Appendix B—Decontamination and Disinfection of Laboratory Surfaces and Items

Purpose and Scope

Appendix B provides basic guidance for the decontamination or disinfection of environmental surfaces and items in the laboratory with antimicrobial substances and other practices to mitigate the possibility of transmission of pathogens to laboratory workers, the public, and the environment. The selection of an appropriate antimicrobial product and adherence to the product label instructions are critical to ensuring the product's performance against the target microorganism. Regulatory oversight, terminology, factors necessary for environmentally-mediated transmission of infection (e.g., aerosol generation, contact, indirect contact), methods for sterilization and disinfection, and the levels of antimicrobial activity associated with liquid chemical disinfectants are reviewed in this appendix. One must remember that aerosol-generating procedures should be conducted in containment. Accidents involving infectious aerosols have been a source of contamination within the laboratory setting and may impact the method chosen for decontamination. General approaches are emphasized instead of detailed protocols and methods. It is important to follow the manufacturer's instructions for use when performing decontamination practices in the laboratory.

Antimicrobial Products—U.S. Regulations

Antimicrobial pesticides (e.g., disinfectants) are classified as pesticides and are regulated by both the United States Environmental Protection Agency under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)[1,2] and the United States Food and Drug Administration, Center for Devices and Radiologic Health by the Food Quality Protection Act (FQPA).[3] The laboratory is responsible for selecting an appropriate EPA-registered product and using it according to the manufacturer's instructions on the product label. The more commonly used public health antimicrobial products are described in the Glossary (e.g., sporicides, disinfectants, and sanitizers). The lists of selected EPA-registered disinfectants are available at https://www.epa.gov/oppad001/chemregindex.htm.

The FDA has defined three types of liquid chemical germicides for processing medical devices, and these germicides are regulated as auxiliary devices (FDA 1977 Policy Manual): (1) sterilant/high-level disinfectant; (2) intermediate-level disinfectant; and (3) low-level disinfectant. See Glossary.

Disinfectants used in the laboratory include those recommended by equipment manufacturers and a broad-spectrum product, typically an intermediate-level disinfectant (i.e., a product with a mycobacteriology claim). Safe use of chemicals within the laboratory falls under the OSHA Laboratory Standard.[4]

Environmentally-Mediated Transmission of Infection

Laboratory-associated infections (LAIs) can be transmitted directly or indirectly from contaminated environmental sources within the laboratory (e.g., air, fomites and laboratory instruments, aerosols, and splashes) to laboratory staff. Fortunately, LAIs are relatively rare events because there are several requirements necessary for environmental transmission to occur;[5,6] this is commonly referred to as the chain of infection.[7,8] The requirements needed for environmental transmission include the presence of a pathogen of sufficient virulence, sufficient dose of a pathogen to cause infection (i.e., infectious dose), a mechanism of transmission of the pathogen from the environment to the host, the correct portal of entry to a susceptible host, and the immune status of the host.

To accomplish successful transmission from an environmental source, all the requirements for the chain of infection must be present. The absence of any one element will reduce and/or prevent the potential for transmission. Additionally, the pathogen in question must overcome environmental stresses to retain viability (e.g., ability to form biofilms in low, nutrient-moist environments or distribution systems, ability to survive dehydration), virulence, and the capability to initiate infection in the host. In the laboratory setting, high concentrations of pathogens are commonplace, and contamination of environmental surfaces (e.g., benchtops, equipment, personal protective equipment) and hands of the laboratorian may occur. Aerosol generation procedures and those that generate splashes may also contaminate surfaces, personnel, and potentially expose workers (e.g., inhalation, contact with mucous membranes) to pathogens. Reduction of environmental microbial contamination by both containment (e.g., performing aerosol-generating procedures in a biological safety cabinet or glove box) and conventional cleaning procedures is often enough to reduce, but not eliminate, the risk of environmentally-mediated transmission. It is the general practice in laboratories to use both cleaning and surface disinfection or sterilization procedures to mitigate the potential for transmission of infection. In addition, proper hand hygiene and appropriate personal protective equipment (e.g., gloves, lab coat/smock, safety glasses, goggles, respirators) use are also important factors in preventing transmission to laboratory personnel.

