Choosing and Using Disinfectants in the Laboratory

TUFTS UNIVERSITY BIOSAFETY OFFICE

OFFICE OF THE VICE PROVOST FOR RESEARCH

ONLINE: HTTPS://VICEPROVOST.TUFTS.EDU/BIOSAFETY/
EMAIL: BIOSAFETY@TUFTS.EDU
This guide will provide an overview of:

- The classes of disinfectants
  - Advantages and disadvantages of each class
- Types of microorganisms
  - General susceptibility of each type to the various classes of disinfectants
- Factors to consider when choosing a disinfectant
- General rules for using a disinfectant
- Links to resources for more information about disinfection
Decontamination terminology

Sterilization
- Destruction or elimination of all forms of life
- At Tufts, this is usually accomplished by autoclaving or incinerating

Disinfection
- Less lethal than sterilization
- Refers to elimination of most pathogenic microbes, but not necessarily spores

Decontamination
- The process of disinfection or sterilization of contaminated material to make it suitable for use
Disinfectants are not one-size-fits-all

4 Main Factors to consider when choosing a disinfectant

1. What type of organism are you handling?
2. What is the susceptibility of the organism?
3. Why are you using the disinfectant?
4. Chemical compatibility
What type of organism are you handling?

- Is it bacterial?
- Is it Viral?
- Is it a spore-former?
- What sort of sample is it?
What type of organism are you handling?

- **Is it** bacterial?
- **Is it** Viral?
- **Is it** a spore-former?
- **What sort of sample is it?**

**Gram-negative or Gram-positive?**
- Gram-negatives are typically less susceptible to disinfectants compared to Gram-positives

**Does it form biofilms?**
- Bacteria within biofilms are typically less susceptible to disinfectants compared to free-living bacteria

**Strain to strain variability**
- There is even strain to strain variation in susceptibility to disinfectants, so mutants may differ from the parental strain
What type of organism are you handling?

- Is it bacterial?
- Is it viral?
- Is it a spore-former?
- What sort of sample is it?

**Enveloped or non-enveloped?**

- Enveloped viruses are more susceptible to disinfectants than non-enveloped viruses because the lipid envelope is easily compromised by most disinfectants.
What type of organism are you handling?

Is it bacterial?  
Is it Viral?  
Is it a spore-former?  
What sort of sample is it?

Spores are meant to withstand environmental hardship and are typically very resistant to chemicals, dessication, UV, and more.
What type of organism are you handling?

Is it bacterial?  
Is it Viral?  
Is it a spore-former?  
What sort of sample is it?

Pure cultures can be easily characterized in terms of susceptibility to disinfectants, while mixed cultures could have range of organisms with differing susceptibility.

Clinical samples, even those that have been screened for select bloodborne pathogens, may still contain pathogens.

One must err on the side of caution when handling clinical and environmental samples.
Classes of Disinfectants

- **High level**: Kills vegetative bacteria, mycobacteria, fungi, viruses (+ or – envelope), but not necessarily bacterial spores.
- **Intermediate level**: Kills vegetative bacteria, most viruses and fungi. Variable activity against bacterial spores.
- **Low level**: Kills most (not all) vegetative bacteria, some fungi, and enveloped viruses. Limited or no activity on mycobacteria and bacterial spores.
What is the susceptibility of the organism?

GENERAL RESISTANCE TO DISINFECTANTS

- Prions
- Bacterial spores (*Bacillus*)
- Protozoal oocysts (*Cryptosporidium*)
- *Mycobacterium*
- Non-enveloped viruses (adenovirus and AAV)
- Fungi (*Cryptococcus, Aspergillus, Candida, Saccharomyces*)
- Gram-negative bacteria (*E. coli, Salmonella, Shigella, Vibrio, Yersinia*)
- Vegetative Gram-positive bacteria (*Staphylococcus, Streptococcus*)
- Enveloped viruses (HIV, Hepatitis B and C, Influenza, Herpes, Dengue)
Quaternary Ammonium Compounds (Quats)

- **Low level disinfectant**
  - Quats have innate detergent action but may be inactivated by some soaps or soap residues
  - Examples: Roccal, Lysol multi-surface cleaner

- **Typically uses**
  - Cleaning non-critical surfaces (floors, walls)
  - Use in conjunction with a stronger disinfectant for contaminated work surfaces and research materials

