



CHEMICAL DISINFECTANTS AND BIOHAZARDOUS MATERIALS

Introduction

The Environmental Protection Agency (EPA) (<https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants>) defines a disinfectant as “a substance, or mixture of substances that destroys or irreversibly inactivates bacteria, fungi and viruses, but not necessarily bacterial spores **in the inanimate environment, such as on hard surfaces**”. Disinfectants are used to treat surfaces/ equipment using physical (wiping, scraping) and chemical means to reduce, inactivate or destroy microorganisms present on inert surfaces.

The efficacy of the chemical disinfectant is based on several factors including: disinfectant concentration, pH, temperature of the disinfectant and/ or the surface, contact time of the disinfectant on the contaminated surface and environmental humidity. These factors determine if the disinfectant is considered a high, intermediate or low-level disinfectant.

Other factors that determine efficacy are: Organic load and condition of the surface (the amount of dirt and other organic material; porous vs. nonporous); microbial concentration and structure (10^4 vs. 10^8 , biofilm, pellicle, prion, endospore, waxy coat) and type of microorganism. The following table provides information regarding chemical disinfectant resistance of various biological agents.

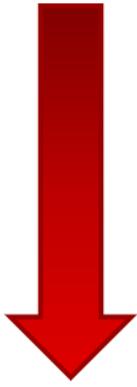
Microbial Resistance to Chemical Disinfectants

Microbial resistance to chemical disinfectants can vary. Refer to the chart provided for examples.

Note: The Environmental Protection Agency (EPA) maintains a registry (<https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants>) of disinfectants that have been tested and shown to be effective against certain biological agents.



Always select an EPA registered disinfectant known to be effective for the microbe(s) in use in your laboratory.

Resistant	Type of agent	Examples
	Prions	Bovine spongiform encephalopathy (Mad Cow) Creutzfeldt-Jakob disease
	Bacterial Spores	<i>Bacillus subtilis</i> ; <i>Bacillus anthracis</i> ; <i>Clostridium sporogenes</i> ; <i>Clostridioides difficile</i>
	Mycobacteria	<i>Mycobacterium tuberculosis</i> , <i>M. bovis</i> , <i>M. terrae</i>
	Non-enveloped or Small Viruses	Poliovirus; Coxsackievirus; Rhinovirus; Adenovirus
	Fungi	<i>Trichophyton spp.</i> ; <i>Cryptococcus sp.</i> ; <i>Candida sp.</i>
	Vegetative Bacteria	<i>Pseudomonas aeruginosa</i> ; <i>Staphylococcus aureus</i> ; <i>Salmonella choleraesuis</i> ; <i>Enterococci</i>
Susceptible	Enveloped or Medium-size Viruses	<i>Herpes Simplex</i> ; <i>Influenza</i> ; <i>CMV</i> ; <i>RSV</i> ; <i>HIV</i> ; <i>HBV</i> ; <i>HCV</i> ; <i>Hantavirus</i> , <i>Ebola Virus</i>

Disinfectant Categories

1.1 Hypochlorite (Bleach):

- Contact Time: 10-60 minutes
- Shelf Live: 1 year from date of manufacture
- Dilution Life: 5000-6000 ppm NaOCl 24 hours, 8000 ppm 14 days, >10000 ppm 30 days

Bleach solution oxidizes molecules and denatures protein in microorganisms and is corrosive to stainless steel. Thorough rinsing must follow its use in the biosafety cabinet, incubator or any stainless-steel surface/ piece of equipment. **DO NOT autoclave bleach solutions!** Commercially available concentrated bleach can contain 5-8.25% (50,000-82,500ppm) sodium hypochlorite. When used as a disinfectant it is recommended to use a solution that contains at least 5000 ppm, but not more than 10,000 ppm available chlorine. See the preparation instructions in the chart below for how to obtain these solution concentrations.