Principles of Cleaning, Disinfection, and Sterilization

To implement a laboratory biosafety program, it is important to understand the principles of cleaning and disinfection or sterilization. The terms are often misused and misunderstood. The definitions and capabilities of each inactivation procedure are discussed with an emphasis on achievement and, in some cases, monitoring of each state.

Cleaning Cleaning is the removal of gross contamination from a surface to the extent necessary for further processing for intended use. In these cases, cleaning

can be used to remove microorganisms and other associated contaminants (e.g., blood, tissues, culture media) from a surface by physical means but may not provide any antimicrobial activity. Cleaning is often an essential pre-requisite to disinfection or sterilization processes to ensure the optimal activity of the antimicrobial effects of disinfectants or sterilization processes. Biofilms may be present in the laboratory (e.g., sinks, plumbing fixtures, fluid-filled lines of laboratory equipment, water containing reservoirs, incubator humidification systems) and are often difficult to treat/disinfect. Most biofilms require physical cleaning (e.g., scrubbing) and the use of compatible oxidative disinfectants (e.g., chlorine dioxide, peroxyacetic acid, ozone). In some situations, replacing tubing and distribution lines may be necessary.

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) present on inanimate objects. Disinfection does not ensure a kill level and lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled by several factors, each one of which may have a pronounced effect on the end results. Factors affecting disinfection include the following:

1. Nature and number of contaminating microorganisms (especially the presence of bacterial spores);
2. Amount of organic matter present (e.g., soil, feces, blood);
3. Type and condition of surfaces, instruments, devices, and materials to be disinfected;
4. Temperature; and
5. Contact (exposure) time.

By definition, chemical disinfection, especially high-level disinfection, differs from chemical sterilization by the lack of sporicidal power. This is an over-simplification of reality because a few chemical disinfectants do 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 disinfectants rapidly kill only the ordinary vegetative forms of bacteria, such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses; others are effective against such relatively resistant organisms as *Mycobacterium bovis* or *Mycobacterium terrae*, non-enveloped viruses, and most forms of fungi.[9]

In general, most laboratories use a disinfectant that has a broad range of activity; thus, most labs should select a product with a tuberculocidal/mycobactericidal claim for routine purposes. Many of these products will also have claims that meet the OSHA Bloodborne Pathogens Standard.[10,11]

Sterilization Any item, device, or solution is sterile when it is completely free of all forms of living microorganisms, including spores and viruses. This definition is categorical and absolute; an item is either sterile or it is not. Sterilization can be accomplished by dry or moist heat, gases and vapors (e.g., chlorine dioxide, ethylene oxide, formaldehyde, hydrogen peroxide, methyl bromide, nitrogen dioxide, ozone, propylene oxide), plasma sterilization technology, and radiation (e.g., gamma, e-beam in industry).

From an operational standpoint, a sterilization procedure cannot be categorically defined because the likelihood that an individual microorganism survives is never zero. 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. This is referred to as a sterility assurance level (SAL) of 10^{-6}.[12–14] Laboratories use sterilization techniques for producing media, sterilizing glassware, and other items, and for decontaminating waste.

