Biosafety Training

Yale University
Environmental Health & Safety

Written and Produced by: Tom Ouimet and Ben Fontes
Learning Objectives

At the end of this training you will be able to describe:

- how microorganisms enter our bodies and the difference between infection and exposure
- the characteristics of the different infectious agent risk groups

You will also be able to:

- Locate agent specific information useful in conducting a risk assessment
- Conduct a risk assessment on a protocol involving microorganisms, toxins or recombinant DNA
- Manage the risks of handling biohazardous agents by selecting appropriate administrative, procedural and equipment controls
Laboratory Acquired Infections

Laboratory acquired infections can result from a wide variety of exposures including:

- needle sticks
- cuts and abrasions from contaminated items
- animal bites
- mucous membrane contact with contaminated items
- ingestion of contaminated items, and
- inhalation of aerosols generated by spills, other accidents and work practices

Being splashed with blood when not wearing appropriate face and eye protection is an example of a laboratory exposure that could lead to an infection. Gloves should also have been worn.
Laboratory Acquired Infections

The studies of laboratory acquired infections (LAIs) demonstrated that many microbial agents are potentially hazardous to individuals within the laboratory as well as those working in surrounding areas if control measures are not applied.

Only 20% if the LAI could be attributable to a recognized accident. In 80% of the LAIs the exposure source was unknown but believed to be due to the inhalation of aerosols.

Sulkin & Pike 1979
Objective of the Biosafety Program

Yale's Biosafety Program began in 1977 with the following objective:

- to control the hazards associated with the handling and use of infectious agents on campus and thereby minimize occupationally acquired infections and laboratory related injuries

The success of the Biosafety Program depends on a high level of awareness of safety issues by laboratory personnel and strict adherence to policies and procedures.
Infectious Disease Process

What is the difference between exposure and infection?

You are exposed to an infectious agent when it...
- is inhaled
- contaminates your mucous membranes
- enters a break in the skin
- is ingested, or
- enters the body through a puncture wound

Exposure does not always lead to infection...

For a microorganism to cause an infection it must...
- gain entry into the body
- migrate to a susceptible organ, tissue or cell and
- establish a colony
Infectious Disease Process

An organism's ability to jump these hurdles is dependent on the...
- virulence and pathogenicity of the pathogen
- the amount of pathogen that enters the body
- route of entry into the body
- ability of the pathogen to find tissues or cells that are susceptible
- ability of the pathogen to evade the body's immune system, and
- health and immunization status of the exposed individual

Again.. exposure does not always lead to infection. Individual factors often come into play.
Infectious Dose

Agent's infectious doses vary widely

Larger doses are generally required to cause infection by ingestion when compared to inhalation

<table>
<thead>
<tr>
<th>Disease or Agent</th>
<th>Dose</th>
<th>Route of Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q fever</td>
<td>10</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>10</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Influenza A2</td>
<td>790</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>$10^5$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Cholera</td>
<td>$10^8$</td>
<td>Ingestion</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>$10^8$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Shigella</td>
<td>$10^9$</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Polio virus 1</td>
<td>2</td>
<td>Ingestion</td>
</tr>
</tbody>
</table>

1 number of viable microorganisms
Risk Groups and Exposure Controls

Infections may result in minor discomfort to death, depending on the agent and individual factors.

