EPA registration of a pesticide for sale into the U.S. interstate commerce. Every U.S. state, Puerto Rico, and the District of Columbia require registration of FIFRA pesticides, accompanied by a registration fee, to allow the product to be sold or used in their locale. For most states, registration is an administrative function with the state working cooperatively with the EPA. The California Department of Pesticide Regulation of the California Environmental Protection Agency (Cal-EPA) requires submission and approval of supporting data along the same lines as the U.S. EPA. There are occasions where Cal-EPA and the U.S. EPA do not arrive at the same conclusions.

The U.S. EPA's authority is based on the FIFRA definitions of *pesticide* and *pest*. According to FIFRA, Section 2 (u), a pesticide is "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest, any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant and any nitrogen stabilizer" ***(20).*** According to FIFRA Section 25 (c) (1), a pest is "any insect, rodent, nematode, fungus, weed or any other form of terrestrial or aquatic plant or animal life or virus, bacteria or other micro-organism (except viruses, bacteria or other micro-organisms on or in living man or other living animals) which the Agency declares to be a pest" ***(20).*** Antimicrobial agents are substances used to destroy or suppress the growth of harmful microorganisms, whether bacteria, viruses, or fungi, on inanimate objects and surfaces.

Registrants of antimicrobial products must demonstrate that the product will not cause unreasonable adverse effects to human health or the environment. Data must be submitted or cited in support of registration, including detailed information on the chemical composition of the product, the chemical characteristics of the formulation, effectiveness data to support their claims against specific microorganisms, and toxicity data. Much of the labeling verbiage is prescriptive and based on the chemistry, safety, and efficacy data.

The EPA recognizes efficacy claims as either public health claims or non-public-health claims. Public health claims are for the control of microorganisms infectious to humans in or on any inanimate environment. Non-public-health claims are for the control and growth of algae; odor-causing bacteria; bacteria that cause spoilage, deterioration, or fouling of materials; and microorganisms infectious only to animals. This general category includes products used in cooling towers, paints, and treatments for textile and paper products. Standard efficacy methods are established to be used to generate data in support of registration.

Registration decisions are made by the EPA on a "risk vs. benefit" approach. Antimicrobials are biocides and often present risk to nontarget organisms, including humans. The EPA examines the safety of the product by assessing worker safety, food safety (when applicable), effects on nontarget organisms, and effects on surfaces by reviewing acute and chronic safety studies and exposure risk assessments of both active ingredients and the finished product. The EPA establishes procedures through product labeling to reduce the inherent risk associated with the use of these biocides.

To assess the efficacy of antimicrobials, the EPA requires manufacturers of these chemical agents to perform specific testing. See Appendix VI for AOAC protocol testing for disinfectant registration.

Although the EPA regulates efficacy data for sanitizers, disinfectants, and sporicides, pharmaceutical, biotech, and medical device manufacturers are not alleviated from FDA and EMA disinfectant validation requirements. Therefore, pharmaceutical, biotech, and medical device companies are required to show performance data against their site-specific isolates as part of their disinfectant validation process. CFR Title 40 registration information is located at http://www.epa.gov ***(21).***

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20.0 Appendix IV: Overview Of The EU Biocidal Regulations

The introduction of the Biocidal Products Regulation 98/8/EC (BPR) brought into enforcement the requirement to gain approval (via registration) to supply biocide products of all types to the EU market. This includes clean room disinfectants. The BPR replaces individual country registration systems for active ingredients.

The BPR takes into consideration new and existing active substances. New active substances can no longer be placed on the market in the EU until full approval is granted—this applies now.

In the case of existing active substances, the European Commission introduced a transitional period to allow suppliers to develop the necessary data for submission to, and evaluation by, the authorities in order to gain approval to supply.

During this transitional period, individual countries can continue with their national approval systems until active substances used in disinfectants are called in for evaluation under the BPR. At this time all national approvals schemes will be phased out. Those that successfully negotiated the evaluation process gained what is known as an Annex 1 to BPR listing, allowing them to be used in formulated products throughout the EU without the need for individual national approvals, as was previously the case.