Decontamination

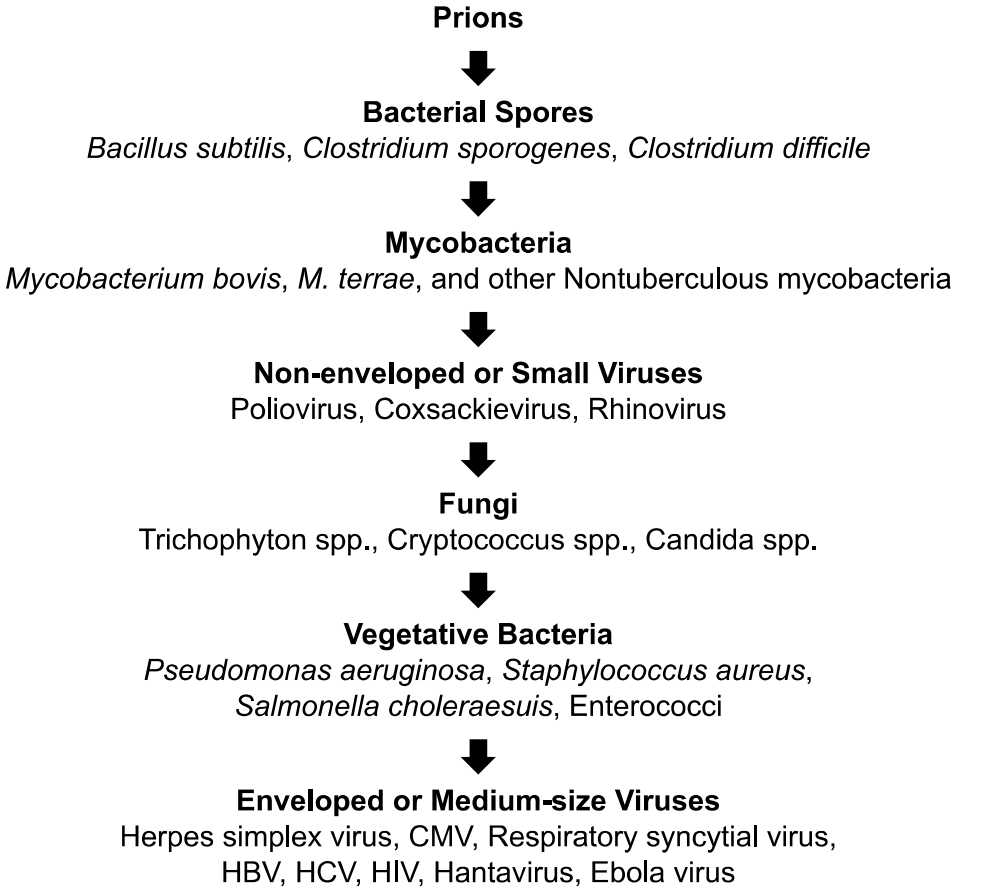
Decontamination renders an area, device, item, or material safe to handle in the context of being reasonably free from a risk of disease transmission. The primary objective of decontamination is to reduce the level of microbial contamination so that transmission of infection is prevented. The decontamination process may involve the cleaning of an instrument, device, or area with ordinary soap and water. In laboratory settings, decontamination of items, used laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, which may be the most cost-effective way to decontaminate 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 can be 20 minutes at 121°C. When steam sterilization is used to decontaminate laboratory waste that contains items that have a high bio-burden and there is no pre-cleaning (i.e., infectious waste), the cycle times are generally longer and should be verified and validated for the typical load. Validation involves the combined use of thermocouples and biological indicators (BIs) placed throughout the load to ensure penetration of steam into the waste. Verification can be accomplished by routine monitoring of the steam sterilization cycles (i.e., cycle times, pressure, temperature) and by placing BIs within the load.[15] In addition to time, temperature may also be increased to ensure inactivation of pathogens.[16–18] Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with steam.

Chemical disinfectants used for decontamination range in activity from high-level disinfectants (e.g., 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 Figure 1. If dangerous and highly infectious agents are present in a laboratory, the methods for decontamination of spills, laboratory equipment, biological safety cabinet, or infectious waste are very significant and may include prolonged autoclave cycles, incineration, or gaseous treatment of surfaces.

Figure 1. Descending Order of Relative Resistance to Disinfectant Chemicals



Note: There are exceptions to this list. *Pseudomonas* spp. are sensitive to high-level disinfectants. However, in biofilms, the protected cells and those within free-living amoeba, or existing as persister cells (viable but not culturable) within the biofilm, 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 *Chaetomium globosum*, and the pink-pigmented Methylobacteria. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this appendix.

Space Decontamination Space decontamination is a specialized activity and should be performed by individuals with proper expertise, training, and personal protective equipment.[19–24] Decontamination requirements for laboratory spaces influence the design of these facilities. The interior surfaces of laboratories must be easy to clean and decontaminate. Penetrations in BSL-3 laboratory surfaces should be sealed or capable of being sealed for decontamination purposes. 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 highly recommended but is usually not required for BSL-3 laboratories. The BSL-4 laboratory design requires interior surfaces that are water-resistant and sealed to facilitate 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 follow. Fumigants that are currently used are either gases, vapors, mists, or fogs (dry mists). Fumigants that are gases obey gas laws, can evenly distribute throughout the room, and are easily scalable by increasing the volume of gases used. Fumigants applied as mists or fogs do not behave like gases and are particles (<1–12 μ in size) that settle onto surfaces being treated.

