

**BIOSAFETY**  
**APPENDIX I**  
**DISINFECTANTS FOR**  
**BIOHAZARDOUS**  
**MATERIALS**  
**SOP**

May, 2024

There are a lot of variables to consider when choosing a decontamination method. The appropriateness of a decontamination procedure will depend on your objective. Do you need to disinfect or sterilize? There are other important factors to consider as well. Will the disinfectant be used on hard, non-porous surfaces, in a biosafety cabinet, on instruments, or on waste? Surgical instruments must be sterile, but this level of microbial killing is generally unnecessary for laboratory surfaces such as floors and walls. When choosing a disinfectant, consider the organism, the item to be disinfected, the disinfectant's cost and ease of use, with a shorter contact time being preferred to help manage workflows.

EPA-registered hospital grade disinfectants can be used in laboratories. Consult the manufacturer's directions to determine efficacy against the biohazards used in your lab. Each product should have an efficacy statement that identifies the agents the product is effective against. Disinfectants include household bleach, quaternary ammonium compounds, and Phenolic compounds.

The following disinfectants are effective against a wide range of infectious agents. Approximate contact times are given for each disinfectant. Disinfectant solutions should be made up and stored according to manufacturer directions. Some need to be made up fresh daily while others are shelf stable. A clean surface is more effectively decontaminated than a soiled surface and multiple applications may be required. While disinfectants are not necessarily detergents, some disinfectants also include a detergent or surfactant. This will be indicated on the product's Safety Data Sheet. Various disinfectants are discussed in detail below.

**Chlorine (Sodium Hypochlorite)**

Chlorine is a fast-acting oxidant that is widely available for use as a broad-spectrum chemical disinfectant. Sold as household bleach, it is an aqueous solution of sodium hypochlorite (5.25% NaOCl) which can be diluted with water to provide various concentrations of available chlorine.

While it can be highly effective as a disinfectant, there are characteristics that make it unsuitable for use in today's research labs. First and foremost, chlorine is highly alkaline and corrosive to metal. Care must be taken when using even 5% bleach on stainless steel such as a biosafety cabinet (BSC); multiple clear water rinses are required to completely remove all residue and prevent rusting. In research, the best use for bleach is in the cell culture lab to decontaminate the contents of the vacuum flask. Because bleach is inherently unstable, flasks should be changed at least once a week to ensure proper decontamination.

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**NOTE: if the pH of this waste is outside the pH 5-9 window, it must be disposed through EHS as chemical hazardous waste.**

The disinfectant activity of chlorine is considerably reduced by organic matter (protein). Stock or working solutions that are stored in open containers, particularly at high temperatures, release chlorine gas thereby reducing their disinfectant potential. Undiluted household bleach, stored at room temperature in the original container, has a shelf-life of approximately six months. However, since the date of manufacture is not stamped on containers, it is impossible to know the age of bleach when it finally reaches the lab. It could have been sitting in a hot warehouse – and decomposing – before it ended up a shelf at Target. Using bleach is therefore a risky proposition as it may not be as effective as it “should be” due to degradation. If used, working solutions of bleach must be prepared fresh daily. Household bleach is diluted 1:10 to obtain a final concentration of 0.5% NaOCl. Industrial strength bleach has a higher sodium hypochlorite concentration (up to 6.25%) and must be diluted accordingly to obtain the correct final concentration. To increase the efficacy of sodium hypochlorite solutions against spores, vinegar may be added to the solution. If your lab works with spore formers, combining 5 ounces of household bleach with one gallon of water and adding 8 ounces of 5% distilled white vinegar yields a disinfectant that is effective against spores.

Chlorine gas is highly toxic. Store and use bleach only in well-ventilated areas. To prevent the rapid release of chlorine gas, do not mix undiluted bleach with acids or other incompatible chemicals, such as ammonia-containing compounds.

All things considered, there are much better choices than bleach for today’s research labs.

### **Phenolic Compounds**

Phenolic compounds were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses, and also show activity against mycobacteria when properly formulated. They are not active against spores and their activity against non-lipid-containing 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. Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water. They may be absorbed by latex gloves and can also penetrate the skin. Phenolic compounds can be irritating to the skin and eyes and may have an associated odor that is irritating to respiratory tissue.

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Hil-Phene is a broad-spectrum phenolic disinfectant typically used at a dilution of 1-5%. Contact time of 10 minutes is recommended. Hil-Phene provides effective disinfection for HIV and many other infectious agents; it is also tuberculocidal.

#### **Quaternary Ammonium Compounds**

Many types of quaternary ammonium compounds are used as mixtures and in combination with other germicides. Depending on the quaternary compound type, germicidal activity can be reduced by organic matter, water hardness, and anionic detergents. Therefore, care should be taken in selecting proper agents for pre-cleaning. When properly diluted, quaternary ammonium compounds have low odor and are not irritating, however potentially harmful bacteria can grow in quaternary ammonium compound solutions.

Vindicator+ is a disinfectant that is cost-effective and has good broad spectrum disinfecting strength. A dilution of 0.1-2% and contact time of 10 minutes is recommended. Vindicator+ provides effective disinfection for HIV, HBV, Adenovirus, many animal viruses, and others.

Lysol I.C. is another quaternary ammonium disinfectant. It is available from Fisher Scientific and various other sources including Amazon. A dilution of 1:256 and contact time of 10 minutes are recommended.

Lysol I.C. is a highly concentrated hospital-approved disinfectant that provides virucidal, fungicidal, and bactericidal protection in the presence of up to 5% organic matter.

#### **Alcohols**

Mixtures of alcohols with other agents may be more effective than alcohol alone; a contact time of at least 10 minutes must be observed. When used alone, alcohols do not leave a residue on treated items. Alcohols are volatile and flammable and must not be used near open flames or sprayed inside a BSC because the lower explosive limit (LEL) is easily reached. If 70% ethanol is used to decontaminate a BSC, it must be wiped on and not sprayed. If sprayed, the vapor will pass over the sparking motor, a potential source of ignition. Alcohol-containing solutions must be clearly labeled to prevent their being autoclaved. Store all working solutions of alcohol in tightly capped containers to prevent evaporation. Include Alcohol in the lab's chemical inventory and monitor its use carefully to minimize the chance of it being used for non-lab use.

Ethanol and isopropyl alcohol have similar disinfectant properties. They are active against vegetative bacteria, fungi, and lipid-containing viruses, but not against spores. They are

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effective against enveloped viruses but their action on non-lipid-containing 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. In addition to being relatively inexpensive, another advantage of aqueous alcohol solutions is that they do not leave any residue on treated items. Alcohol-based hand-rubs and alcohol mixed with emollients are recommended for decontaminating lightly soiled hands in situations where proper handwashing is inconvenient or not possible. Ethanol is ineffective against spores and *Mycobacterium tuberculosis* (TB) and may not kill all types of non-lipid-containing viruses.

**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. However, iodophors and tinctures of iodine are good antiseptics and polyvidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine should not be used on aluminum or copper. Iodine-based products must be stored at 4–10°C to prevent the growth of potentially harmful bacteria. Iodine can be toxic.

**Summary**

**For general lab use a hospital grade EPA-registered product would be a good choice. Always check the pH if using in a BSC. If alkaline or highly acidic, do not use in a BSC as it will cause the stainless steel cabinet to rust. 70% Ethanol would be a better choice.**

**References:**

World Health Organization Laboratory Biosafety Manual, 4<sup>th</sup> ed.

<https://www.who.int/publications/i/item/9789240011311> (free download)

EPA Approved Disinfectants Website

<https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants>