Culture Contamination Follow-Up and Decontamination

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Introduction

When biological cultures become contaminated, follow these steps to identify, remove, and prevent reintroduction of the contamination.

General Decontamination

To prevent contamination from spreading, focus decontamination efforts on the bays or equipment, like biosafety cabinets and incubators, where the outbreak was first observed.

To reduce the chance of reintroduction during decontamination efforts:
• Try to identify potential contamination sources, such as improperly sterilized media, inconsistently disinfecting equipment and surfaces between tasks, or using gloves across multiple spaces.

• Change your gloves frequently throughout cleanup.

• Wash your hands after you remove personal protective equipment (PPE) and between tasks.

To dispose of cleanup materials:

• Dispose of all cleanup materials following biological waste disposal procedures, unless they include hazardous chemicals. If hazardous chemicals are present, dispose of them as hazardous waste.

• Remove biological waste immediately after cleanup.

• Send out all used cloth lab coats for laundering through a laundry service; do not take used lab coats home.

• Dispose of disposable PPE in biological waste.

**Surface Decontamination**

To disinfect surfaces, you should typically use either bleach (bleach wipes or standard bleach diluted to 10%) or hydrogen peroxide-based disinfectants.

Do not mix disinfectants, and use all products as directed on their labels and safety data sheets (SDS) for appropriate concentrations and contact times. Refer to PPE compatibility guidance charts and SDSs.

Do not spray bleach, as some people may be sensitive to the odor and vapors. Spraying bleach further aerosolizes particles and does not provide complete coverage of the surface area.

**Decontamination by Material and Surface Type**

Use the information in this table to identify the appropriate disinfectant and decontamination process for each type of material and surface.
<table>
<thead>
<tr>
<th>Material or Surface Type</th>
<th>Disinfectant and Decontamination Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture waste</td>
<td>To decontaminate liquid culture waste, disinfect with bleach (final liquid volume of 10% bleach). Dispose of solid cultures in biological waste.</td>
</tr>
<tr>
<td>Equipment surfaces</td>
<td>To wipe down sensitive equipment surfaces, you may use hydrogen peroxide-based disinfectants (for example Oxivir or Clorox Healthcare® Hydrogen Peroxide). For more information, see <a href="#">Equipment Decontamination</a>.</td>
</tr>
<tr>
<td>Surfaces, such as bench tops</td>
<td>Disinfect surfaces using either bleach wipes or hydrogen peroxide-based disinfectants.</td>
</tr>
</tbody>
</table>

### Cold and Warm Room Decontamination

When decontaminating cold and warm rooms, follow these requirements.

**During chemical decontamination:**

- Stop all operations in the warm or cold room.
- Work with Facilities to shut off the ventilation, heating, and cooling. This prevents heat from further volatilizing chemical vapors and the fan preventing aerosols from settling.
- Ensure the door to the room remains open for the entire duration of chemical disinfection. This is important because cold and warm rooms don’t have exhaust ventilation.
- Start the decontamination process by decontaminating surfaces.

  If chemical surface decontamination doesn’t work, contamination persists, and your lab decides to pursue more rigorous measures, contact a vendor to perform a full room decontamination. Don’t try to decontaminate a full room on your own.

**After chemical decontamination:**
- Ensure the door to the room remains open for approximately 1 hour and 2 hours after decontamination or until the odor is undetectable.

**Equipment Decontamination**

When decontaminating equipment, follow these requirements.

- Begin by chemically decontaminating equipment surfaces.
- If chemical surface decontamination doesn’t work, contamination persists, and your lab decides to pursue more rigorous measures, contact a vendor to decontaminate a whole piece of equipment through gaseous or vapor decontamination.
- Don’t try to decontaminate a whole piece of equipment on your own.

**Decontamination by Equipment Type**

For more information about disinfectants and decontamination, see [Surface Decontamination](#).