Paraformaldehyde and Formaldehyde Gas

Paraformaldehyde and solutions of formaldehyde have been used to generate formaldehyde gas and mists; historically, they have been used in laboratory settings for decontamination of large spaces and biological safety cabinets.[25,26] When using formaldehyde and paraformaldehyde, take safety precautions,[27,28] federal regulations, state regulations, and local regulations into consideration.[29] Formaldehyde is also recognized as a known human carcinogen.[30] There is at least one EPA-registered paraformaldehyde product available for the decontamination of laboratories. It is important that paraformaldehyde is used per labeling instructions and that a fumigation management and safety plan that meets federal, state, and local regulations is prepared in advance of application and is implemented during application. For use as a space decontamination agent, the standard concentration of formaldehyde is 0.3g/ft^3 (approximately 8,000 ppm) with a relative humidity of between 60 and 85%.[31] Increasing the amount of paraformaldehyde is not advised, as the lower explosive limit for formaldehyde gas is 7% (70,000 ppm).[32] It is recommended that formaldehyde gas decontamination be performed only by highly experienced individuals.

Hydrogen Peroxide Vapor

Hydrogen peroxide can be vaporized and used for the decontamination of glove boxes and 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 and oxygen) are not toxic. Low relative humidity can be used.[33–36]

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 a contact time of one to two hours.[37–40]

Chlorine dioxide possesses the bactericidal, virucidal, and sporicidal properties of chlorine, but unlike chlorine, it does not lead to the formation of trihalomethanes and does not combine with ammonia to form chlorinated organic products (chloramines). The gas cannot be compressed and stored in high-pressure cylinders, but it 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 humidity is optimal. Although most often used in closed sterilizers, the destination enclosure for the chlorine dioxide gas does not 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.[40] 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 disinfectants may be used for decontamination of large surface areas. The usual procedure is to flood the area with a disinfectant for periods up to several hours. This approach is messy, and some of the disinfectants used represent a toxic hazard to laboratory staff. For example, most of the high-level disinfectants on the United States market are formulated for use on instruments and medical devices rather than on environmental surfaces. Intermediate and low-level disinfectants are formulated for use on fomites and environmental surfaces but lack the potency of high-level disinfectants. For the most part, intermediate and low-level disinfectants can be safely used and, as with all EPA-registered disinfectants, the manufacturer’s instructions

should be followed.[41] 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. Procedures for the use of chemical disinfectants should include safety precautions, the use of appropriate personal protective equipment, hazard communication, and training on spill response.

Concentrations and exposure times vary depending on the disinfectant formulation and the manufacturer's instructions for use. See Table 1 for a list of chemical disinfectants and their activity levels. A spill control plan must be available in the laboratory. This plan should include the rationale for selection of the disinfectant, the approach to its application, contact time, and other parameters. Biological agents requiring BSL-3 and BSL-4 containment pose a high risk to workers and possibly to the environment, and these agents should be managed by trained, professional staff who are equipped to work with concentrated material.

Table 1. Activity Levels of Selected Liquid Chemical Disinfectants

Chemical[a]	Concentration	Activity level
Glutaraldehyde	Variable	Sterilization
Glutaraldehyde	Variable	Intermediate to high-level disinfection
Ortho-phthalaldehyde (OPA)	0.55%	High-level disinfection
Hydrogen peroxide	6–30%	Sterilization
Hydrogen peroxide	3–6%	Intermediate to high-level disinfection
Formaldehyde[b]	6–8%	Sterilization
Formaldehyde	1–8%	Low- to high-level disinfection
Chlorine dioxide	Variable	Sterilization
Chlorine dioxide	Variable	High-level disinfection
Peracetic Acid	0.08%–0.23% with peroxide concentrations of 1–7.35%	Sterilization
Peracetic acid	Variable	High-level disinfection
Hypochlorites[c]	500–6000 mg/L Free available	Intermediate to high-level disinfection
Alcohols (ethyl, Isopropyl)[d]	70%	Intermediate-level disinfection
Phenolics	0.5–3%	Low- to intermediate-level disinfection

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Chemical[a]	Concentration	Activity level
Iodophors[e]	30–50 mg/L Free	Low- to intermediate-level disinfection
Quaternary Ammonium Compounds	Variable	Low-level disinfection

a. This list of chemical disinfectants 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. Users can search for EPA-registered products at https://www.epa.gov/pesticide-labels.

