

Chapter 10

Decontamination and Disinfection

This chapter describes basic strategies for decontaminating surfaces, items, and areas in laboratories to eliminate the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment. Factors necessary for environmentally mediated infection transmission are reviewed as well as methods for sterilization and disinfection and the levels of antimicrobial activity associated with liquid chemical germicides. General approaches are emphasized, not detailed protocols and methods. The principles of sterilization and disinfection are stated and compared.

ENVIRONMENTALLY MEDIATED INFECTION TRANSMISSION

Environmentally associated laboratory infections can be transmitted directly or indirectly from environmental sources (e.g., air, contaminated fomites and laboratory instruments, and aerosols) to laboratory staff. Fortunately, LAI are rare events¹ because there are a number of requirements necessary for environmental transmission to occur.² Commonly referred to as the “chain of infection” they include: presence of a pathogen of sufficient virulence, relatively high concentration of the pathogen (i.e., infectious dose), and a mechanism of transmission of the pathogen from environment to the host, a correct portal of entry to a susceptible host.

To accomplish successful transmission from an environmental source, all of these requirements for the “chain of infection” must be present. The absence of any one element will prevent transmission. Additionally, the pathogen in question must overcome environmental stresses to retain viability, virulence, and the capability to initiate infection in the host. In the laboratory setting, high concentrations of pathogens can be common. Reduction of environmental microbial contamination by conventional cleaning procedures is often enough to prevent environmentally mediated transmission. However, it is the general practice in laboratories to use sterilization methods to remove the potential for infection transmission.

PRINCIPLES OF STERILIZATION AND DISINFECTION

In order to implement a laboratory biosafety program it is important to understand the principles of decontamination, cleaning, sterilization, and disinfection. We review here the definitions of sterilization, disinfection, antisepsis, decontamination, and sanitization to avoid misuse and confusion. The definitions and implied capabilities of each inactivation procedure are discussed with an emphasis on achievement and, in some cases, monitoring of each state.

Sterilization

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either

sterile or it is not). A sterilization *procedure* is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10^{-6}). This is referred to as the “sterility assurance level.”^{3,4}

Disinfection

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an “overkill” and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are:

- the nature and number of contaminating microorganisms (especially the presence of bacterial spores);
- the amount of organic matter present (e.g., soil, feces, and blood);
- the type and condition of instruments, devices, and materials to be disinfected;
- the temperature.

Disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other. By definition, chemical disinfection and in particular, high-level disinfection differs from chemical sterilization by its lack of sporicidal power. This is an over simplification of the actual situation because a few chemical germicides used as disinfectants do, in fact, kill large numbers of spores even though high concentrations and several hours of exposure may be required. Non-sporicidal disinfectants may differ in their capacity to accomplish disinfection or decontamination. Some germicides rapidly kill only the ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses, whereas others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. *bovis*, non-lipid viruses, and most forms of fungi.

Spaulding Classification

In 1972, Dr. Earl Spaulding⁵ proposed a system for classifying liquid chemical germicides and inanimate surfaces that has been used subsequently by CDC, FDA, and opinion leaders in the United States. This system, as it applies to device surfaces, is divided into three general

categories based on the theoretical risk of infection if the surfaces are contaminated at time of use. From the laboratory perspective, these categories are:

- critical - instruments or devices that are exposed to normally sterile areas of the body require sterilization;
- semi-critical - instruments or devices that touch mucous membranes may be either sterilized or disinfected;
- non-critical - instruments or devices that touch skin or come into contact with persons only indirectly can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant, or simply cleaned with soap and water.

In 1991, microbiologists at CDC proposed an additional category, environmental surfaces (e.g., floors, walls, and other “housekeeping surfaces”) that do not make direct contact with a person’s skin.⁶ Spaulding also classified chemical germicides by activity level:

High-Level Disinfection

This procedure kills vegetative microorganisms and inactivates viruses, but not necessarily high numbers of bacterial spores. Such disinfectants are capable of sterilization when the contact time is relatively long (e.g., 6 to 10 hours). As high-level disinfectants, they are used for relatively short periods of time (e.g., 10 to 30 minutes). These chemical germicides are potent sporicides and, in the United States, are classified by the FDA as sterilant/disinfectants. They are formulated for use on medical devices, but not on environmental surfaces such as laboratory benches or floors.⁷

Intermediate-Level Disinfection

This procedure kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. Chemical germicides used in this procedure often correspond to Environmental Protection Agency (EPA)-approved “hospital disinfectants” that are also “tuberculocidal.” They are used commonly in laboratories for disinfection of laboratory benches and as part of detergent germicides used for housekeeping purposes.

Low-Level Disinfection

This procedure kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses. The EPA approves chemical germicides used in this procedure in the US as “hospital disinfectants” or “sanitizers.”

DECONTAMINATION IN THE MICROBIOLOGY LABORATORY

Decontamination in the microbiology laboratory must be carried out with great care. In this arena, decontamination may entail disinfection of work surfaces, decontamination of equipment so it is safe to handle, or may require sterilization. Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory. Reduction of cross-contamination in the laboratory is an added benefit.

Decontamination and Cleaning

Decontamination renders an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated. The decontamination process may be ordinary soap and water cleaning of an instrument, device, or area. In laboratory settings, decontamination of items, spent laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item.

The presence of any organic matter necessitates longer contact time with a decontamination method if the item or area is not pre-cleaned. For example, a steam cycle used to sterilize pre-cleaned items is 20 minutes at 121°C. When steam sterilization is used to decontaminate items that have a high bioburden and there is no pre-cleaning (i.e., infectious waste) the cycle is longer. Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with the decontaminating agents.

Chemical germicides used for decontamination range in activity from high-level disinfectants (i.e., high concentrations of sodium hypochlorite [chlorine bleach]), which might be used to decontaminate spills of cultured or concentrated infectious agents in research or clinical laboratories, to low-level disinfectants or sanitizers for general housekeeping purposes or spot decontamination of environmental surfaces in healthcare settings. Resistance of selected organisms to decontamination is presented in descending order in Table 1. If dangerous and highly infectious agents are present in a laboratory, the methods for decontamination of spills, laboratory equipment, BSC, or infectious waste are very significant and may include prolonged autoclave cycles, incineration or gaseous treatment of surfaces (see below).

TABLE 1
DESCENDING ORDER OF RESISTANCE TO GERMICIDAL CHEMICALS

<p><u>BACTERIAL SPORES</u> <i>Bacillus subtilis, Clostridium sporogenes</i> ↓</p>
<p><u>MYCOBACTERIA</u> <i>Mycobacterium tuberculosis</i> var. <i>bovis</i>, Nontuberculous mycobacteria ↓</p>
<p><u>NONLIPID OR SMALL VIRUSES</u> Poliovirus, Coxsackievirus, Rhinovirus ↓</p>
<p><u>FUNGI</u> <i>Trichophyton</i> spp., <i>Cryptococcus</i> spp., <i>Candida</i> spp. ↓</p>
<p><u>VEGETATIVE BACTERIA</u> <i>Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella choleraesuis, Enterococci</i> ↓</p>
<p><u>LIPID OR MEDIUM-SIZE VIRUSES</u> Herpes simplex virus, CMV, Respiratory syncytial virus, HBV, HCV, HIV, Hantavirus, Ebola virus</p>

Note: There are exceptions to this list. *Pseudomonas* spp are sensitive to high-level disinfectants, but if they grow in water and form biofilms on surfaces, the protected cells can approach the resistance of bacterial spores to the same disinfectant. The same is true for the resistance to glutaraldehyde by some nontuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Cheatomium globosum*, and the pink pigmented *Methylobacteria*. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this chapter.

Decontamination of Large Spaces

Space decontamination is a specialized activity and should be performed by specialists with proper training and protective equipment.⁸ Decontamination requirements for BSL-3 and BSL-4

laboratory space have an impact on the design of these facilities. The interior surfaces of BSL-3 laboratories must be water resistant in order for them to be easily cleaned and decontaminated. Penetrations in these surfaces should be sealed or capable of being sealed for decontamination purposes. Thus, in the BSL-3 laboratory, surface decontamination, not fumigation, is the primary means of decontaminating space. Care should be taken that penetrations in the walls, floors and ceilings are kept to a minimum and are "sight sealed." Verification of the seals is usually not required for most BSL-3 laboratories. The BSL-4 laboratory design requires interior surfaces that are water resistant AND sealed to facilitate fumigation. These seals must be tested and verified to ensure containment in order to permit both liquid disinfection and fumigation. Periodic fumigation is required in the BSL-4 suit laboratory to allow routine maintenance and certification of equipment. Procedures for decontamination of large spaces such as incubators or rooms are varied and influenced significantly by the type of etiologic agent involved, the characteristics of the structure containing the space, and the materials present in the space. The primary methods for space decontamination are:

Formaldehyde—Paraformaldehyde

Formaldehyde gas at a concentration of 0.3 grams/cubic foot for four hours is often used for space decontamination. Gaseous formaldehyde can be generated by heating flake paraformaldehyde (0.3 grams per cubic foot) in a frying pan, thereby converting it to formaldehyde gas. The humidity must be controlled and the system works optimally at 80% relative humidity. This method is effective in killing microorganisms but toxicity issues are present.^{1,9} Additional information on environmental and safety issues related to paraformaldehyde is available from the EPA at: www.epa.gov/pesticides.

Hydrogen Peroxide Vapor

Hydrogen peroxide can be vaporized and used for the decontamination of glove boxes as well as small room areas. Vapor phase hydrogen peroxide has been shown to be an effective sporicide at concentrations ranging from 0.5 mg/L to <10 mg/L. The optimal concentration of this agent is about 2.4 mg/L with a contact time of at least one hour. This system can be used to decontaminate glove boxes, walk in incubators and small rooms. An advantage of this system is that the end products (i.e., water) are not toxic. Low relative humidity can be used.¹⁰⁻¹⁴

Chlorine Dioxide Gas

Chlorine dioxide gas sterilization can be used for decontamination of laboratory rooms, equipment, glove boxes, and incubators. The concentration of gas at the site of decontamination should be approximately 10 mg/L with contact time of one to two hours.