<table>
<thead>
<tr>
<th>Equipment Type</th>
<th>Decontamination Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosafety cabinets</td>
<td>Clean and disinfect the entire interior of the biosafety cabinet, including underneath the grate.</td>
</tr>
<tr>
<td>Glassware</td>
<td>Soak in disinfectant solution for between 20 minutes and 30 minutes.</td>
</tr>
<tr>
<td>Equipment Type</td>
<td>Decontamination Process</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Incubators</td>
<td>Dispose of water in incubator trays, disinfect the trays through autoclaving or chemical decontamination, and replace trays with sterile water (also potentially treated). If the incubator has a built-in disinfectant cycle, run the disinfectant cycle. Otherwise, check the HEPA filters and replace them if necessary. Ensure the incubator is shut off and then completely wipe down the interior of the incubator with an appropriate disinfectant. Autoclave the racks and trays or wipe them down with an appropriate disinfectant.</td>
</tr>
<tr>
<td>Water baths</td>
<td>Drain water baths and wipe the interior with a disinfectant. If using metal beads in place of water, autoclave the beads. Replace with fresh water or metal beads.</td>
</tr>
</tbody>
</table>

**Working with Cultures**

Follow these work practices and PPE measures to reduce the risk of future contamination:

- Wear appropriate PPE, including lab coats.
  - Change your gloves frequently.
  - Never reuse gloves or walk between rooms while wearing gloves. You could easily pick up contamination on a single surface and transfer it throughout the entire facility.
  - Launder and change your PPE when necessary.
  - Wash your hands after you remove PPE and between tasks.
- Don't use or touch personal items while working in a lab.
Never touch cellphones and headphones while wearing gloves, which can carry and transmit sources of contamination.

- Decontaminate all surfaces before and after work with fresh 10% bleach (0.525% sodium hypochlorite or greater) or hydrogen peroxide-based disinfectant wipes.

  Decontaminate shared equipment as often as you would clean your own.

- Autoclave all media before use.
  - Perform spore testing to document autoclave effectiveness.
  - If possible, treat media with agents to prevent new growth.

- When feasible, use disposable loops, culture dishes, and flasks to reduce reintroducing contamination.

  Avoid flaming loops, which can lead to aerosolization of bacteria on benches. Instead, use disposables and dispose of them following biological waste disposal procedures.

- Routinely test cultures for contamination, especially new, non-commercial cultures.

**Resources**

- [America Biological Safety Association Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets](#).
- [EH&S Disinfection Guide](#).
- [EH&S Working Safely in a Biosafety Cabinet Training](#).
- [Selected Environmental Protection Agency-Registered Disinfectants](#).
- [Thermo Fisher Scientific Aseptic Technique](#).
Appendix 1: Ultraviolet Light Disinfection is Prohibited

Don’t use ultraviolet (UV) light for full room or surface disinfection.¹

UV lights pose a high risk to people in the area and are mostly ineffective for decontamination because:

- UV lights can cause damage to both your skin and your eyes, even after their intensity drops below the effective level.
- UV lights inactivate microorganisms by destroying nucleic acid by inducing thymine dimers. This process is most effective on naked nucleic acids and isn’t as effective on materials like phages and bacteria.²
- Because of the limited germicidal effectiveness of UV radiation, you must clean and disinfect surfaces before using UV light, and even then, its usefulness is unreliable.³
  - UV light disinfection only works at very specific wavelengths.⁴
  - The further away a UV light is from a surface, the less effective it is at disinfecting the surface.
  - UV light can’t disinfect areas with high surface complexity, like benchtops, because shadows prevent the light from reaching all the necessary areas for decontamination.

¹ Before installing or using any room UV lights, contact Radiation Safety Services.

² For more information, review Labconco: 2 biosafety cabinet UV lamp rules and Centers for Disease Control and Prevention Infection Control: Miscellaneous Inactivating Agents.

³ The germicidal effectiveness and use of UV radiation is influenced by organic matter, wavelength, type of suspension, temperature, type of microorganism, and UV intensity, which is affected by distance and surface textures and cleanliness.

⁴ For example, mercury vapor lamps emit more than 90% of their radiation at 253.7 nm, which is near the maximum microbicidal activity. However, this effectiveness starts degrading immediately.