University of Massachusetts/Amherst
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 "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. *M. bovis*, non-lipid viruses, and most forms of fungi.

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.

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 following).

TABLE 1

DESCENDING ORDER OF RESISTANCE TO GERMICIDAL CHEMICALS

BACTERIAL SPORES
Bacillus subtilis, Clostridium sporogenes

⇓

MYCOBACTERIA
Mycobacterium tuberculosis var. *bovis*, Nontuberculous mycobacteria

⇓

NONLIPID OR SMALL VIRUSES
Poliovirus, Coxackie virus, Rhinovirus

⇓

FUNGI
Trichophyton spp., *Cryptococcus* spp., *Candida* spp.

⇓

VEGETATIVE BACTERIA
Pseudomonas aeruginosa, *Staphylococcus aureus*, *Salmonella choleraesuis*, Enterococci

⇓

LIPID OR MEDIUM-SIZE VIRUSES
Herpes simplex virus, Cytomegalovirus, Respiratory syncytial virus, HBV,
HCV, HIV, Hantavirus, Ebola virus

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 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 A spill control plan should 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.

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 equipment. There are no FDA cleared liquid chemical sterilant/disinfectants that contain formaldehyde.

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).

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).

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.

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Chemical Disinfectants

Chemical Disinfectants and Their Use in Laboratories

Disinfectant	Concentration (active ingredients)	Contact Time (min)	Effective Against*					
			B	F	LV	TB	HV	S
Quaternary Ammonium	0.1 - 0.2%	10 - 30	++	++	++	-	-	-
Phenolic compounds	0.2 - 3.0%	10 - 30	++	++	++	++	+	-
Chlorine Compounds ** (available chlorine)	0.01 - 5.0%	10 - 30	++	++	++	++	++	+
Iodophor Compounds)	0.5%	10 - 30	++	++	++	++	+	-
Alcohol (ethyl or isopropyl)	70 - 85%	10 - 30	++	++	++	-	+	-
Formaldehyde	4 - 8%	10 - 30	++	++	++	++	++	+
Glutaraldehyde	2%	10 - 600	++	++	++	++	++	++

* B = Vegetable bacteria; F = fungi and asexual spores but not necessarily chlamydospores or sexual spores; LV = lipophilic viruses; TB = tubercle bacillus; HV = hydrophilic viruses; S = spores. + = positive response; ++ = very positive response; - = negative response.

** Household bleach contains 5% available chlorine.

*** References: "Decontamination, Sterilization, Disinfection, and Antisepsis in the Microbiology Laboratory," in Laboratory Safety: Principles and Practices and "Sterilization, Disinfection, and Antisepsis in the Hospital," in Manual of Clinical Microbiology.