A competent authority in any EU member country can approve a formulated product containing an active substance listed in Annex 1. Once a formulated product has been authorized in one member country, it will be possible for it to be mutually recognized and approved for sale in other member countries, although there may be some specific local requirements that must be met. An application must be made to each member country in which the formulated product will be sold, and fees will be payable for these processes. Achieving Annex 1 listing triggers the next phase of registration, which involves the systematic evaluation of all formulated products.

A major part of the dossier for each formulated product is the efficacy assessment. Although there is no ranking for the efficacy test methods, at the top of the commission wish list are the EN test methods. Over the past few years there has been an aggressive program to develop new EN test methods applicable for use to support biocide product testing. It is expected that as these become available, they will eventually replace current national standards. See Appendix VII for EN test method information.

The new BPR regulations are not industry specific, nor are they very specific to clinical health-care requirements covering hospital ward and theater situations. They have little to do with EU GMP or pharmaceutical production in clean room environments.

The BPR is a major task, which will take time to be brought into full effect. Further details about the BPR can be found at http://ec.europa.eu/environment/biocides/index.htm.

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21.0 Appendix V: EPA-Related Safety Labeling Information

Labeling requirements for antimicrobial products vary by country. For those antimicrobial products registered in the United States, the EPA provides prescriptive precautionary label verbiage ***(22)***. This verbiage is typically determined by the results of six acute toxicity studies performed with the product formulation. The acute oral, acute dermal, and acute inhalation studies evaluate systemic toxicity via the designated routes of exposure. The primary eye irritation and primary skin irritation studies measure irritation or corrosion, while the dermal sensitization study evaluates the potential for allergic contact dermatitis. With the exception of dermal sensitization, each acute study is assigned to a toxicity category based on the study results (See **Table 21.0-1** below). The results of these six acute toxicity studies must be known in order for the appropriate labeling language to be determined. **Table 21.0-2** provides the required precautionary language based on the assigned toxicity category.

Table 21.0-1 Toxicity Categories ***(22)***

Study	Category I	Category II	Category III	Category IV
Acute oral	Oral LD_{50} up to and including 50 mg/kg	>50 through 500 mg/kg	>500 through 5,000 mg/kg	>5,000 mg/kg
Acute dermal	Dermal LD_{50} up to and including 200 mg/kg	>200 through 2,000 mg/kg	>2,000 through 5,000 mg/kg	>5,000 mg/kg
Acute inhalation (4-hour exposure)	Inhalation LD_{50} up to and including 0.05 mg/liter	>0.05 through 0.5 mg/liter	>0.5 through 2 mg/liter	>2 mg/liter
Primary eye irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8–21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Primary skin irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or slight erythema)

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Table 21.0-2 Precautionary Statements by Route of Entry

Acute Oral Toxicity	
Toxicity category	Statements
I	Fatal if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet.
II	May be fatal if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet.
III	Harmful if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet.
IV	No statements are required. However, the registrant may choose to use category III labeling.
Acute Dermal Toxicity	
Toxicity category	Statements
I	Fatal if absorbed through skin. Do not get in eyes, on skin, or on clothing. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Wear [specify appropriate protective clothing]. Remove and wash contaminated clothing before reuse.
II	May be fatal if absorbed through skin. Do not get in eyes, on skin, or on clothing. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Wear [specify appropriate protective clothing]. Remove and wash contaminated clothing before reuse.
III	Harmful if absorbed through skin. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse. Wear [specify any appropriate protective clothing, if appropriate].
IV	No statements are required. However, the registrant may choose to use category III labeling.
Acute Inhalation Toxicity	
Toxicity category	Statements
I	Fatal if inhaled. Do not breathe (dust, vapor, or spray mist).* Wear [specify appropriate respiratory protection from Table 4, Chapter 10 of EPA Label Review Manual]. Remove and wash contaminated clothing before reuse.
II	May be fatal if inhaled. Do not breathe (dust, vapor or spray mist).* Wear [specify appropriate respiratory protection from Table 4, Chapter 10 of EPA Label Review Manual]. Remove and wash contaminated clothing before reuse.
III	Harmful if inhaled. Avoid breathing (dust, vapor or spray mist).* Remove and wash contaminated clothing before reuse.
IV	No statements are required. However, the registrant may choose to use category III labeling.
* Choose the word which appropriately describes the product during use.	