Bleach Solution Preparation Table

Starting NaOCl %	Ending NaOCl %	Bleach Solution Ratio	Bleach Dilution	Chlorine concentration	Shelf life*
5.25%	0.53%	1:10	1 part bleach, 9 parts water	5,250 ppm	7 days
5.25%	1.05%	1:5	1 part bleach, 4 parts water	10,500 ppm	30 days
6.15%	0.62%	1:10	1 part bleach, 9 parts water	6,150 ppm	7 days
8.25%	0.55%	1:15	1 part bleach, 14 parts water	5,500 ppm	7 days
8.25%	0.825%	1:10	1 part bleach, 9 parts water	8,250 ppm	14 days

*Shelf life If stored in an opaque container away from heat and light. Translucent or clear containers will speed up decomposition of NaOCl into salt and water.

Two things you must know about the commercial concentrated bleach you use:

The concentration of sodium hypochlorite. This must be >5.25%.

The manufacture date of the solution. Some manufacturers print this date on the bottle, others like Clorox print a code on the bottle, which must be deciphered.

<https://www.clorox.com/learn/how-to-tell-when-a-bleach-bottle-was-made/>

Sodium hypochlorite solutions **are unstable and are easily inactivated by organic material**. When opened, chlorine evaporates at a high rate from the solution, rapidly reducing the concentration of free chlorine. When stored at room temperature and away from sunlight, bleach loses 20-50% of its sodium hypochlorite concentration after 6 months; breaking down into salt and water. **Commercial bleach containers must be disposed of within 1 year of the date of manufacture to ensure that the sodium hypochlorite concentration is always at an effective level.**



If you are unable to determine the manufacture date, it is acceptable to label the bottle with the date the bottle was received from the supplier. Then use or dispose of the solution within 1 year of that date.

Hypochlorite solutions are classified as irritant and corrosive. Take appropriate precautions when using hypochlorite products: read labels carefully, adhering to cautionary warnings

and following usage directions. Chlorine solutions should never be mixed or stored with cleaning products containing ammonia, ammonium chloride, or phosphoric acid. Combining these chemicals will result in the release of chlorine gas, which can cause nausea, eye irritation, tearing, headache, and shortness of breath. These symptoms may last for several hours. If you are exposed to an unpleasantly strong odor following the mixing of a chlorine solution with a cleaning product, leave the room or area immediately until the fumes have cleared completely.

1.2 Alcohol

- Contact Time: 30 seconds to 5 minutes
- Shelf Life: Undiluted Alcohol approximately 3 years; See manufacturer's label for purchased solutions.
- Dilution Life: 1 year for lab-made solutions

Ethanol kills organisms by denaturing their proteins and dissolving their lipids and their action on non-lipid containing viruses is variable. 70% ethanol or 70% isopropyl alcohol is better than 80% - 95% ethanol or isopropyl alcohol as a disinfectant and ethyl alcohol is considered a better broad-spectrum disinfectant than isopropyl alcohol. Higher or lower concentrations of either may not be germicidal. Alcohol can be purchased already diluted or you can prepare your own solutions. A 70% ethanol or isopropyl solution is made by adding 2.5 parts water to 7.5 parts 95% ethanol. Methanol should not be substituted for ethanol or isopropyl, because it is not as effective and is a health hazard. Alcohol evaporates rapidly, so extended contact times are difficult to achieve without immersion. Ensure surfaces stay wet for the minimum contact time. When a longer contact time is required, select a different disinfectant.

These solutions are flammable. Always keep ethanol and isopropyl solutions away from potential sources of ignition. Prolonged and repeated use of alcohol as a disinfectant can also cause discoloration, swelling, hardening and cracking of rubber and certain plastics. Check with the manufacturer for the effective shelf life if buying undiluted solutions. It is recommended that lab-made solutions be labeled and dated, with an expiration date of 1 year from the date it is made. If the solution drops below 70%, it will no longer be an effective disinfectant. Discontinue use if the solution changes color from clear.

1.3 Oxidizing Agents

- Contact Time: 1-60 minutes (depending on biological agent); check label
- Shelf Life: 3 years concentrate; check label

- Dilution Life: <6% - varies depending on the properties of the agent; example, accelerated hydrogen peroxide has stabilizers and greater efficacy than hydrogen peroxide alone; check label

Like chlorine, hydrogen peroxide (H₂O₂) and peracetic acid are strong oxidants. They are also safer than chlorine to humans and the environment. However, dilute solutions have a short shelf life. In their diluted form, these agents are relatively safe but may be irritating and damage clothing when concentrated. **When stored properly in dark containers, the decomposition rate is less than 2% per year.**

1.3.1 Hydrogen peroxide

Hydrogen peroxide works by producing destructive hydroxyl free radicals that attack proteins, membrane lipids, DNA and other essential cell components. It can be purchased as a ready-to-use disinfectant, over the counter (3-10% concentration), or as an industrial concentration of 30% or greater aqueous solution. Hydrogen peroxide solutions alone are generally unstable and can break down quickly, so fresh solutions should be used.

Concentrations of hydrogen peroxide from 6% to 25% show promise as a chemical sterilant; however, dilute solutions of <6% hydrogen peroxide alone are relatively slow and limited as germicides. Accelerated hydrogen peroxide products (e.g., Accel Rescue/ Intervention) incorporate additional compounds, such as stabilizers and surfactants to minimize the degradation after mixing and to enhance the disinfectant's cleaning ability.

Hydrogen peroxide is effective against bacteria, viruses (non-enveloped viruses may be resistant), and fungus and higher concentrations (>15%) are sporicidal. It has limited activity against mycobacteria, but there are products that are tuberculocidal [PREempt™ RTU (Ready to Use) and Accel TB]- check the label. Hydrogen peroxide can be used for the decontamination of work surfaces and laboratory equipment, including biosafety cabinets.

1.3.2 Peracetic acid

Peracetic acid is a strong oxidizing agent and is a formulation of hydrogen peroxide and acetic acid. It is effective against bacteria, fungi, spores and viruses. It is also effective against mycobacteria and algae and has some activity in the presence of organic material. Example: Spor-Klenz, OxySept 333®, Peridox RTU

Hydrogen peroxide and peracetic acid can be corrosive to metals such as aluminum, 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. A 1% solution loses half its strength through hydrolysis in 6 days, whereas 40% peracetic acid loses 1%–2% of its active ingredients per month.

1.3.3 *Virkon® S (potassium peroxymonosulfate and sodium chloride)*

This disinfectant is a peroxygen molecule, organic acid and surfactant combination, with a wide microbial spectrum of activity and some efficacy in the presence of organic material. This comes in powder or tablet form and mixed solutions are good for up to 7 days. The powder is corrosive; use appropriate PPE when preparing solutions. The maximum contact time required is 10 minutes.



WARNING! These compounds have a strong vinegar odor, are acidic, and can cause irritation to skin, eyes, and mucous membranes. Use in well-ventilated areas and/or with appropriate respiratory protection.

1.4 Phenolic Compounds:

- Contact Time: 5-10 minutes; check label
- Shelf Life: 2 years
- Dilution Life: Working solution 14 days

Phenolic compounds denature proteins and inactive membrane bound enzymes to alter cell wall permeability. At a concentration of 5%, phenolic compounds are effective against vegetative bacteria, fungi, mycobacteria and lipid-containing/ enveloped viruses. Phenolic compounds are not suitable for bacterial spores and some hydrophilic viruses. They have a strong pine odor, can easily be absorbed through the skin and are irritants to the mucous membrane and respiratory tract. Examples: Vesphene IIIse, CiDecon TB

1.5 Quaternary Ammonium Compounds (Quats):

- Contact Time: 10 minutes
- Shelf Life: Approximately 1-5 years
- Dilution Life: Working solution 14 days

Quats irreversibly bind phospholipids in the cell membrane and denature proteins impairing permeability. These disinfectants are available as pre-made solutions containing one or more quaternary ammonium compounds at concentrations ranging from 0.1 – 2%. This

concentration is effective against vegetative bacteria and lipophilic (lipid enveloped) viruses. They are not effective against non-lipid viruses or mycobacteria. Quaternary ammonium compounds are not effective against spores and may be neutralized by anionic detergents and organic material (e.g., dirt, blood, etc.). Examples of quats: Cavicide 1, Neutral Q, Morning Mist, Lysol I.C., Envirocare, Conflikt

