

Laboratory Safety

Common Practices



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Agenda

- Definitions
- Biosafety Levels
- Laboratory safety practices at FORTH
- Decontamination
- What to do in the case of a spill?

Definitions



Biosafety

The combined use of

- Laboratory practices,
- Laboratory facilities and
- Safety equipment

**to work with potentially infectious
microorganisms**

Why Biosafety Practices?

To protect:

- Workers/students
- Co-workers/visitors
- Lab support personnel

- **Products/Experimental results**

- Environment/Laboratory classroom

Biohazard

- An *agent of biological origin* that can cause disease in humans
 - Microorganism
 - Toxin
 - Allergen



Laboratory Biohazards

- **Infectious agents and pathogens:**
 - Bacteria, viruses, parasites, fungi
 - Human tissues, cells, body fluids
 - Primate tissues non-human, cells, body fluids
 - Animals - wild or laboratory
- **Biological toxins:**
 - Botulinum, tetrodotoxin, ricin, etc.
- **Recombinant DNA, RNAi:**
 - Plasmids, linear naked DNA, synthetic oligonucleotides etc.
- **Viral vectors:**
 - Adenoviruses, MuLV, lentivirus, etc.
They are all designed to express transgenes. Some are integrated in the genome.

Examples of Laboratory hazards

Equipment	Hazard
<ul style="list-style-type: none">• Needles	<ul style="list-style-type: none">■ Accidental inoculation, aerosol, spillage
<ul style="list-style-type: none">• Centrifuge	<ul style="list-style-type: none">■ Aerosols, splashing, tube breakage
<ul style="list-style-type: none">• Water baths	<ul style="list-style-type: none">■ Growth of microorganisms

Routes of Entry

- **Adsorption through the skin**
 - Wear gloves and lab coat, closed toed shoes, no application of make up
- **Splash to the eyes**
 - Wear safety glasses
- **Ingestion into Digestive Tract**
 - NO food or drinks, no chewing on gum or pens
- **Injection to the blood stream**
 - Proper use of sharps
- **Inhalation**
 - Prevent aerosols
 - Use Biological Safety Cabinets

Safety practices should be applied throughout the testing process:

- **Pre-analytical**
 - Specimen collection
 - Specimen preparation
 - Specimen transport
- **Analytical**
 - Testing
- **Post-analytical**
 - Disposal

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Biosafety Levels



Biosafety Levels (BSL/BL)

BL1 – *agents not known to cause disease*

(*B. subtilis*, *E. coli*)

BL2 – *agents associated with human disease*

(hepatitis B, *Salmonellae*, *Toxoplasma*)

BL3 – *exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences*

(*M. tuberculosis*, *C. burnetii*)

BL4 – *dangerous/exotic agents which pose high risk of life-threatening disease*

(Marburg and Ebola virus)

Biosafety Level 1

BL-1

Biosafety Level 1 (BL-1)

Use BL-1 when working with:

- Well characterized agents
- Agents that are **NOT** known to cause disease in human health
- Agents that are of minimal hazard to lab personell and the environment

Examples of BL-1 agents:

- *E. coli* JM109, DH5a
- *Saccharomyces cerevisiae*

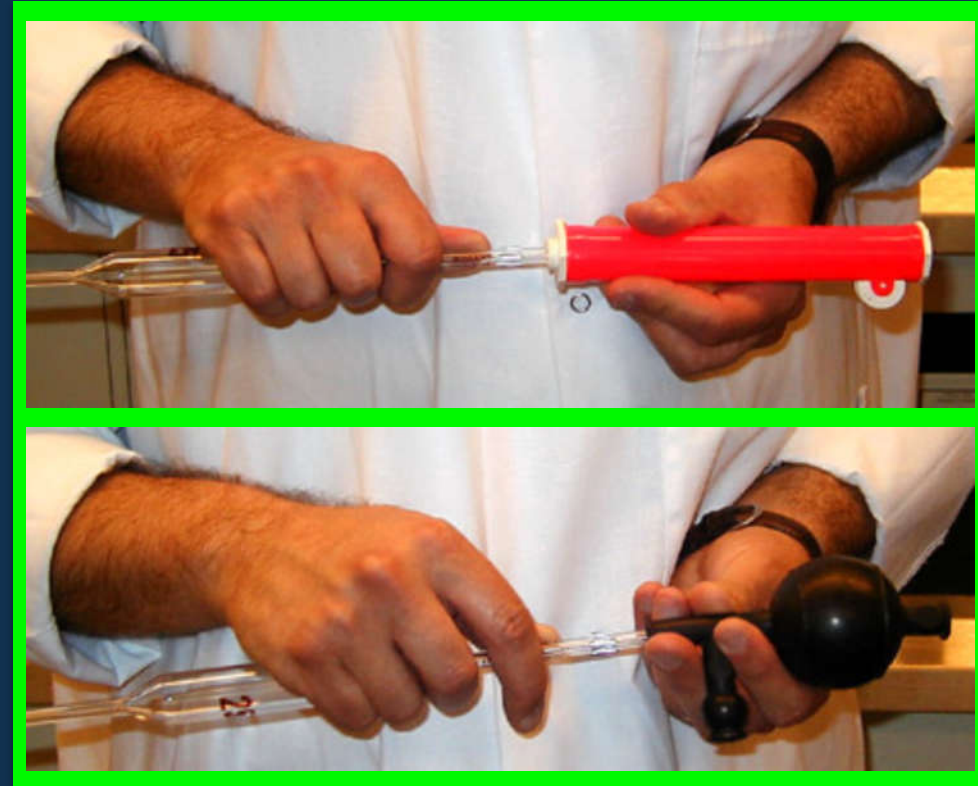
Biosafety Level 1 (BL-1)

Standard Work Practices

- Pipetting by mouth strictly forbidden



NO



YES

Biosafety Level 1 (BL-1)

Standard Work Practices

- Use mechanical pipetting devices
- Prohibit eating, drinking and smoking in the laboratory



No food in lab refrigerator!!!

Biosafety Level 1 (BL-1)

Standard Work Practices

- Use mechanical pipetting devices
- NO eating, drinking or smoking
- Wash hands frequently



Biosafety Level 1 (BL-1)

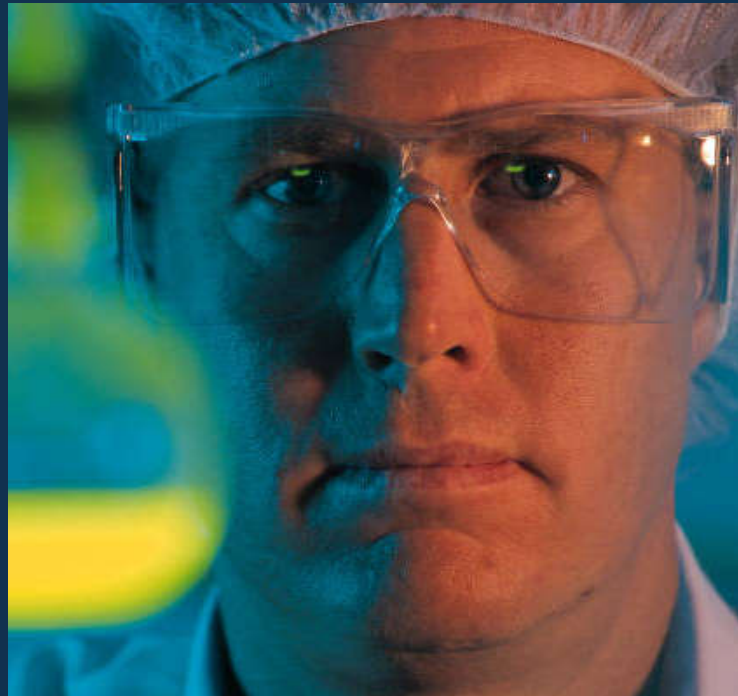
Standard Work Practices

- Use mechanical pipetting devices
- NO eating, drinking or smoking
- Wash hands frequently
- Minimize splashes and aerosols
- Decontaminate work surfaces daily
- Handle wastes properly
- Maintain insect & rodent control programme (mouse room, insectarium)

Biosafety Level 1 (BL-1)

Personal Protective Equipment (PPE)

- Lab coat
- Safety glasses or goggles
- Gloves as needed



Biosafety Level 2

BL-2

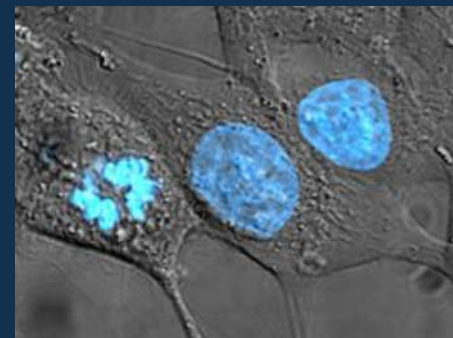
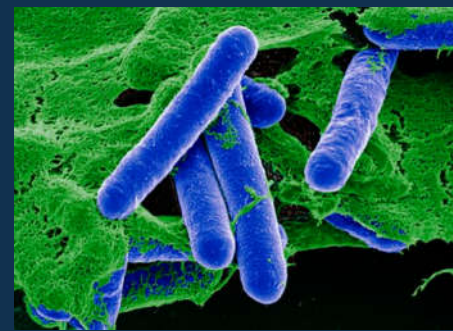
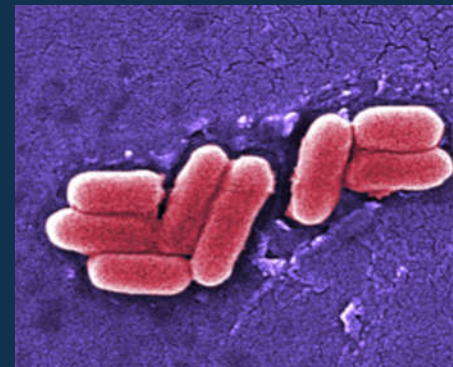
Biosafety Level 2 (BL-2)

Use BL-2 practices when working with:

Agents of moderate potential hazard to personnel and the environment

Examples of BL-2 agents:

- Human blood or body fluids
- *E. coli* 0157:H7
- *Clostridium botulinum*
- Retroviral vectors
- Human cells in cell culture



Biosafety Level 2 (BL-2)

Laboratory Facilities

- Adequate illumination
- Eyewash facility



Biosafety Level 2 (BL-2)

Laboratory Facilities (Secondary Barriers)

- Adequate illumination
- Eyewash facility
- Negative air pressure
- Autoclave available



Biosafety Level 2 (BL-2)

Laboratory Facilities

- Adequate illumination
- Eyewash facility
- Negative air pressure
- Autoclave
- Biological safety cabinet



Biosafety Level 2 (BL-2)

Laboratory Facilities

- Location
(Tissue culture facility)
(separation from public areas)



Biosafety Level 2 (BL-2)

Standard Work Practices (as in BL-1)

- Use mechanical pipetting devices
- Wash hands frequently
- Minimize splashes and aerosols
- Decontaminate work surfaces daily
- Handle wastes properly
- Maintain insect and rodent control programme

Biosafety Level 2 (BL-2)

Special Practices

- Place used slides and coverslips in sharps containers, never in any other receptacle



Sharps containers

- Marked
- Puncture resistant
- Leak proof

Biosafety Level 2 (BL-2)

Special Practices

- **DO NOT place** needles or sharps in office waste containers



Biosafety Level 2 (BL-2)

Special Practices

- **DO NOT** touch broken glass with hands



Biosafety Level 2 (BL-2)

Special Practices

- Remove gloves, lab coats before leaving work area
- **NO** animals in laboratories

Biosafety Level 2 (BL-2)

Personel Protective Equipment

- Lab coat
- Safety glasses or goggles
- Gloves
- Biosafety cabinet



Biosafety Level 3

BL-3

Biosafety Level 3 (BL-3)



Biosafety Levels (BSL)

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Biosafety Level 4

BL-4

Biosafety Level 4 (BL-4)



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- Decontamination
- What to do in the case of a spill?