- **Contact time**
  - Usually 10 minutes

- **Check labels!**
  - Not all are approved for use with human source material due to limited activity

- **Advantages**
  - Odorless, deodorizing, non-irritating
  - Readily available
  - Stable in storage

- **Disadvantages**
  - Not effective on non-enveloped viruses, mycobacteria, or bacterial spores
Phenolics

Intermediate to low level disinfectant
• Examples: Vesphene, Lysol® Concentrate Disinfectant* see note below

Typical uses
• Surface disinfection

Contact time
• Usually 10 minutes

Check labels!
• Different formulations vary in activity against classes of microbes

Advantages
• Readily available
• Stable in storage

Disadvantages
• Variable activity on non-enveloped viruses and mycobacteria (check label)
• Does not work on bacterial spores
• Toxic and can be absorbed through skin
• Leaves a residue

*Lysol products have different formulations with varying disinfectant properties. Check the label to confirm activity against your microbe.
Iodophores

Intermediate to low level disinfectant

- An iodophor is iodine complexed with a carrier to increase solubility and allow sustained release

Available as

- Antiseptic for use on skin, example: Betadine
  - NOT FOR USE AS A LABORATORY DISINFECTANT due to lower iodine concentration
- Laboratory disinfectants for surface disinfection, example: Wescodyne

For surface disinfection:

- Use 1-3% iodophore solution (check label for specific information)

Contact time

- Usually 10 minutes on hard surfaces

Advantages

- Readily available, non-corrosive, low toxicity
- Long shelf life

Disadvantages

- For surface disinfection only
- Not effective on spores
Alcohols

**Intermediate level disinfectant**
- Examples: ethanol, isopropanol

**For surface disinfection only**
- 70% ethanol is most effective
- Higher concentrations of alcohol are LESS effective

**Contact time**
- Usually 10 minutes on hard surfaces
- Reapply as needed to keep surfaces wet for the full contact time

**Not for use with human source material due to limited activity**

**Advantages**
- Inexpensive, non-toxic and readily available

**Disadvantages**
- Extended contact times required for fungi and mycobacteria
- Not effective on spores or non-enveloped viruses
- Long contact times difficult to achieve due to evaporation
- Flammable
## Chlorine

### Intermediate level disinfectant

- **Examples:** Household bleach, Bleach-Rite, Austin’s A1, Clidox

### For general disinfection:

- Use ~5,000 ppm chlorine
- That is a 1:10 dilution of household bleach or Austin’s A1
- Bleach-Rite is already diluted to working concentration

### To disinfect material with high organic load:

- 20% household bleach is recommended
- Organic material reacts with chlorine, so there is less chlorine available

### Contact time varies

- Usually 1-10 minutes on hard surfaces
- Up to 20 minutes for cultures or spills with high organic load

### Chlorine dissipates over time

- Fresh solutions of dilute bleach must be made at least weekly (daily is best)

### Advantages

- Broadly effective on bacteria, mycobacteria, viruses, fungi
- Some activity against bacterial spores
- Inexpensive and readily available

### Disadvantages

- Limited shelf life unless stabilizers are present (such as in Bleach-Rite)
- Corrosive (rinse metal surfaces with water or alcohol to remove residue)
- Mixing chlorine with ammonium compounds creates poisonous gas (either avoid it or perform in chemical fume hood)
Hydrogen Peroxide

<table>
<thead>
<tr>
<th>High level disinfectant</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Examples: 3% hydrogen peroxide available from drug stores, Clorox Hydrogen Peroxide Spray and Wipes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For routine surface disinfection:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Check manufacturer labels for details since contact time varies according to the microbe and the concentration of hydrogen peroxide.</td>
</tr>
<tr>
<td>• Usually 10 minutes or less works for 1-3% hydrogen peroxide as a disinfectant (not sporicidal)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>As a chemical sterilant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hydrogen peroxide can be sporicidal when used at higher concentrations and extended contact times.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Broadly effective on bacteria, mycobacteria, viruses, fungi, and spores</td>
</tr>
<tr>
<td>• No residue (breaks down to H₂O and O₂)</td>
</tr>
<tr>
<td>• Short contact time</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Incompatible with rubber, some plastics, aluminum, copper, zinc, brass</td>
</tr>
</tbody>
</table>
Aldehydes