1.6 Iodophor Disinfectant:

- Contact Time: 10-30 minutes
- Shelf Life: Approximately 3 years

Iodine compounds are broad spectrum and considered effective for a variety of bacteria, mycobacteria, fungi and viruses. Iodines function by denaturing proteins to interfere with the enzymatic systems of microorganisms. Iodine compounds are often formulated with soaps and considered relatively safe. Concentrated iodine compounds can be irritating to the skin; can stain clothes, damage rubber, and some metals. Iodine agents are inactivated by organic material. Prepare iodine solutions according to the instructions on the label. Shelf life is approximately 3 years but should be marked on the bottle by the manufacturer.

Iodophors are iodine complexes that have increased solubility and sustained release of iodine. One of the more commonly used iodophors is povidone-iodine. They are good for general use and are less readily inactivated by organic matter than elemental iodine compounds. The dilution of iodophors increases free iodine concentration and antimicrobial activity. The final concentration listed in the table reflects the active iodine concentration, which is commonly 1% titratable iodine. Example: Wescodyne, Betadine, Povidone-iodine

1.7 Aldehydes

1.7.1 Formalin:

- Contact Time: 10-30 minutes
- Shelf/Dilution Life: 10% 7 days

Formalin is a 37% solution of formaldehyde gas in water. A 10% formalin solution is roughly equivalent to 4% formaldehyde; at this concentration it is an effective disinfectant and is usually used to fix human and animal tissue samples. Formaldehyde (formalin) has good disinfectant properties against vegetative bacteria, spores and viruses. It has an irritating odor and **is a human carcinogen**. Formaldehyde is not recommended for daily disinfection. Use only with proper ventilation control (e.g., chemical fume hood; Thimble

connected Class IIA2 BSC, Class IIB2 BSC or ducted Class IIC1). The shelf life for 10% formalin solution is about 1 week; shelf life is moderately extended in brands that use methanol to prevent polymerization. Breakdown of the solution can be determined by the appearance of precipitate forming.

1.7.2 Glutaraldehyde: (also called a "cold disinfectant"):

- Contact Time: 15-30 minutes
- Shelf/Dilution Life: 2-3% 14 days

Two percent (2%) solutions exhibit good disinfectant activity against vegetative bacteria, spores and viruses. Glutaraldehyde **is toxic**, a sensitizer and is generally not used for laboratory surface disinfectant and is capable of eye damage. Concentrated glutaraldehyde maintains its concentration for up to one (1) year. Temperature, pH and contamination can adversely affect shelf life. Working solutions of 2-3% glutaraldehyde can be used for up to 14 days. Store solutions at or below room temperature. Use only with proper ventilation control, such as a chemical fume hood, thimble connected Class IIA2 BSC, Class IIB2 BSC or a ducted Class IIC1. Cidex is an example of a glutaraldehyde disinfectant.



IMPORTANT: Always refer to the product label for directions and for the list of agents the chemical is effective against. **Always** review the Safety Data Sheet for chemical disinfectants for hazards, recommended PPE and proper disposal methods. Corrosive, flammable or oxidizing chemicals should never be autoclaved.

Disinfectants and Inactivation Methods for Other Biological Materials

Human Materials (Blood, tissues, body fluids, cell lines, etc.)

OSHA has stated that any EPA-registered disinfectant effective against HIV and HBV or *Mycobacterium tuberculosis* will be effective in disinfecting and inactivating human materials under the Bloodborne Pathogens Standard (29 CFR 1910.1030). Refer to the product label for dilution instructions and contact times for effective disinfection. Liquid/ porous material needs to be autoclaved before disposal.

Prions:

Current recommendations from both the CDC and World Health Organization (WHO) for inactivation of prions include:

Instruments

Immerse in 1N NaOH or 2.5% NaOCl (sodium hypochlorite; 20,000 ppm available chlorine) for 1 hour; remove and rinse in water and then transfer to open pan and treat in a gravity displacement (121°C) or porous load (134°C) sterilization time 1 hour; clean; and subject to routine sterilization.