Biosafety Practices @ FORTH



Biosafety

- Gloves
- Safety glasses
- Proper footwear
- Hair back
- NO food or drink in the laboratory



Biosafety Practices

- Leave the bio in the lab
 - Wash hands after removing gloves and before leaving
 - **DO NOT** wear lab coat and gloves outside the lab



Biosafety Practices

- Use disposable sharps
- Use plastic instead of glass when possible



Biosafety Practices

Reduce aerosols

- Gently expel fluids against the walls of tubes or flasks
- Use contaminated container in Biological Safety Cabinet to reduce drips to the biohazard bag



Biosafety Practices

- Prevent turbulent air flow within the cabinet
 - Keep sash pulled down
 - Do not block grill
 - Keep materials towards the back of the cabinet
 - Move arms slowly



Biosafety Practices

Helpful Hints

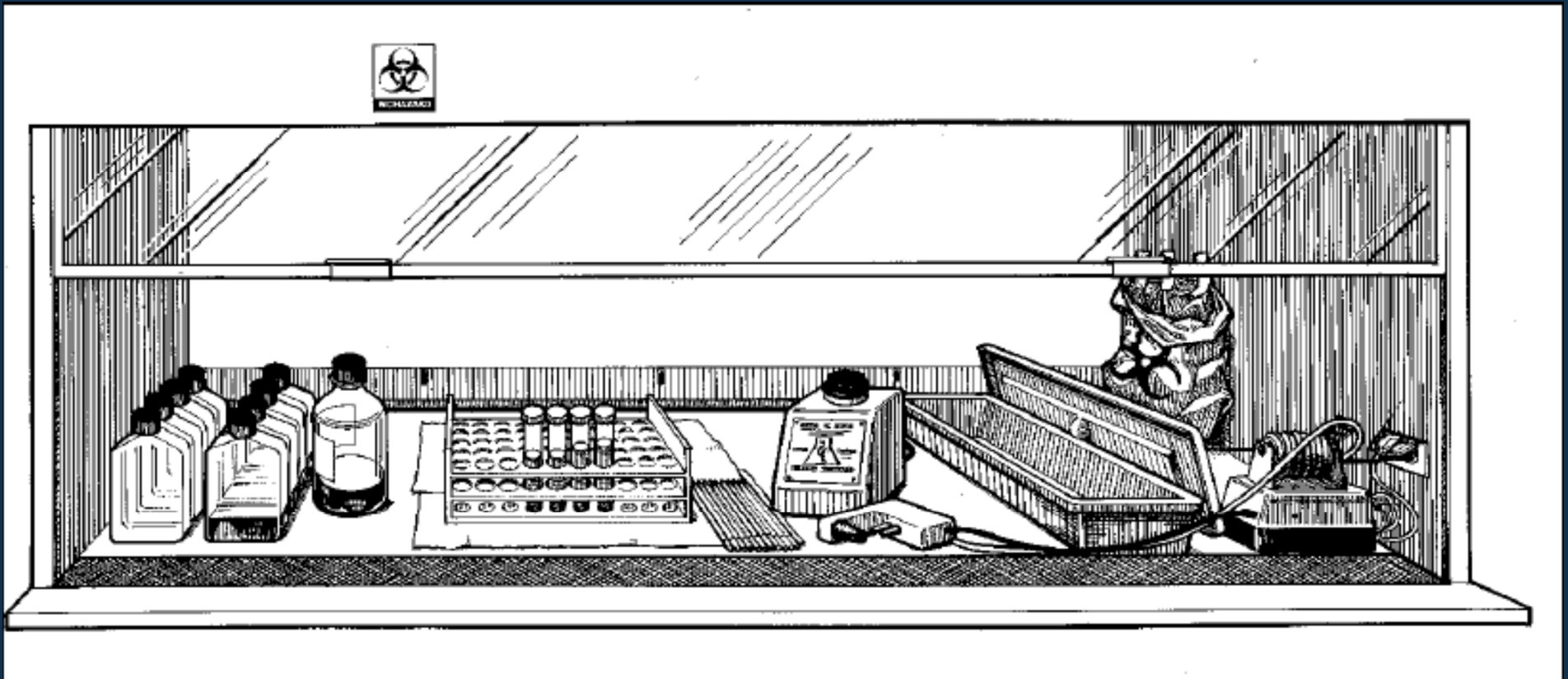
- Enter straight into the cabinet, avoid sweeping motions
- Don't place materials on the grill
- Keep discard pan or bag inside the cabinet
- Decon materials before removal from the cabinet