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Table 21.0-2 (Continued)

Primary Eye Irritation	
Toxicity category	Statements
I	Corrosive.* Causes irreversible eye damage. Do not get in eyes or on clothing. Wear [specify appropriate protective eyewear such as goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.
II	Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear [specify appropriate protective eyewear such as goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.
III	Causes moderate eye irritation. Avoid contact with eyes or clothing. Wear [specify protective eyewear, if appropriate]. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet.
IV	No statements are required. However, the registrant may choose to use category III labeling.
* The term "corrosive" is not required if corrosive effects were not observed during the study.	
Primary Skin Irritation	
Toxicity category	Statements
I	Corrosive. Causes skin burns. Do not get in eyes, on skin, or on clothing. Wear [specify appropriate protective clothing and gloves]. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.
II	Causes skin irritation. Do not get on skin or on clothing. Wear [specify appropriate protective clothing and gloves]. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.
III	Avoid contact with skin or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Wear [specify protective clothing and gloves, if appropriate].
IV	No statements are required. However, the registrant may choose to use category III labeling.
Dermal Sensitization	
Study result	Statement
Product is a sensitizer or is positive for sensitization	Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.
Product is not a sensitizer or is negative for sensitization	No labeling is required for this result.

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22.0 Appendix VI: AOAC Protocol Testing For Disinfectant Registration

Firms registering antimicrobial products are required by the U.S. EPA or other international authority to submit or cite, in support of registration, detailed information on the formula of the product, the chemical or physical characteristics of the formulation, effectiveness data to support claims against specific microorganisms, and safety or toxicity data. While this type of testing is required by the U.S. EPA for manufacturers of marketed antimicrobial products, it should not be considered a mandatory requirement for GMP operations. Furthermore, GMP operations do not utilize an AOAC protocol for their antimicrobial effectiveness; however, the U.S. EPA approval should be verified by the user during the selection process of an appropriate disinfection agent. See http://www.eoma.aoac.org for specific AOAC methods ***(23)***.

Performance claims require the generation of efficacy data under controlled testing conditions. Data generated in support of claims for microorganisms that are pathogenic to humans must be submitted to the EPA for review and approval. Data generated for microorganisms not considered pathogenic to humans under certain conditions do not always need to be submitted to the agency but must be on file and available to the EPA upon request.

Generation of data to support label claims and product registration may be conducted under the following approaches:

- Recognized consensus methods
- EPA-approved protocols
- Published peer-reviewed data (rarely used)

Test data are generated against specific microorganisms or surrogate organisms identified by the EPA as acceptable marker organisms. The EPA has recognized or established standard methodology and continues to work cooperatively with interested parties to develop improved consensus methods.

The general efficacy label claims (indications) recognized by the EPA are as follows:

- Sporicides (also termed "cold sterilants") are used on hard inanimate surfaces and objects to eliminate all forms of microbial life, including fungi, viruses, and all forms of bacteria and spores. Spores are considered the most difficult form of microorganism to destroy. Therefore, the EPA and other chemical registration organizations consider the term *sporicide* to be synonymous with cold sterilant
- Disinfectants are used on hard inanimate surfaces and objects to eliminate or irreversibly inactivate infectious bacteria but not necessarily their spores. The EPA treats the terms *germicide* and *bactericide* as synonymous with *disinfectant*. Disinfectant products are divided into two major categories:
  1. Hospital Use: Hospital-type disinfectants are the most critical to infection control and are used in health-care settings.
  2. General Use: General disinfectants are the major source of products used in households, swimming pools, and water purifiers.
- Fungicides are agents used to reduce, but not necessarily eliminate, microorganisms from the inanimate surfaces to levels considered safe as determined by public health codes or regulations. Sanitizers include food-contact and non-food-contact surfaces.
- Tuberculocides are agents that destroy or irreversibly inactivate tubercle bacilli in the inanimate environment.
- Virucides are agents that destroy or irreversibly inactivates viruses in the inanimate environment

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The U.S. EPA test requirements for registering label claims or indications are summarized below. Depending on the country and registration requirements, other tests and acceptance criteria may be required.