Research with Risk Group 1, 2, and 3 agents are conducted on campus.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Risk Group 1</th>
<th>Risk Group 2</th>
<th>Risk Group 3</th>
<th>Risk Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Does not cause disease in healthy individuals</td>
<td>Can cause infection of varying severity, rarely lethal</td>
<td>Agents associated with moderate to severe disease outcome – can be lethal</td>
<td>Capable of causing severe disease with lethal outcome</td>
</tr>
<tr>
<td>Availability of Treatment</td>
<td>Not applicable</td>
<td>Treatment usually available or host immune system is capable of controlling</td>
<td>Treatment may not be available</td>
<td>Treatment is generally not available – experimental treatment regimens possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routes of Transmission</td>
<td>Not applicable</td>
<td>Ingestion, through the skin and via facial mucous membranes</td>
<td>Same as RG2 plus inhalation</td>
<td>Same as RG3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease Severity to Individual</td>
<td>None to healthy adults</td>
<td>Low to moderate</td>
<td>Moderate to high – higher mortality and morbidity</td>
<td>High – highest mortality rates in this category (RG)</td>
</tr>
<tr>
<td>Community Risk</td>
<td>Low</td>
<td>Low</td>
<td>Low to moderate</td>
<td>High – perception of risk is also very high</td>
</tr>
<tr>
<td>Infectious Dose</td>
<td>Not applicable</td>
<td>Generally high (variable)</td>
<td>Low doses capable of infection</td>
<td>Can be as low as one organism</td>
</tr>
<tr>
<td>Example Agents</td>
<td>Non-conjugative strains of E. Coli, rodent cell lines, Saccharomyces cerevisiae</td>
<td>Trypanolsomes, Leishmania, Shigella, Salmonella, HBV, HCV, Borrelia</td>
<td>M. tuberculosis, West Nile virus, Yellow Fever virus, Rickettsia rickettsi</td>
<td>Ebola virus, Sabia virus, Marburg virus, Equine Morbillivirus</td>
</tr>
</tbody>
</table>
## Risk Groups and Exposure Controls

<table>
<thead>
<tr>
<th>Route of Transmission</th>
<th>How Exposure Typically Occurs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td>Release of infectious aerosols by lab procedures or equipment</td>
</tr>
<tr>
<td>Contact with non-intact skin, mucous membranes;</td>
<td>Contamination of work surfaces, skin, mucous membranes, hands, and objects placed in the mouth through spills, splashes, splatter or settled aerosols of infectious materials</td>
</tr>
<tr>
<td>Ingestion</td>
<td>Poor personal hygiene, eating/drinking/smoking in lab</td>
</tr>
<tr>
<td>Puncture</td>
<td>Injection of infectious material through skin via needle stick, puncture with a contaminated sharp object or animal bite or scratch</td>
</tr>
</tbody>
</table>
Risk Groups and Exposure Controls

The policies and procedures developed by the biological safety program are intended to prevent exposures. The level of control used is related to the risk posed by the infectious agent. The greater the risk the more extensive the exposure controls applied. The types of controls incorporated into protocols may include:

- following specific work practices
- working within containment equipment (such as the biological safety cabinet)
- working in specially designed laboratories

The level of control is determined by the risk posed by the agent.
Risk Groups and Exposure Controls

Exposure controls used with lower Risk Group agents are followed and expanded on when working with higher Risk Group agents.
Risk Groups and Exposure Controls

So, how does one select the procedures to be followed and exposure controls used during a specific protocol?

- Each protocol must undergo a comprehensive risk assessment
- Identified risks are addressed with appropriate procedures and controls in the risk management step

Protocols must undergo a risk assessment to identify the hazards associated with them.
Summary

In this section of the training program we learned that...

- not all exposures lead to infections
- infectious agents are categorized by Risk Group (1-4) with Risk Group 1 agents representing low risk and Risk Group 4 agents representing the highest risk
- we may be exposed to infectious agents through inhalation, ingestion, contact with non-intact skin and mucous membranes or through direct injection into the body
- we establish procedural and engineering controls to prevent these exposures and the higher the risk group of the agent handled the more controls put in place
Risk Assessment & Risk Management
Overview - Agent Classification

The risk of a protocol is defined by the:
- agent handled
- procedures followed
- experience and health status of personnel

The first step is to learn as much as possible about the agent including its pathogenicity, communicability and survival in the environment.

Risk of handling an agent increases with increasing pathogenicity, communicability and survival in the environment.
Procedure Related Factors

The procedures followed are the second factor that contributes to risk.

Some procedures are more inherently hazardous due to their potential to:
- release aerosols
- spread contamination
- result in skin punctures or inoculations

Centrifuges can release aerosols if proper work practices are not followed.
Personnel Factors

The personnel conducting the work also may introduce risks.

Personnel working with pathogens should be:
- experienced, well trained and capable of performing the necessary tasks safely
- healthy and particularly have a healthy immune system
- comfortable in performing the proposed work
Risk Management Overview

During the risk management phase the risks identified in the risk assessment are controlled. To control these risks...
- personnel may require more training, vaccination or additional experience
- specialized personal protective equipment (PPE) may be specified
- work may be performed in specialized containment equipment
- work may be performed in specially designed facilities that prevent the release of the agent into the environment

You must make certain the appropriate controls are in place before beginning your work.
CDC's Risk Management Guidelines

The CDC/NIH guidelines can serve as a starting point for determining a protocol's risk management requirements.