Chlorine dioxide possesses the bactericidal, virucidal and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia

to form chlorinated organic products (chloramines). The gas cannot be compressed and stored in high-pressure cylinders, but is generated upon demand using a column-based solid phase generation system. Gas is diluted to the use concentration, usually between 10 and 30 mg/L. Within reasonable limits, a chlorine dioxide gas generation system is unaffected by the size or location of the ultimate destination for the gas. Relative humidity does need to be controlled and high humidities are optimal. Although most often used in closed sterilizers, the destination enclosure for the chlorine dioxide gas does not, in fact, need to be such a chamber. Because chlorine dioxide gas exits the generator at a modest positive pressure and flow rate, the enclosure also need not be evacuated and could be a sterility-testing isolator, a glove box or sealed BSC, or even a small room that could be sealed to prevent gas egress.¹⁵ Chlorine dioxide gas is rapidly broken down by light; care must be taken to eliminate light sources in spaces to be decontaminated.

Decontamination of Surfaces

Liquid chemical germicides formulated as disinfectants may be used for decontamination of large areas. The usual procedure is to flood the area with a disinfectant for periods up to several hours. This approach is messy and with some of the disinfectants used represents a toxic hazard to laboratory staff. For example, most of the “high-level” disinfectants on the United States market are formulated to use on instruments and medical devices and not on environmental surfaces. Intermediate and low-level disinfectants are formulated to use on fomites and environmental surfaces but lack the potency of a high-level disinfectant. For the most part intermediate and low level disinfectants can be safely used and, as with all disinfectants, the manufacturer’s instructions should be closely followed.⁷ Disinfectants that have been used for decontamination include sodium hypochlorite solutions at concentrations of 500 to 6000 parts per million (ppm), oxidative disinfectants such as hydrogen peroxide and peracetic acid, phenols, and iodophors.

Concentrations and exposure times vary depending on the formulation and the manufacturer’s instructions for use.^{6,16} See Table 2 for a list of chemical germicides and their activity levels. A spill control plan must be available in the laboratory. This plan should include the rationale for selection of the disinfecting agent, the approach to its application, contact time and other parameters. Agents requiring BSL-3 and BSL-4 containment pose a high risk to workers and possibly to the environment and should be managed by well-informed professional staff trained and equipped to work with concentrated material.

TABLE 2
ACTIVITY LEVELS OF SELECTED LIQUID GERMICIDES ^a

PROCEDURE/PRODUCT	AQUEOUS CONCENTRATION	ACTIVITY LEVEL
STERILIZATION		
glutaraldehyde	variable	
hydrogen peroxide	6-30%	
formaldehyde	6-8% ^b	
chlorine dioxide	variable	
peracetic acid	variable	
DISINFECTION		
glutaraldehyde	variable	High to intermediate
<i>ortho</i> -phthalaldehyde	0.5%	High
hydrogen peroxide	3-6%	High to intermediate
formaldehyde	1-8%	High to low
chlorine dioxide	variable	High
peracetic acid	variable	High
chlorine compounds ^c	500 to 5000 mg/L free/ available chlorine	Intermediate
alcohols(ethyl,isopropyl) ^d	70%	Intermediate
phenolic compounds	0.5 to 3%	Intermediate to low
iodophor compounds ^e	30-50 mg/L free iodine up to 10,000 mg/L available iodine	Intermediate to low
quaternary ammonium compounds	0.1 - 0.2%	Low

- a. This list of chemical germicides centers on generic formulations. A large number of commercial products based on these generic components can be considered for use. Users should ensure that commercial formulations are registered with EPA or by the FDA.
- b. Because of the ongoing controversy of the role of formaldehyde as a potential occupational carcinogen, the use of formaldehyde is limited to certain specific circumstances under carefully controlled conditions, e.g., for the disinfection of certain hemodialysis equipment. There are no FDA cleared liquid chemical sterilant/disinfectants that contain formaldehyde.
- c. Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite). Although the indicated concentrations are rapid acting and broad-spectrum (tuberculocidal, bactericidal, fungicidal, and virucidal), no proprietary hypochlorite formulations are formally registered with EPA or cleared by FDA. Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and

1000 mg/L chlorine are appropriate for the vast majority of uses requiring an intermediate level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory).

- d. The effectiveness of alcohols as intermediate level germicides is limited because they evaporate rapidly, resulting in short contact times, and also lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal and fungicidal, but may vary in spectrum of virucidal activity (see text). Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time (e.g., 10 minutes).
- e. Only those iodophors registered with EPA as hard-surface disinfectants should be used, closely following the manufacturer's instructions regarding proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

SPECIAL INFECTIOUS AGENT ISSUES

Transmissible Spongiform Encephalopathy Agents (Prions)

The major exception to the rule in the previous discussion of microbial inactivation and decontamination is the causative agent of CJD or other prion agents responsible for transmissible spongiform encephalopathies of the central nervous system in humans or animals. Studies show that prions are resistant to conventional uses of heat and/or chemical germicides for the sterilization of instruments and devices (See Section 9).

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