- **Sterilizer claim requirements:** AOAC Sporicidal Test [60 Carriers each on two surfaces (porcelain penicylinders and silk suture loops)] against spores of *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) [three samples representing three lots, one lot 60 days old, killing all 720 carriers]. One lot tested independently is required by the U.S. EPA.

- **Disinfectant (limited efficacy) requirements:** AOAC Use-Dilution Method or AOAC Germicidal Spray Products Test against *Salmonella choleraesuis* (ATCC 10708) or *Staphylococcus aureus* (ATCC 6538) [60 carriers testing three samples representing three different lots, one lot 60 days old killing 59 out of each set of 60 carriers].

- **Disinfectant (hospital or medical environment) requirements:** AOAC Use-Dilution Method or AOAC Germicidal Spray Products Test against *S. choleraesuis* (ATCC 10708), *S. aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) [60 carriers testing three samples representing three different lots, one lot 60 days old killing 59 out of each set of 60 carriers].

- **Fungicide requirements:** AOAC Fungicidal Test or versions of the AOAC Use-Dilution Method or Germicidal Spray Products Test modified with appropriate elements in the AOAC Fungicidal Test against *Trichophyton mentagrophytes* (ATCC 9533) [10 carriers testing two samples representing two different lots killing all fungal spores].

- **Virucide requirements:** Carrier methods as modifications of either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test against the particular virus with a recoverable virus titer of at least 10^4 from the test surface [two different lots of four determinations per each dilution showing inactivation of virus at all dilutions when no cytotoxicity is observed and at least a three-log reduction in viral titer for both samples when cytotoxicity is present].

- **Tuberculocide requirements:** Tuberculocidal Activity Method or the AOAC Germicidal Spray Products Test modified to meet the requirements of the Tuberculocidal Activity Method against *Mycobacterium tuberculosis* var. bovis (BCG) [two samples representing two different lots killing the entire test microorganism on all carriers and no growth in any of the inoculated tubes of two additional media]. Alternative method—Quantitative Tuberculocidal Activity Test (four log kill required). Products with tuberculocidal claims that are formulated with quaternary ammonium compounds may be evaluated for tuberculocidal efficacy using any one of the test methods listed above. However, validation data are required for any test method chosen. Validation data must be developed by testing one additional sample of the product by a laboratory of the registrant's choice (other than the laboratory that developed the original efficacy data) using the *same* optional test procedure and test conditions as the original laboratory.

- **Non-food-contact sanitizer requirements:** Guideline 91-30 Method No. 8 against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048) on representative surfaces depending on the proposed uses, including but not limited to glass, metal, unglazed or glazed ceramic tile, or vitreous china showing a bacterial reduction of at least 99.9% over the parallel control count within five minutes.

- **Food-contact sanitizer requirements:** For Halide Chemical Products: AOAC Available Chlorine Germicidal Equivalent Concentration Method against *Salmonella typhi* (ATCC 6539) [One test on each of three samples representing three lots, one that is at least 60 days old showing product

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concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine]. For other chemical products, such as quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations: AOAC Germicidal and Detergent Sanitizers Method against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) [one sample from each of three different lots, one of which is at least 60 days old, demonstrating 99.999% reduction in the number of each test organism within 30 seconds].

- **Additional organism requirements:** (applies to specific microorganisms other than those named by the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method and not including viruses): AOAC Use-Dilution Method or AOAC Germicidal Spray Products Test against the specific organism [10 carriers testing two samples representing two different lots killing all carriers].

- Other, more specific claims include residual self-sanitizing activity of dried chemical residue, towelettes, air sanitizers, laundry additives, carpet sanitizers, drinking water, swimming pool water, and preservatives.

- Other circumstance and variables to consider are confirmatory efficacy testing, organic soil load (one-step application) claim, and hard water claim (400 ppm).