Biosafety Practices

Use of Biosafety cabinet

Prevent cross-contamination



Clean
area

Working
area

Contaminated
area

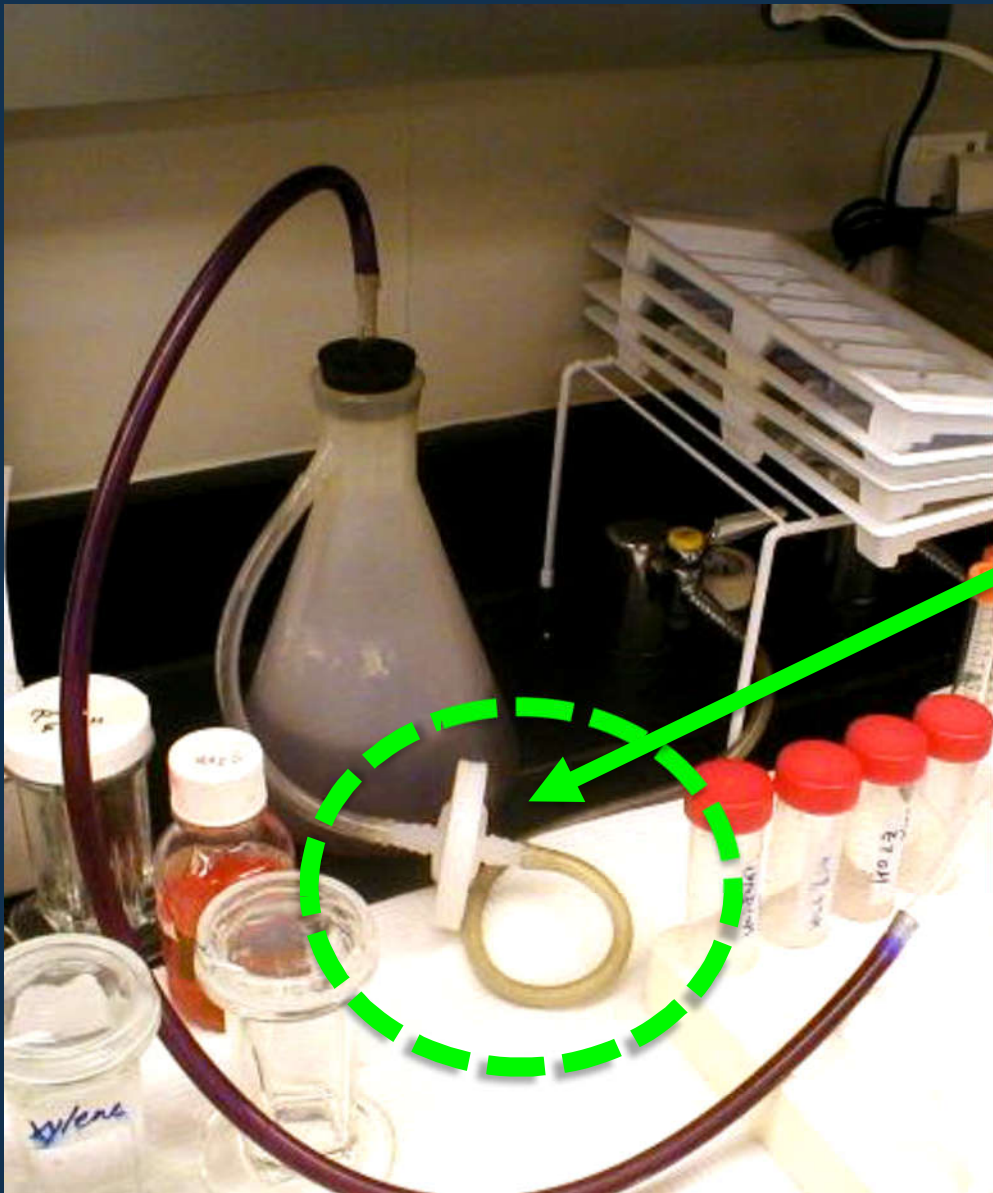
Biosafety Practices

Biological Safety Cabinets are **NOT** fume hoods



Biosafety Practices

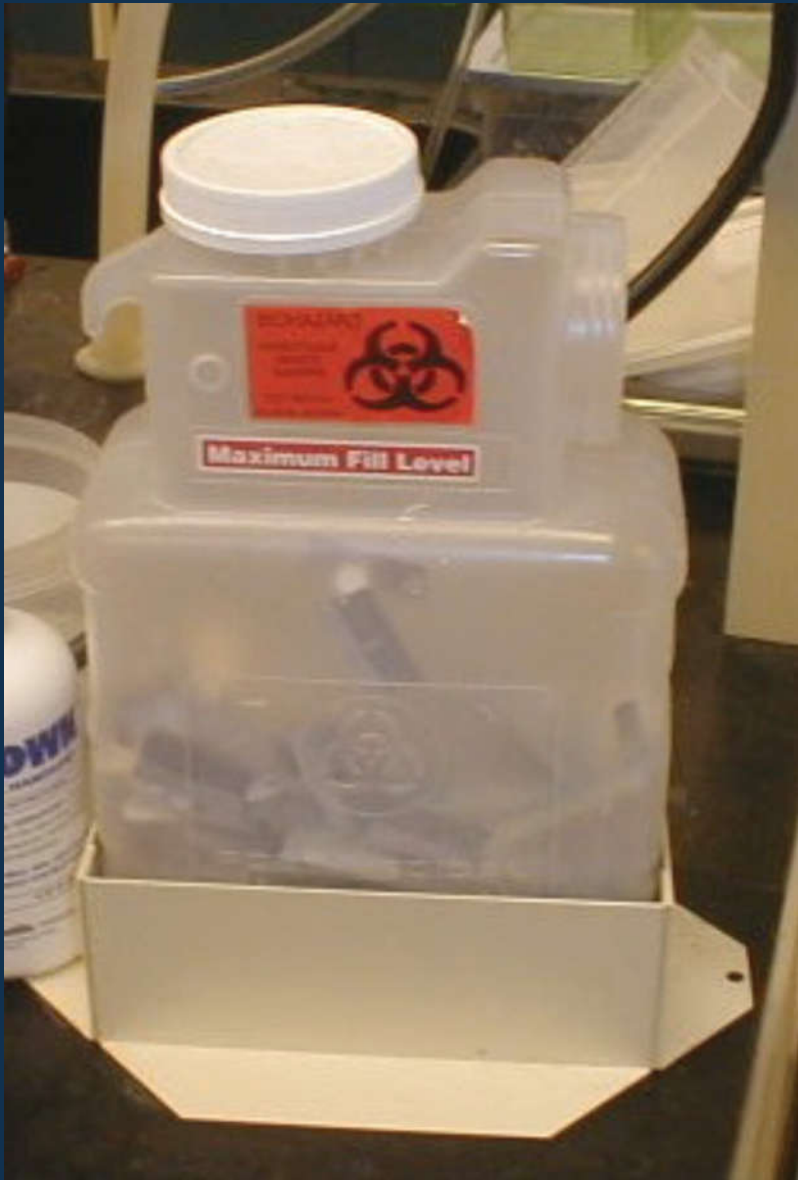
Protection of Vacuum lines



- Protect the vacuum lines from aspiration flask by using filter

Biosafety Practices

Sharps



- Collect in approved sharps container
- Do not overfill sharps container
- When filled secure lid and place in biohazard box

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Decontamination



Decontamination

- **Sterilization:**
destroy all microbial life, including spores
- **Disinfection:**
destroy a majority of microbial life, but not necessarily spores
- **Antiseptic:**
germistat used on skin to inhibit growth of microorganisms

Decontamination

Methods

- **Heat:**

steam heat, dry heat, incineration

- **Chemical:**

bleach, ethanol, H_2O_2 , ethylene oxide, PFA

- **Radiation**

Decontamination

Disinfectants **do not** replace standard
microbiological practices and good
hygiene!!!

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- **What to do in the case of a spill?**

In case of a spill



In case of a spill

- Wear disposable gloves
- Small spill – wipe with paper towel soaked in 10% bleach
- Discard contaminated towels in infective waste containers
- Wipe down the area with clean towels soaked in a same solution of household bleach
- Wash hands

