

Disinfectants are characterized into 3 activity levels according to *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*:

Classes of chemical disinfectants	Alcohols	Aldehydes	Chlorine compounds	Iodophor	Oxidizing agents	Phenolic compounds	Quaternary ammonium compounds (QUATS)
<b>Examples</b>	-70%ethyl alcohol -isopropyl	-Formaldehyde -Paraformaldehyde Glutaraldehyde -Calgocide 14 -Cidex -Vespore	Bleach Clorox® ChloramineT Purex	Betadyne® Providon®	Hydrogen peroxide Peroxyacetic acid Trifectant® Virkon S® Oxy-Sept 333®	One-Stroke Environ® Pheno-Tek II® Tek-Trol® Pine-Sol, Lysol®	Hi-Tor Roccal® DiQuat® Parvosol® Zepharin® D-256®
<b>Aqueous Concentration</b>	70%	Variable	10% (500 to 5000 mg/L)	0.1-0.2%	3-6%	0.5 to 3%	
<b>Activity Level</b>	Intermediate	High to Intermediate	Intermediate	Intermediate to Low	High to Intermediate	Intermediate to low	Low

### 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."

Commonly used classes of chemical germicides are described below inclusive of generic information on their applications and safety profiles:

### Alcohols

Ethanol (ethyl alcohol, C<sub>2</sub>H<sub>5</sub>OH) and 2-propanol (isopropyl alcohol, (CH<sub>3</sub>)<sub>2</sub>CHOH) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on non-lipid viruses is variable. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde, and alcohol containing 2 g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses. Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labeled to avoid autoclaving.

### Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of

available chlorine. Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g. with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week. A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above. Granules or tablets of calcium hypochlorite ( $\text{Ca}(\text{ClO})_2$ ) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5 g/l available chlorine, respectively. Bleach is not recommended as an antiseptic, but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. Chlorine gas is highly toxic. Bleach must therefore be stored and used in well ventilated areas only. Also, bleach must not be mixed with acids to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

### **Iodine and iodophors**

The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. On the other hand, iodophors and tinctures of iodine are good antiseptics. Polyvidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine are generally unsuitable for use on medical/dental devices. Iodine should not be used on aluminium or copper. Iodine can be toxic. Organic iodine-based products must be stored at 4–10 °C to avoid the growth of potentially harmful bacteria in them.

### **Hydrogen peroxide and peracids**

Like chlorine, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment. Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid ( $\text{CH}_3\text{COOOH}$ ) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment. Hydrogen peroxide and peracids can be corrosive to metals such as aluminium, copper, brass and zinc, and can also decolorize fabrics, hair, skin and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

### **Phenolic compounds**

Phenolic compounds, a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against nonlipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (e.g. triclosan and chloroxylenol) are among the more commonly used antiseptics. Triclosan is common in products for hand-washing. It is active mainly against vegetative bacteria and safe for skin and mucous membranes. However, in laboratory based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics. The significance of this finding in the field remains unknown. Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water. Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin. National chemical safety regulations must be followed.

### **Quaternary ammonium compounds**

Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics. The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness and anionic detergents. Care is therefore needed

in selecting agents for pre-cleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

### **Formaldehyde**

Formaldehyde (HCHO) is a gas that kills all microorganisms and spores at temperatures above 20 °C. However, it is not active against prions. Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about 370 g/l (37%), containing methanol (100 ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms (see section on Local environmental decontamination in this chapter). Formaldehyde (5% formalin in water) may be used as a liquid disinfectant. Formaldehyde is a suspected carcinogen. It is a dangerous, irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well ventilated area. National chemical safety regulations must be followed.

### **Glutaraldehyde**

Like formaldehyde, glutaraldehyde (OHC(CH<sub>2</sub>)<sub>3</sub>CHO) is also active against vegetative bacteria, spores, fungi and lipid- and nonlipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores. Glutaraldehyde is generally supplied as a solution with a concentration of about 20 g/l (2%) and some products may need to be “activated” (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid. Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. National chemical safety regulations must be followed.

References: World Health Organization Laboratory Biosafety Manual 3rd edition, 2004  
<http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>