Alternative methods necessary for special application methods or unique organism testing where standard methods are not appropriate require EPA review and approval of protocols prior to generation and submission of the data.

Several end users in the pharmaceutical, biotech, and medical device industries have modified the log reduction requirements for AOAC methods. This has been done to reflect normally lower bioburden levels in controlled manufacturing environments. End users have utilized these methods against their environmental isolates. Additionally, end users will typically look for a log reduction (at a 10^4 inoculate level) of three logs for vegetative bacteria on hard surfaces and two logs for spore-forming bacteria on hard, nonporous surfaces. The log reduction may vary depending on organisms and conditions tested. Worst-case environmental monitoring data should be the guide for deciding the required effectiveness of the chemical agents.

The AOAC methods are normally used for U.S. EPA registration purposes only. Typically, pharmaceutical and biotechnology operations utilize a carrier surface study or a suspension study or both.

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23.0 Appendix VII: EN Tests For Disinfection Efficacy

The acceptance criteria for registration efficacy depend on which EU standard is being met. This is the basis for the efficacy claims on the product.

Test methods follow a three-phase evaluation:

- **Phase 1:** Suspension test to determine basic bactericidal, fungicidal, or sporicidal activity. The test protocol gives no specific contact time and does not require interfering substances to be added.
- **Phase 2:** Tests for defined applications:
  - Step 1: Suspension test to determine bactericidal, fungicidal, virucidal, or sporicidal activity. The test protocol specifies a contact time (see **Table 23.0-1**). Bovine serum albumin (BSA) is added as the interfering substance at 0.3% to simulate clean conditions and 3.0% to simulate dirty conditions.
  - Step 2: Tests attempting to simulate practical conditions, for example, surface tests.
- **Phase 3:** Field trial tests (in-situ field studies).

The following list describes the typical EN tests currently used by vendor companies evaluating disinfectants for registration purposes:

- **EN 1276:2009 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 (phase 2, step 1).
- **EN 1650:1998 Chemical disinfectants and antiseptics.**
  Quantitative suspension test for evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas (phase 2, step 1).
- **EN 13704:2002 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).
- **EN 13697:2001 Chemical disinfectants and antiseptics.**
  Quantitative nonporous surface test for the evaluation of bactericidal or fungicidal activity of chemical disinfectants used in food, industrial, domestic, and institutional areas (phase 2, step 2).

A brief overview of the criteria for each EN test is outlined in the table that follows:

Table 23.0-1 Summary of EN Test Criteria for Registration for Established Claims

Organism Type	Test Method	Test Type	Contact Time (minutes)	Log Reduction Pass Criteria
Vegetative bacteria	EN 1276:1997	Suspension	5	5
Vegetative bacteria	EN 13697:2001	Surface	5	4
Vegetative fungi	EN 1650:1998	Suspension	15	4
Vegetative fungi	EN 13697:2001	Surface	15	3
Bacterial spores	EN 13704:2002	Suspension	60	3

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Users' Protection: Safety Data Sheets (SDS)

Wearing gloves is a critical safety procedure when handling and using sanitizers, disinfectants, and sporicides. Typically rubber or nitrile gloves are recommended for the hands, as well as chemically compatible gowning materials, when diluting or using disinfectants. Operators that are applying the disinfectants to ceilings should wear hoods or smocks and goggles with an ocular cavity fit so that the disinfectant or sporicide does not get into the ocular cavity. Generally, a rubber apron should be used when diluting a disinfectant product. Finally, sporicides and alcohol products should be used in well-ventilated areas, or a breathing apparatus should be used, to prevent overexposure to any volatile actives.

In the United States, the Safety Data Sheets (SDS) and Environmental Protection Agency (EPA) registered labels, as well as any additional toxicological studies from the vendor, are the primary source for additional safety information. See Appendix V (**Section 21.0**) for the EPA's safety labeling requirements.

Companies that are considering fogging a disinfectant or a sporicide should be sure to have adequate ventilation or ancillary breathing apparatus while applying in the clean room before operators return to their workstations. The levels of the active in the clean room should be below permissible exposure limits before operators return to their workstations.