Surfaces

Spray or pour 2N NaOH or 2.5% NaOCl [sodium hypochlorite; 20,000 ppm available chlorine; 40% bleach solution (solution good for 30 days)] on surface and let sit for 1 hour. Ensure surfaces stay wet for the entire period and then rinse twice with water. Surfaces should be clean of any gross contamination as organic material can reduce the effectiveness of the solutions.



CAUTION: Wear appropriate PPE when using and be aware that hypochlorite damages stainless steel surfaces if not rinsed properly.

Biological Toxins:

Allow at least a 30-minute contact time for complete inactivation of toxin.

<i>Toxin</i>	2.5% NaOCl + 0.25 N NaOH ⁽¹⁾	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl
<i>Abrin</i>	YES	YES	YES	NO
<i>Botulinum neurotoxins A-G</i> ⁽²⁾	YES	YES	YES	YES
<i>Brevetoxin (PbTx-2)</i>	YES	YES	NO	NO
<i>Conotoxins</i>	YES	YES	YES	NO
<i>Diacetoxyscirpenol (DAS)</i>	YES	NO	NO	NO
<i>Microcystin</i>	YES	YES	YES	NO
<i>Palytoxin</i>	YES	YES	YES	YES
<i>Ricin</i> ⁽²⁾	YES	YES	YES	NO
<i>Saxitoxin</i> ⁽²⁾	YES	YES	YES	YES
<i>Shigatoxin</i>	YES	YES	YES	NO
<i>Staphylococcal enterotoxins</i> ⁽²⁾	YES	YES	YES	NO
<i>Tetrodotoxin</i> ⁽²⁾	YES	YES	YES	NO
<i>T-2 Toxin</i> ⁽³⁾	YES	YES	NO	NO

1. This is a caustic and corrosive material and may damage surfaces and equipment. Wear appropriate PPE. Neutralize after an appropriate contact time.
2. Inactivation for Saxitoxin, Tetrodotoxin, Ricin, Botulinum toxin, or Staphylococcal Enterotoxins B (SEB), exposure of 60 minutes to 10% sodium hypochlorite is an effective procedure for working solutions, equipment, working area and spills.
3. For complete inactivation of T-2 mycotoxins, all liquid samples, accidental spills, and non-burnable waste be soaked in 2.5% sodium hypochlorite with 0.25 N sodium hydroxide for 4 hours. Cages and bedding from

animals exposed to T-2 mycotoxin or brevetoxin should be treated with 2.5% sodium hypochlorite with 0.25 N sodium hydroxide for 4 hours.

Container Labeling Requirements

Disinfectant containers must be labelled just like any other chemical container, see the **EHS SOP Chemical Container Labelling** for general guidance. There are a few additional labelling requirements for containers of disinfectant solutions:

- The concentration of the disinfectant must be included on the label.
- The date the solution was prepared should be included on the label.
- The date the solution expires must be included on the label.

1:64 Neutral Q: Water
Made: 5/1/19, Exp: 5/14/19

Example Label

The dilution life of a disinfectant is important to its effectiveness as a disinfectant (see definition at the beginning of the SOP).

Disinfectant Selection and Preparation

Use the information in this document to assist you in selecting an appropriate disinfectant for the microbes or materials you are working with. Approved disinfectants for the agents used in your lab are listed in the approved IBC protocol for your lab. If you are unsure of the appropriate disinfectant to use or do not have access to the IBC protocol, contact EHS at 402.472.4925 or ehs@unl.edu.

Labs should establish procedures for regularly checking disinfectant solutions and ensuring they are made at the correct interval and concentration to be effective.

Disposal of Expired Disinfectants

Disinfectants that have lost their effectiveness due to use or expiration must be properly disposed. Most disinfectants are hazardous chemicals in their concentrated form, and EHS hazardous waste management procedures must be followed. Some disinfectants are safe to dispose of down the sanitary sewer at the working solution concentration; others must be collected by EHS for disposal. Please review the EHS SOPs **Sewer Disposal List** and **Hazardous/Radioactive Material Collection Procedures** to determine the appropriate means of disposal.