In many cases you can simply follow the guidance in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* or BMBL.

In other cases the procedures used or factors associated with the infectious agent will modify the risk and allow or require deviations from the BMBL.
Worked Examples - Rabies and TB

Assume that you would like to work with the Rabies virus and TB... What practices should be followed?
Worked Examples - Rabies and TB

Assume that you would like to work with the Rabies virus and TB... What practices should be followed?

This laboratory will grow large volumes of rabies virus and isolate the virus in a centrifuge.

These laboratory procedures can create aerosols and airborne concentrations of the virus that have resulted in infections by inhalation, a route of transmission not found in nature.

In this case the work culturing and isolating rabies should be performed with BSL3 containment even though rabies is a Risk Group 2 agent.
Worked Examples - Rabies and TB

Assume that you would like to work with the Rabies virus and TB... What practices should be followed?

Suppose a laboratory was handling an attenuated strain of TB, rendered non-infectious to humans.

Does this risk group 3 agent have to be handled with BL3 controls?

This work could be conducted at BSL1 or BSL2 after confirming that the strain of TB handled was indeed non-infectious.
Worked Examples - Rabies and TB

Assume that you would like to work with the Rabies virus and TB... What practices should be followed?

Take home lesson....

Although the agent (its Risk Group) typically defines the containment level (Biosafety Level) of the work, the procedures followed and factors related to the agent can modify this decision. This should be identified during the risk assessment process.
### Risk Management - Additional Resources

<table>
<thead>
<tr>
<th>rDNA</th>
<th>Toxins</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHS poster describing experiments which must be approved prior to initiation</td>
<td>EHS poster describing safe work practices when handling toxins</td>
<td>CDC/NIH guidelines (BMBL) vertebrate animal Biosafety Level criteria</td>
</tr>
<tr>
<td>EHS overview of experiments covered by NIH Guidelines</td>
<td>CDC/NIH guidelines (BMBL) for work with toxins of biological origin</td>
<td>Working safely with laboratory animals (training videos)</td>
</tr>
<tr>
<td>Animal experiments covered under NIH guidelines</td>
<td>Animal experiments covered under NIH guidelines</td>
<td>Animal experiments covered under NIH guidelines</td>
</tr>
<tr>
<td>Link to complete NIH guidelines</td>
<td>Link to complete NIH guidelines</td>
<td>Link to complete NIH guidelines</td>
</tr>
</tbody>
</table>
Summary

The types of exposure controls will vary based on the Risk Group of the agent handled, the procedures followed and health status of individuals involved.

The CDC/NIH BMBL defines appropriate work practices, safety equipment and facility requirements for work with infectious agents and is a good starting point for determining a protocol's risk management requirements.

Certain agents, termed select agents and USDA regulated agents, are highly regulated and require very special procedures and approvals before being brought on campus.
Learning Objectives
At the end of this training you will be able to describe:

- the labeling and signage requirements when working with risk group 2 agents
- the rational for a medical surveillance program
- the importance of the three "Cs" of chemical disinfection
- the laboratory equipment features that limit the release of aerosols

You will also be able to:
- Properly select, put on and take off BL2 PPE
- Work safely with sharps
- Work safely in a biological safety cabinet
- Properly dispose of biomedical waste
- Respond appropriately to infectious material exposures
Overview

The requirements at BSL2 are composed of those of those required for work with all biological material plus the additional requirements necessary when working with Risk Group 2 agents.

We will review the requirements in each of the following topics:

- Containment
- Medical evaluation/surveillance
- Labeling and signage
- Personal protective equipment
- Practices and procedures
  - personal hygiene
  - aerosol containment
  - use of lab equipment
  - Use of biological safety cabinet
  - disinfection and cleaning
  - use of sharps
  - Waste disposal
  - Emergency response and reporting of incidents
Facility, Personnel & PPE
Surfaces are easily cleaned and hand washing sinks are readily available.
At BSL2 most work is conducted in a biological safety cabinet.
BSL3 Laboratory

Special ventilation system

Autoclave immediately available

Special anterooms required

(Graphic from WHO Laboratory Biosafety Manual and provided by CUH2A, Princeton, NJ, USA)
Medical Evaluation and Screening

Employee Health oversees Yale's medical surveillance program for personnel engaged in biological research.