The individual firm is responsible for complying with environmental, health, and safety regulations for disposal, storage, and personnel protection within the laws of their respective countries, states, cities, counties, and townships. Regulations may vary between authoritative bodies within a region, and appropriate due diligence should be exerted to meet all federal, state, and local requirements.

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24.0 Appendix VIII: Large-Scale Gassing Or Fogging Of Clean Rooms

Vaporization or fogging of disinfectants for large-scale decontamination is being used or considered by many firms in the pharmaceutical industry. A significant amount of published data show the efficacy of this type of disinfectant system against vegetative bacteria, fungi, and bacterial spores. Chemical agents commonly used for this technology include the following:

- Paraformaldehyde
- Peracetic acid/hydrogen peroxide
- Phenols
- Bleach
- Quaternary ammonia
- Vapor phased hydrogen peroxide (VHPH)
- Gaseous chlorine dioxide
- Ozone

While gassing and fogging systems can provide excellent destruction of microorganisms present in areas that are contaminated, such systems do not clean surfaces. Therefore, cleaning is considered to be a mandatory and routine step in addition to gassing or fogging.

• Paraformaldehyde

Paraformaldehyde gas has been used as a large-scale clean room decontamination methodology for many years. Paraformaldehyde is a white, crystalline powder with the odor of formaldehyde that has been used for more than thirty years to decontaminate laboratory facilities and to disinfect sickrooms, clothing, linen, and sickroom utensils. The process involves heating of the paraformaldehyde to release formaldehyde gas, which is the actual decontaminant. (See http://www.epa.gov/pesticides/factsheets/chemicals/paraformaldehyde_factsheet.htm for more information)

The gas is created inside the room, normally by use of a pan and a heating element. The gas is then spread throughout the room by portable fans that are set up prior to the creation of the gas. Paraformaldehyde gas microbial destruction claims through the U.S. EPA are limited to what would be termed a high-level disinfectant, sanitizer, or fungicide. However, as with many other agents, sporicidal reduction has been obtained in clean rooms by many firms worldwide.

While effective, Paraformaldehyde is an older methodology that has been replaced by most GMP firms with current more modern chemistries and systems. Characteristically, Paraformaldehyde decontamination leaves concerning residuals on all surfaces (as defined by FDA) and requires the utmost safety concerns for its implementation realting to human health. For these reason this methodology is declining in use in the marketplace.

• Wet Droplet Fogging

Wet droplet fogging has been employed in a variety of industries for many years and is a proven technology. This method involves the generation or vaporization of small liquid droplets from a chemical agent that is placed into an air steam by a generator that is linked to a fogging device. Droplets usually range in size from 10.0 to 25.0 microns. The chemical agent is slowly dripped into the stream of air in the fogging device. Various fogging devices are placed strategically throughout the room, and portable fans are used to circulate the droplets throughout the room. Chemical agents such as peracetic acid and hydrogen peroxide, sodium hypochlorides, phenols, and quaternary ammoniums are normally used and chosen based on the type of antimicrobial action that is required. Efficacy is based on the fogging time and the chemical agent used. The method is versatile, as end users can decide on what agent and what fogging time should be employed. Antimicrobial

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claims can range from sanitization to sporicidal, depending on the chemical agent used, and have been obtained by both registering companies and GMP forms worldwide.

The goal of the fogging method is to lightly coat all surfaces with a thin but constant layer of chemical agent for an extended period. This type of decontamination is considered a wet or aerosol process rather than a gaseous process. Wetting of surfaces reduces decontamination times, dry times, and release times. Normal fogging times range from fifteen minutes to one hour, and release times are normally very short, also ranging from fifteen minutes to one hour, or when all surfaces are dry. Once inhalation concerns are acceptable, end users could enter areas and dry any surfaces that are not completely dried. But this should be done with the utmost concern for contamination of such surfaces.

Depending on the chemical agent used, corrosion and residual can be controlled. However, with overuse and without manual cleaning procedures, residues can build up over time and corrosion can occur.