In case of a spill

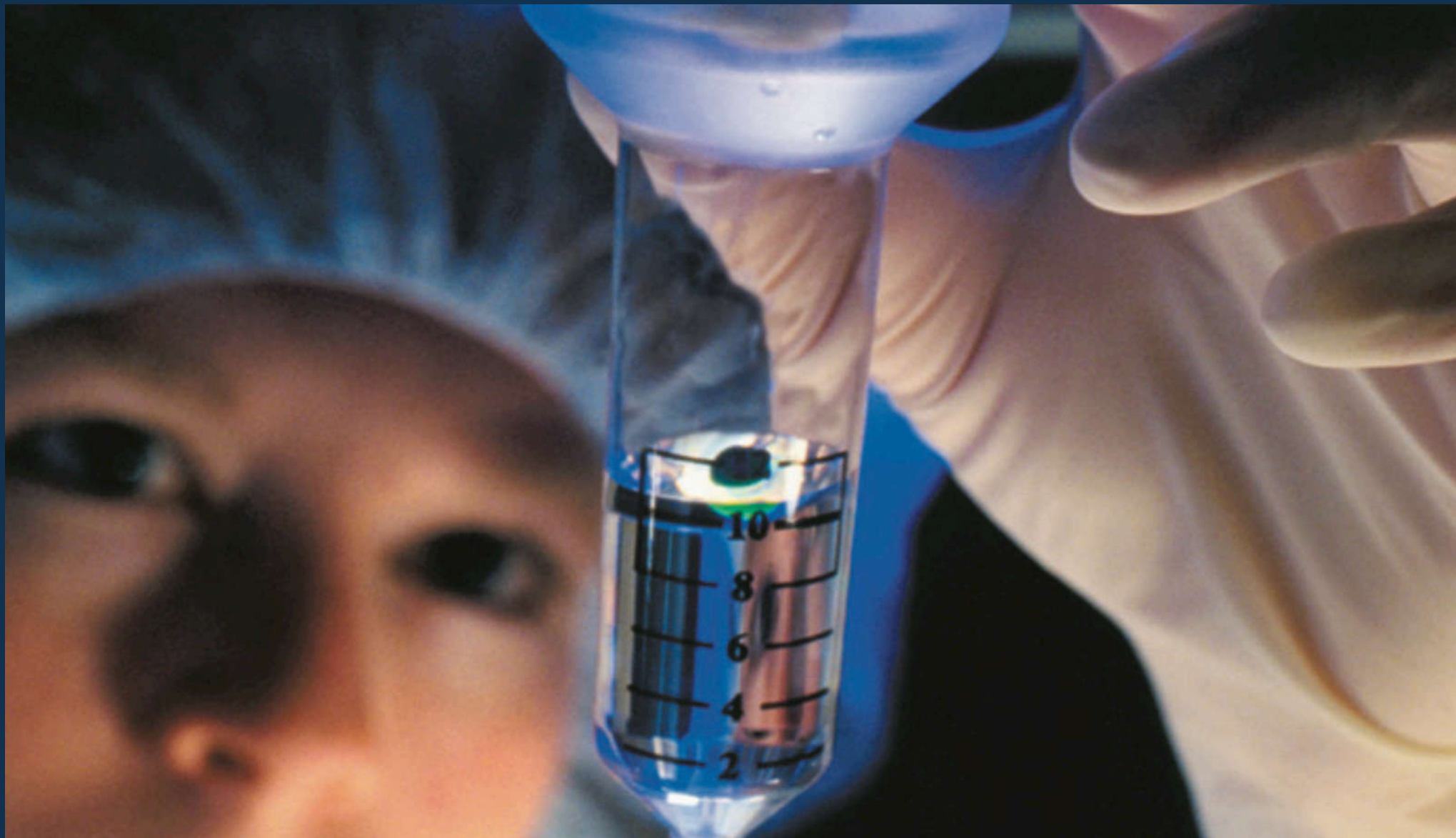
For large spill

1. Alert coworkers and contain spill
 - If hazardous aerosols are possible evacuate room for 30 minutes
2. Replace contaminated Personal Protective Equipment
3. Cover with adsorbent saturated with fresh 10% bleach
4. Wait 20-30 minutes then dispose of materials in biohazard box
5. Change Personal Protective Equipment and wash hands
6. Call Biosafety for assistance in cleaning up spill
7. Notify PI of spill

Decontamination of Work Spaces

- Liquid Disinfectants
 - 70% Isopropyl alcohol or ethyl alcohol
 - Volatile and flammable
 - Act quickly with no residue
 - 10% bleach solution
 - Must be prepared daily
 - Effective against wide range of agents
 - Requires contact time to deactivate agent
 - Leaves residue

Disinfect work surfaces daily and after spill !!!



Thank you!!!