Sources:



- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th ed.; Meehan, P., Potts, J., Eds.; U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Institutes of Health, 2020.
- Rutala, W. A. (1996). APIC guideline for selection and use of disinfectants. *American Journal of Infection Control*, 24(4), 313-342.
- Rutala W. A., Cole G., Thomann, C. A., Weber, D. J. (1998). Stability and Bactericidal Activity of Chlorine Solutions. *Infection Control and Hospital Epidemiology* 19(5):323-7
- “Disinfection with Bleach”, 3M Tech Talk, June 2011
<https://multimedia.3m.com/mws/media/735976O/disinfection-with-bleach-tech-talk.pdf>
- Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute Respiratory Infections in Health Care. Geneva: World Health Organization; 2014. Annex G, Use of disinfectants: alcohol and bleach. Available from:
<https://www.ncbi.nlm.nih.gov/books/NBK214356/>
- <https://www.cdc.gov/prions/cjd/infection-control.html>
- J Hosp Infect. 2013 Dec;85(4):268-73. doi: 10.1016/j.jhin.2013.08.003
- “Disinfection 101,” Center for Food Security & Public Health, Iowa State University
www.cfsph.iastate.edu
- Guideline for Disinfection and Sterilization in Health Care Facilities (2008)
<https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html>

Liquid Disinfectant Comparison Table 1

<i>Disinfectant</i>	Quaternary ammonium compounds	Phenolic compounds	Chlorine compounds [5]	Peroxygen Compounds (Virkon® S)	Alcohol (ethyl or isopropyl)	Formaldehyde (liquid) (Formalin)	Glutaraldehyde	Hydrogen peroxide (liquid)
Use Requirements								
<i>Final Concentration for use</i>	0.1-2%	0.2-5%	500-10000 ppm available chlorine	1-2%	70-85%	10% formalin solution	2%	6-30%
Contact Times								
<i>Lipo viruses Only</i>	10 min	10 min	10 min	10 min	2-10 min	10 min	15 min	10 min
<i>Broad spectrum</i>	N/E	N/E	30 min	10 min	N/E	30 min	30 min	60 min
Inactivates								
<i>Vegetative bacteria</i>	X	X	X	X	X	X	X	X
<i>Enveloped viruses</i>	X	X	X	X	X	X	X	X
<i>Tubercle bacilli</i>		X	X	X	X	X	X	X
<i>Non-enveloped viruses</i>		[1]	X	X	[1]	X	X	X
<i>Bacterial spores</i>			X, [6]			X	X	X
Important Characteristics								
<i>Effective Shelf Life [2]</i>	>1 year See mfg label.	up to 1 week	24h (5000 ppm); 14d (8000 ppm) 30d (10,000 ppm)	Solution: 7 days	Check with mfg. or 1-3 years	>1 week	14 days	5-7 days
<i>Inactivated by organic matter</i>	X		X		X			
<i>Residual</i>		X	X			X	X	
<i>Corrosive</i>		X	X	X				X
<i>Flammable</i>					X			
<i>Irritant: Skin/eye/respiratory</i>	X / X / X	X / X / X	X / X / X	X / X / X	* / X / *	X / X / X	X / X / X	X / X / X
<i>Toxic</i>	X	X	X		X	X	X	
Applications								
<i>Work Surfaces</i>	X	X	X	X	X	X	X	X
<i>Equipment Surfaces</i>	X	X	X & [4]	X	X	X	X	X
<i>Lens Compatible [3]</i>	X						X	
Other considerations	[8]	[7], [8] Unpleasant odor	[7]	Can be used in foot baths		Carcinogen	[9]	[4] Oxidizer

KEY:

N/A = Not applicable	[3] = Refers to microscope and camera lenses	[7] = Effectiveness reduced by alkaline pH
N/E = Not effective	[4] = Will corrode stainless steel and other metals	[8] = Effectiveness influenced by hard water and detergents
X = Effective disinfectant/characteristic	[5] = 10:1 dilution of 5.25% bleach = 5000 ppm	[9] = Usable on plastics, rubber, lenses, and other items that cannot be autoclaved.
[1] = Variable results dependent on virus	[6] = >2500 ppm	
[2] = When protected from light and air		