- **Vaporized Phased Hydrogen Peroxide (VPHP)**

The vapor of hydrogen peroxide is noncarcinogenic and breaks down to water and oxygen, therefore eliminating corrosive residues that are inherent with other traditional methods of large-scale decontamination such as paraformaldehyde gassing.

VPHP systems generate a low level of vaporized 35% hydrogen peroxide (250–1200 ppm) into manufacturing areas through portable or fixed distribution systems. In the vapor phase, disinfection may require longer times compared to the traditional method, depending on the number of VPHP generators, the size of the area to be treated, and the required contact and clearance times. Vapor is continuous is emitted through dispensing heads in an attempt to distribute the vapor and provide sufficient vapor in all areas to destroy microorganisms.

The distribution of vapor within the room can be verified with hydrogen peroxide sensors or chemical indicators.

The VPHP process of decontamination, while effective, is still considered a disinfection step rather than a sterilization process. Implementing this type of disinfection system should be done with appropriate safety precautions. Leaks to the external environment and clearance time should be tested and assessed properly to assure safety. The VPHP system may require air-handling systems to be shut off and doorways and return vents sealed. Release times for human intervention may run from two to four hours, depending on the size of the area and the length of the gassing process.

The effectiveness of VPHP systems has been well documented in isolator operations for many years. However, isolator operations are smaller, are sealed, and have evacuation systems that can remove and scrub the volatile gases. The use of VPHP in a large-scale, open manufacturing environment has also been very successful and unlike UV is effective in the presence of shadowing. However, conclusive studies proving validation of the system in this venue are specific to the operation and the setup where it will be used. Each area should be assessed for effectiveness in its own validation study. Although large-scale VPHP has proved effective, it is not a cleaning step. Residues, particulates, foreign matter, and pyrogens are not cleaned or removed from the environment in the VPHP process. Therefore, routine cleaning of surfaces and equipment is considered mandatory even when VPHP is used. VPHP then serves as an additional bioburden reduction step either before or after cleaning. A large-scale VPHP system used before cleaning would be considered a sanitization step and should be followed by a mechanical-action (wipe and mop) application to the surface. A large-scale VPHP system that is implemented after a mechanical cleaning step (wipe and mop) would be considered a final disinfection step.

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- **Gaseous Chlorine Dioxide**

  Gaseous chlorine dioxide is another available alternative for gassing. Of the methods already discussed, it is most similar to paraformaldehyde or VPHP rather than wet droplet fogging, as it is a gas product. Application to the area to be decontaminated and to the surface is accomplished much the same way as the VPHP gas processes. Basic differences in the products relate to corrosion, residual, safety, and setup.

  To disinfect a room, gaseous chlorine dioxide is precipitated into the area to be decontaminated via a generator and dispersion heads. These systems have been employed in many industry settings and are now being considered as a possible alternative for GMP operations.

- **Ozone Gas**

  The use of Ozone Gas ia another alternative for gassing small or large scale operations. Ozone is made by adding high voltage to oxygen. The system uses a high concentration of ozone gas that bintegrates a gas generator to emit the Ozone to the area to be decontaminated. Normally the design specifications for the system included an ozone gas concentration of 200 ppm or more, relative humidity of 80% or more, and a treatment time that is determined by the size of the area, the inherent bioburden and the obstructions contained within the area. These systems have been employed in many industry settings and are now being considered as a possible alternative for GMP operations.

Whenever chemical agents are used for large-scale gassing or fogging of clean rooms, safety concerns must be addressed. All of the agents discussed can result in injury or death of personnel if proper precautions are not taken to ensure the containment of the chemical agent to the intended areas. For many of the agents discussed, residues that are left behind on product-contact surfaces are also a significant concern and must be evaluated.

Although these methods of decontamination are effective, they should not be used to replace a routine program for cleaning and disinfecting the clean room areas. If they are used as the standard practice, they should be validated to demonstrate their ability to achieve an appropriate level of bioburden reduction. This should be performed taking into consideration the material of construction present in the clean room areas.

It is also important to consider the source of these organisms, for gassing will only remove what is present and may leave behind moisture, allowing for further proliferation if the causative agents have not been removed from the area.

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