b. Because formaldehyde is classified as a known human carcinogen and has a low permissible exposure limit (PEL), 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). The indicated concentrations are rapid-acting and broad-spectrum (i.e., tuberculocidal, bactericidal, fungicidal, and virucidal). Note: Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and 1000 ppm 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 may be spores or there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory). In situations where there is an excessive amount of organic material present, the surfaces should be thoroughly cleaned to remove as much organic material as possible before applying sodium hypochlorite solution to disinfect the surface (see product label instructions). The concentration of the sodium hypochlorite should be determined in advance of use and the solution should be made fresh each day.

d. The effectiveness of alcohols as intermediate-level germicides is limited because they evaporate rapidly, resulting in short contact times, and because they lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal, and fungicidal, but may vary in spectrum of virucidal activity. Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time.

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.

Transmissible Spongiform Encephalopathy Agents (Prions) Prions are exceptionally difficult to inactivate and decontaminate and are the causative agent of Creutzfeldt-Jakob disease (CJD) and other 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.[12,42,43] Treatment of tissues and contaminated tissues is based on tissue infectivity.[44] See Section VIII-H: Prion Diseases for additional information.

Inactivation of Select Agents Select agents can be inactivated using conventional disinfection and sterilization procedures appropriate to the type of agent (e.g., virus, spore-forming bacteria). Inactivation procedures typically leave cell components intact that can then be used as reagents for assay development or other studies while the purpose of disinfection is to kill and damage pathogens with no attention to preserve cell components. Once inactivated, the agents are no longer subject to the Select Agent Regulations. Problems have arisen when spore-forming Select Agents such as *Bacillus anthracis* have not been completely inactivated. This was highlighted in 2015 when irradiated spores were shipped to non-select, agent-approved laboratories but were later found to be only partially inactivated.[45] The Select Agent Regulations require that the inactivation process

used for these agents be validated. Select Agent guidance is available at https://www.selectagents.gov/resources/Inactivation_Guidance.pdf and at https://www.selectagents.gov/resources/Biosafety_Guidance.pdf.

Chemical Safety When using chemical agents for decontamination, pay attention to instructions for their use and Safety Data Sheets (SDS); ensure they are used safely and that appropriate precautions and protections are used. Exposures to disinfectants have resulted in occupational injuries such as cancer, hypersensitivities, dermatitis, and asthma.[46,47]

Hand Hygiene Handwashing and hand decontamination are an underappreciated part of risk mitigation for handling pathogens. Gloves should be worn when handling biohazardous materials and hazardous chemicals, including those used in disinfection and decontamination; this does not replace the need for regular hand hygiene by laboratory personnel.[48] Hand hygiene should be performed after removing gloves, after touching potentially contaminated surfaces with bare hands, after completing work, and before exiting the laboratory. The main method of hand hygiene in the laboratory is handwashing with soap and water.

When handwashing facilities are not available, an alcohol-based hand sanitizer (ABHS) with an alcohol concentration between 60–95% may be used in conjunction with or in lieu of immediate handwashing, based on agent type and a risk assessment that accounts for potential reduced efficacy of hand sanitizers for soiled hands and inactivating some microorganisms (i.e., bacterial spores, parasites, and non-enveloped viruses). ABHS may be used for immediate hand hygiene until a handwashing facility can be accessed only if hands are not grossly contaminated. The limitations of AHBS should be communicated to staff. Handwashing with soap and water remains the preferred method of performing hand hygiene.[49] ABHS should be applied to cover the skin and nails (including underneath the nail) of the hands for 20–30 seconds. Posters are available to assist in demonstrating the proper method of hand sanitizing using ABHS at https://www.cdc.gov/features/handhygiene.

If hands are grossly contaminated when exiting the laboratory, they should be washed with soap or soap containing an antiseptic agent (i.e., antimicrobial soap) and water.[49,50] When using soap and water, the entire procedure should last 40–60 seconds from wetting hands to drying with a paper towel. Posters are available to assist in demonstrating the proper method of handwashing at https://www.cdc.gov/handwashing/posters.html. Posters are available to assist in demonstrating the proper method of handwashing and use of an ABHS at https://www.who.int/gpsc/tools/GPSC-HandRub-Wash.pdf.

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