High-level disinfectants

• Examples: Glutaraldehyde and Formaldehyde
  • Glutaraldehyde must be activated (made alkaline) to kill bacterial spores
  • Formalin is 37% formaldehyde in water with methanol as stabilizer

Typical use:

• Formalin and glutaraldehyde are often used for tissue fixation, and in the process of fixing tissues microbes are also destroyed.
• Typically 2-4% of formalin or activated glutaraldehyde, freshly made, is used to fix tissues.
• Contact times range (minutes to hours) depending on concentration, nature of material, and microbe.

Avoid exposure!

• Glutaraldehyde is an irritant and suspected carcinogen, while formaldehyde is a known carcinogen and OSHA has set exposure limits.
• See EHS website to review the SOP for Formaldehyde, Paraformaldehyde and Formalin for more information

Advantages

• Broadly effective on bacteria, mycobacteria, viruses, fungi, and spores

Disadvantages

• Corrosive, toxic, carcinogenic
• Must be used with adequate ventilation and PPE
**Peracetic Acid**

### High level disinfectant
- Concentrated peracetic acid may be diluted and mixed with stabilizer using specialized equipment/sprayers
- SporGon and Spor-Klenz are available as ready-to-use mixtures of peracetic acid and hydrogen peroxide

### For general disinfection:
- Check manufacturer labels for concentration and contact times
- Usually 10-15 minutes for disinfection (not sporicidal)

### As a chemical sterilant:
- Can be used to kill spores, but requires higher concentrations and/or longer contact times (several hours to overnight)

### Advantages
- Broadly effective on bacteria, mycobacteria, viruses, fungi, and spores
- Breaks down to acetic acid, water, oxygen, hydrogen peroxide and leaves no residue

### Disadvantages
- Unstable; mixtures or dilutions must be made fresh immediately before use
- Can be corrosive
Why are you using the disinfectant?

- Liquid waste disposal
- Spill cleanup
- Surface decontamination
If handling human source materials, the disinfectant must be OSHA-approved:

- Bleach solutions (≥ 10% household bleach)
- EPA registered high-level disinfectants or sterilants (List A)
- EPA-registered disinfectants labelled as effect against tuberculosis (List B); both HIV and HBV (List D); tuberculosis, HIV and HBV (List E)
- See [EPA website](https://www.epa.gov) for lists
- See [OSHA](https://www.osha.gov) for more information
Liquid Waste Disposal

Choose effective product
- At Tufts, mercury-free bleach (Clorox or Austin’s A1) is most common.
- Remember, disinfectants are not one-size-fits-all!
- Must be effective on the organism and the material.
- Must be able to maintain proper concentration upon dilution into the liquid waste.
- Must be chemically compatible with the liquid. Some combinations (bleach plus ammonium compounds) produce dangerous fumes.

Apply disinfectant
- For bleach, add undiluted bleach until you reach 10-20% final concentration (0.5-1% sodium hypochlorite).
- More may be needed for high organic content, such as blood or serum.

Allow sufficient contact time
- Wait 30 minutes before disposing in sink.
- Longer contact time may be needed depending on your microbe and choice of disinfectant.
Spill Cleanup: Preparation

- Click here to access the Institutional Biosafety Manual or find it on the Biosafety website at https://viceprovost.tufts.edu/biosafety/resources/
- Give consideration to injuries, size of spill and/or generation of infectious aerosols before proceeding.
- Call for assistance if needed:
  - Call TUPD (617-627-6911) if it is a very large or dangerous spill or if there is an emergency situation.
  - Call Biosafety Manager (617-201-2479) to report the spill and/or receive advice on spill clean up.

Consult Institutional Biosafety Manual for general guidance on handling spills

- At Tufts, mercury-free bleach (Clorox or Austin’s A1) is most common.
- Remember, disinfectants are not one-size-fits-all!
- Must be effective on the organism and compatible with surface (ie- metal, plastic, etc).
- Must be able to maintain proper concentration upon dilution into the spilled liquid.

Choose effective product
Spill Cleanup: Procedure Overview

**WAIT!**
If the spill generated hazardous aerosols, leave the area and wait at least 30 minutes to allow the aerosols to settle.