The purpose of the medical surveillance program is to:

- recommend appropriate medical precautions when working with specific biological agents, and
- periodically re-evaluate employees to determine if any medical conditions related to work with biological agents are present, and if so, address them
Medical Evaluation and Screening

Medical evaluation and screening is generally not required when working at Biosafety Level 1 (immunodeficient or allergic individuals may wish to discuss their health status with Employee Health)

When working at Biosafety Level 2 the medical surveillance program might call for:
- immunizations
- periodic examination
- collection of a serum specimen

The required medical surveillance will vary for a specific employee and will be dependent on:
- the nature of the research project
- the biological agents handled
- the health status of the individual

An immunization may be required when working with some agents.
Medical Evaluation and Screening

Employee Health is also a source of information and counseling for:

- pregnant women
- men and women working with reproductive biological hazards and considering conceiving children, and
- individuals who may be immunocompromised

Examples of biological agents capable of causing adverse reproductive effects:

- Hepatitis B
- Brucella
- Rubella virus
- Cytomegalovirus
- Herpes simplex virus I and II
- Human immuno-deficiency virus
- Human pavovirus B19
- Toxoplasmosis
- Venezuelan equine encephalitis virus
- Syphilis
- Varicella
- LCMV

Employee Health can assist you evaluate the risk of working with an agent if special health conditions apply.
Laboratory Signage and Labeling

Laboratory signage and labels warn us that a biohazardous material may be present in a laboratory, in a container or in a piece of equipment.
Laboratory Signage and Labeling

Biohazard labels warn of potential contamination and must be applied to:
- containers used to store, transport or ship Risk Group 2 materials
- equipment containing or contaminated with Risk Group 2 materials

The label should contain the name of the infectious agent or "human materials" if it is just human cells, blood or similar material.

Equipment decontaminated between uses may be labeled temporarily when being used with Risk Group 2 agents.

Labels must be applied to sample containers with RG2 agents and equipment contaminated with RG2 agents.
Personal Protective Equipment

PPE adds a barrier to your body and prevents contamination of your eyes, mouth and skin (as well as your street clothes)

PPE should not be worn outside the laboratory which can spread contamination

PPE is selected based on the specific task being performed and level of risk associated with the research

Remember... PPE serves as a second line of defense. Good laboratory technique and use of appropriate containment equipment should always be the primary barriers to exposure and used to the extent feasible before relying on PPE.
Personal Protective Equipment - BSL 1

The following PPE should be worn when working with any biological material:
- gloves
- a lab coat
- protective eyewear

Skin surfaces below the waist should be covered when working in the laboratory.

PPE can also be worn to protect your product from contamination.
Personal Protective Equipment - BSL 2

The following additional PPE is typically worn when working at Biosafety Level 2.

In laboratories - a face shield is added to gloves and lab coat to provide mouth, nose and eye protection.

When working with laboratory animals - a surgical mask is added to gloves and lab coat or gown to protect the nose and mouth and a face shield protects the eyes.

Double gloving is also recommended when working at BSL2.

When there is a high potential for splashing of potentially infectious materials the following additional PPE may be recommended:

- Tyvek coveralls
- booties
- sleeve guards
- plastic aprons
- thicker, longer rubber gloves
Personal Protective Equipment - BSL2

Care must be taken when removing your PPE to prevent contamination of yourself or your surroundings.

The most contaminated items are always removed first.

Always wash your hands thoroughly after removing PPE.
Personal Protective Equipment - Special Issues

Cover any minor cuts, abrasions or other disruptions of the skin on the hand with a waterproof bandage and double glove.

If you cannot do this, do not work with Risk Group 2 agents until healed or obtain a clearance to work from Employee Health.

Avoid wetting gloves with disinfectants, select a glove that is resistant to the disinfectants and solvents handled and examine gloves regularly for deterioration.

Check gloves for breaks when you don them, change them regularly and consider double gloving.

If you have a latex allergy, wear nitrile gloves or gloves composed of a "non-latex" polymer.

Wear nitrile gloves in place of latex if you are allergic.
Personal Protective Equipment - final thoughts

To prevent contaminating objects in the laboratory or adjacent areas:
- avoid touching shared clean surfaces with gloved hands
- remove your gloves as well as other PPE and wash your hands before exiting the lab

Don't contaminate objects in your lab by touching them with gloves
Summary

In this section of the training program we learned that...

- Work with RG2 agents is performed in a facility designed to BSL2 specifications.
- Immunizations, a periodic physical examination and other medical procedures may be required when working with RG2 agents.
- Biohazard signs must be posted at the entrances of laboratories working with RG2 agents.
- All equipment and containers in which RG2 agents are handled or stored must have a biohazard label.
- Additional personal protective equipment is worn when working with RG2 agents and it must be removed in sequence to minimize contamination.

BL2 researcher preparing to work with animals.
Review of Good Work Practices
Personal Hygiene - Hand Washing

When washing your hands use soap and rub your hands together for at least 30 seconds

Rinse thoroughly with water

Turn the facet off with a paper towel to prevent re-contaminating yourself

Rub your hands with Purell if a sink is not immediately available for hand washing and wash your hands as soon as possible.
Aerosols

Infectious aerosols are airborne particles that contain microorganisms

Aerosols are released during spills and other accidents as well as by some laboratory procedures

Infectious aerosols will vary by size, ranging from one to several hundred microns

Aerosols greater than 50 microns settle quickly

\[ V_s = \frac{2}{9} \left( \frac{\rho_p - \rho_f}{\mu} \right) g R^2 \]

Stoke's settling velocity equation

Spills and many lab procedures can create aerosols
### Aerosols

<table>
<thead>
<tr>
<th>Particle Diameter (microns)</th>
<th>Settling Velocity (ft/min)</th>
<th>Settling Time From Five Feet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.006</td>
<td>13.8 hours</td>
</tr>
<tr>
<td>4</td>
<td>0.094</td>
<td>52 minutes</td>
</tr>
<tr>
<td>16</td>
<td>1.51</td>
<td>3.3 minutes</td>
</tr>
<tr>
<td>64</td>
<td>24.2</td>
<td>12 seconds</td>
</tr>
</tbody>
</table>
Aerosols

Pipetting
Use of a Bunsen burner
Streaking plates
Opening tubes/plates
Hand homogenizing tissue
Filling syringe
Many exposures have been associated with the opening of vacutainer or eppendorf tubes by hand where slight pressure and the energy involved in the process has led to creation of splash, splatter, or aerosols, usually directed upward at the face of the worker.

Such tubes should be opened away from the operator, preferably within a biological safety cabinet. Alternatively, tubes can be covered with a cotton pledgelet or a disinfectant-wetted gauze to completely cover the top of the tube while opening to confine any splash or splatter.
Aerosols

Following good laboratory practices reduces but does not eliminate the generation of aerosols.

When bioaerosols may be infectious, laboratory procedures are conducted in containment devices such as the biological safety cabinet.
Aerosol Released From Equipment

Standard laboratory equipment also has the potential to release high concentrations of bioaerosols.
# Aerosol Containment Equipment

## Viable Particles Released Into The Laboratory While Operating Equipment

<table>
<thead>
<tr>
<th>Type Equipment</th>
<th>Viable particles/ft³ of air</th>
</tr>
</thead>
<tbody>
<tr>
<td>High speed blender – normal operation</td>
<td>119.6</td>
</tr>
<tr>
<td>High speed blender – top removed after operation</td>
<td>1,500</td>
</tr>
<tr>
<td>Sonicator</td>
<td>6.3</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>1.9</td>
</tr>
<tr>
<td>Mechanical mixing Vortex (and opening tube cap)</td>
<td>9.4</td>
</tr>
</tbody>
</table>

*Kenny and Sabel, 1968*
Aerosol Containment Equipment

Laboratory equipment can generate sufficient concentrations of aerosols to cause infections by inhalation even when inhalation is not a natural route of exposure.