**Put on PPE**
Lab coat, gloves, safety glasses are minimum; more may be needed depending on nature of spill and the type of disinfectant.

**Cover spill**
Use paper towels or other absorbent material to prevent splashing, and place a ring of paper towels around the spill to prevent spreading.

**Disinfect**
Pour undiluted bleach or other disinfectant over the spill.

**Allow contact time**
Wait 30 minutes to allow sufficient contact time before wiping up the spill.
- Longer contact time may be needed depending on your microbe and choice of disinfectant.

**Repeat!**
Repeat disinfection step to ensure thorough decontamination.
Rinse area with water or alcohol, if needed to remove disinfectant residue.
Surface Disinfection

Clean

• If the area is heavily soiled (dirty or has high organic load such as blood), you will need to clean before you can disinfect.
• Cleaners may include low-level disinfectants or soaps, but these may not be sufficient to disinfect depending on your microbe. The area should still be considered contaminated until proper disinfection has been completed.

Choose effective disinfectant

• At Tufts, mercury-free bleach (Clorox or Austin’s A1) or 70% ethanol is most common.
• Remember, disinfectants are not one-size-fits-all!
• Must be effective on the organism and the surface (metal, plastic, etc).

Apply disinfectant

• Thoroughly saturate the surface by spraying the disinfectant on the area, or use a paper towel, cloth, or wipe that is saturated with disinfectant.

Allow sufficient contact time

• Wait for designated time before wiping up the disinfectant (usually 10 minutes, but consult product label or ask Biosafety Office).
• You may need to reapply disinfectant to achieve proper contact time, especially if using alcohol-based products that evaporate quickly.
# Do’s and Don’ts

<table>
<thead>
<tr>
<th>Do</th>
<th>Don’t</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Consult the disinfectant product label, technical bulletin, and/or Safety Data Sheet</td>
<td>• Use 70% ethanol with human source material, adenovirus, spores</td>
</tr>
<tr>
<td>• Disinfect after any spill</td>
<td>• Use diluted bleach that is more than a week old</td>
</tr>
<tr>
<td>• Disinfect equipment and work surfaces at least daily</td>
<td>• Use a product that is chemically-incompatible with the liquid waste</td>
</tr>
<tr>
<td>• Disinfect work surfaces and equipment between experiments to prevent cross-contamination</td>
<td>• Use UV light as a disinfectant</td>
</tr>
<tr>
<td>• Check the shelf-life of your product</td>
<td></td>
</tr>
</tbody>
</table>
Remember

Avoid spreading contamination!

Always remove your PPE
- After cleaning up a spill
- After handling waste
- When finished working in the lab
- Before leaving the lab

And wash your hands!
## Important Resources

<table>
<thead>
<tr>
<th>Important Resource</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Click here to access the BMBL</td>
<td>Appendix B describes principles of disinfection</td>
</tr>
<tr>
<td>Click here to access the Chemical Disinfectants page</td>
<td>Modes of action, uses, data and literature references are included</td>
</tr>
<tr>
<td>Click here to access lists of EPA-registered disinfectants</td>
<td></td>
</tr>
<tr>
<td>Click here to access the Bloodborne Pathogens Regulations</td>
<td>Click here for a general overview of Bloodborne Pathogens, safety precautions and the regulations. Click here to access a quick reference guide for the regulations. Click here to access a very helpful FAQ.</td>
</tr>
<tr>
<td>Click here to access the Tufts Biosafety Office website</td>
<td>Contains Biosafety contacts, the Institutional Biosafety Manual and other resources, and information on handling accidents</td>
</tr>
<tr>
<td>Click here to access Tufts EHS Biosafety Support page</td>
<td>Includes links to Bloodborne Pathogen training and the Exposure Control Plan for Bloodborne Pathogens</td>
</tr>
<tr>
<td>Click here to access the Tufts Emergency Response Guide</td>
<td>Includes emergency contacts, instructions for handling biohazardous spill as well as other urgent scenarios</td>
</tr>
</tbody>
</table>

This is an excellent resource on chemical disinfectants
When in doubt, give us a shout!

Reach the Biosafety Manager any time at 617-201-2479

During business hours, call or email your campus Biosafety Officer

• Find us online at https://viceprovost.tufts.edu/biosafety/staff/