Case Study

Rabies in nature is transferred by skin punctures or animal bites (it is not an airborne pathogen)

In 1973 a researcher contracted rabies following the inhalation of an aerosol generated by a blender
Aerosol Containment Equipment

The laboratory acquired infections that resulted from aerosols generated by laboratory equipment stimulated:

- the development of specialized equipment capable of containing aerosols
- new procedures for working with this equipment

In 1973 a rabies infection resulted from the inhalation of an aerosol generated by a blender.
Aerosol Containment Equipment

Non-infectious samples are centrifuged on a bench-top unit in eppendorf tubes or, if slightly higher risk, sealed tubes.
Aerosol Containment Equipment

Non-infectious samples are centrifuged on a bench-top unit in eppendorf tubes or, if slightly higher risk, sealed tubes.

Infectious samples are placed in sealed secondary containers prior to centrifugation and the secondary container may be a sealed rotor.
Aerosol Containment Equipment

Non-infectious samples are centrifuged on a bench-top unit in eppendorf tubes or, if slightly higher risk, sealed tubes.

Infectious samples are placed in sealed secondary containers prior to centrifugation and the secondary container may be a sealed rotor.

The secondary container is then opened in a biological safety cabinet after waiting 2-5 minutes for aerosols to settle.

Some centrifuges are equipped with a local exhaust ventilation system that removes aerosols at their point of release.
Biological Safety Cabinet

The biological safety cabinet (BSC) is an example of primary containment equipment.

It contains and removes aerosols that are generated within it.

Its surfaces are easily cleaned and disinfected.

When exposure to bioaerosols may be infectious, it is the engineering control of choice and all procedures should be conducted within it.

BSCs come in a variety of types and selection is based on if your work can tolerate contamination by room air and if hazardous chemicals will be released in the cabinet.
Working Safely In a Biological Safety Cabinet

If you work with infectious materials in a BSC, you must know how to use it effectively.

This video describes:
- what to wear while work in a BSC
- how to prepare the cabinet for work
- how to work within the cabinet
- how to respond to spills within the cabinet
- how to remove your material, disinfect the cabinet and remove your PPE
Decontamination And Cleaning

It is essential that a chemical disinfectant be selected that has proven effectiveness against the family of biological agents you wish to inactivate.
Decontamination And Cleaning

Finally, a disinfectant must have sufficient contact time to be effective - they do not work instantaneously.

A contact time of ten minutes is used in evaluating the effectiveness of disinfectants.

Use a contact time of at least ten minutes when disinfecting objects or surfaces with a chemical disinfectant.

The disinfectant must have sufficient contact time to be effective.
Decontamination And Cleaning

Work surfaces, tools and equipment that become visibly contaminated during your procedures should be immediately decontaminated.

Work surfaces should also be cleaned and sanitized after you have completed your work.

When working at BSL2, work surfaces should be disinfected with disinfectant specific to the organism being studied:
- before beginning work, and
- when your work is completed.
Decontamination And Cleaning

Biohazardous samples transported outside the laboratory must be decontaminated and packaged in the following way:

- the primary container should have a screw top and all sides top and bottom must be decontaminated before placing it into a labeled secondary container
- the secondary container should also have a screw top. All outer surfaces of this container must be decontaminated before it is removed from the biological safety cabinet
Equipment, such as this biological safety cabinet (contaminated or potentially contaminated with infectious materials) must be decontaminated and certified free from any biological hazard before being disposed of or sent out for repair.
Decontamination And Cleaning

BIOSAFETY NOTICE

This equipment's exterior and interior surfaces were decontaminated to prevent the spread of Biological Hazards. This notice does not apply to radiation or chemical hazards.

This equipment is released for: (Circle one)
Service/Repair
Relocation
Discard

Decontamination performed by: ________________________________
Chemical or disinfectant used: ________________________________
Date of decontamination: ________________________________
Location of equipment: ________________________________
Lab telephone number: ________________________________

Note: The following areas ________________ of this equipment remain contaminated and a biohazard warning label has been attached near the contaminated area.

Additional forms are available through the Office of Environmental Health and Safety.

Yale University
Office of Environmental Health and Safety 785-3550
Correct Use Of Sharps

When working at BSL2 avoid the use of sharps whenever possible

If a sharp must be used select the safest sharp possible and follow safe sharp practices
Summary

In this section of the training program we learned its important to...

- wash your hands whenever you think they may be contaminated and after removing your gloves
- recognize that aerosols are released from the procedures you follow and the equipment you use. Work in containment equipment such as the biological safety cabinet and select laboratory equipment with the appropriate safeguards.
- follow the three “Cs” of chemical disinfection - select the appropriate chemical disinfectant, use it in the appropriate concentration and allow for sufficient contact time
- substitute sharps with non-sharp items or safer sharps whenever possible
Waste Disposal & Emergency Response
Waste Disposal

Proper disposal of infectious material prevents exposure to co-workers and members of the community.

Sharps must be placed in needle boxes.
Waste Disposal

Proper disposal of infectious material prevents exposure to co-workers and members of the community.

Sharps must be placed in needle boxes.

Waste that could puncture a bag and break the skin are placed in a red-bag lined medical waste cardboard box.
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Waste that could puncture a bag and break the skin are placed in a red-bag lined medical waste cardboard box.

Higher risk waste must be treated or disinfected prior to disposal by the laboratory generating it.

Only the high risk biomedical waste must be autoclaved by the lab before being removed by EHS.
Waste Disposal

Proper disposal of infectious material prevents exposure to co-workers and members of the community.

Sharps must be placed in needle boxes.

Waste that could puncture a bag and break the skin are placed in a red-bag lined medical waste cardboard box.

Higher risk waste must be treated or disinfected prior to disposal by the laboratory generating it.

All infectious waste containers (those that contain waste contaminated with RG2 agents or materials) must be labeled with a biohazard sticker (or symbol printed on box) and closed when transported out of the laboratory.
Waste Disposal

Infectious waste is often autoclaved before leaving the laboratory.

The high temperature and moisture denatures proteins killing the microorganisms.

Wear PPE when potentially exposed to high temperature and steam.

Items to be autoclaved should be prepared as follows:
- containers should be loosely covered or capped to avoid over pressurization.
- loads should be kept small and left partially open to facilitate steam penetration.

Infectious waste is autoclaved before being removed from the laboratory.
Emergency Response and Reporting of Incidents

If you are exposed to infectious material by any exposure route immediately remove your gloves and other PPE as necessary and treat the affected area

**Needle sticks and other sharp injuries**
Wash the affected area with antiseptic soap and water for 15 minutes

**Splashes to the face**
Flush affected area in eyewash for 15 minutes

**Aerosol exposure**
- Hold your breath and immediately leave the room
- Remove your PPE without contaminating yourself
- Wash your hands with soap and water
- Prevent entry into the lab for 30 minutes (post sign)
- Contact EHS for information on lab decontamination
Emergency Response and Reporting of Incidents

Immediately report all exposure incidents to your Principal Investigator, Manager or Supervisor.

Depending on the agent involved you may need immediate therapy and should seek medical assistance from one of the following locations:

**Campus wide**
- University Health Services, Urgent Visit (432-0123)

**Medical Area**
- Yale-New Haven Hospital Occupational Health Services, East Pavilion 1 - Room 40 (7:30 am to 4:00 PM)
- Yale-New Haven Hospital Emergency Room (4:00 PM to 7:30 am)

Yale Employee Health will provide all follow up care.
**Spill Response**

Spills of RG1 materials can be cleaned up with paper towels by lab personnel wearing standard laboratory clothing - a lab coat, gloves and eye protection.

Spills or RG2 materials are cleaned up using an appropriate spill clean up kit by individuals wearing appropriate PPE.

**PPE And Supplies**

**Spill Clean Up Of RG2 Biological Materials**

- **PPE**
  - lab coat
  - eye, nose and mouth protection
  - two pairs of gloves
  - disposable shoe covers

- **Spill kit supplies**
  - PPE
  - paper towels
  - biohazard bags
  - dust pan and brush
  - disinfectant
  - forceps

Make up a fresh dilution of 10% bleach and place it in the empty spray bottle to clean up the spill.
Summary

When working at Biosafety Level 2 it is important to...

- segregate and dispose of your biomedical waste according to the guidance provided for your section of campus
- decontaminate those areas of the body that have become contaminated immediately, notify your Principal Investigator or supervisor and seek medical attention
- clean up spills wearing the appropriate personal protective equipment and following the procedures outlined in this program