

Science at the heart of medicine

# Biosafety Training

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<http://einsteinmed.edu/ehs>

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# Biosafety

- Biosafety is defined as “The discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials” that have the potential to cause disease in humans.
- The practice of safe handling of pathogenic microorganisms and their toxins in the biological laboratory is accomplished through the application of containment principles and the risk assessment.

# Regulations

- OSHA Bloodborne Pathogen Rule requirements:
  - Exposure Control Plan
    - ✓ List of job titles exposed/not exposed
  - Annual training
  - Offer the Hepatitis B Vaccine
  - Personal protective equipment
  - Utilize engineering controls
  - Provide exposure follow-up/counseling



# Other Regulatory Guidance

- NIH Recombinant Guidelines
  - Apply to all institutions receiving NIH funding
  - Covers rDNA work, but also includes risk group listing
- CDC's BMBL -Biosafety in Microbiological and Biomedical Laboratories
  - Guidelines that are not optional
- Select Agent law (covers high consequence agents and toxins)
- Dangerous goods shipping regulations
- Animal Welfare Act
  - IACUC review of animal protocols

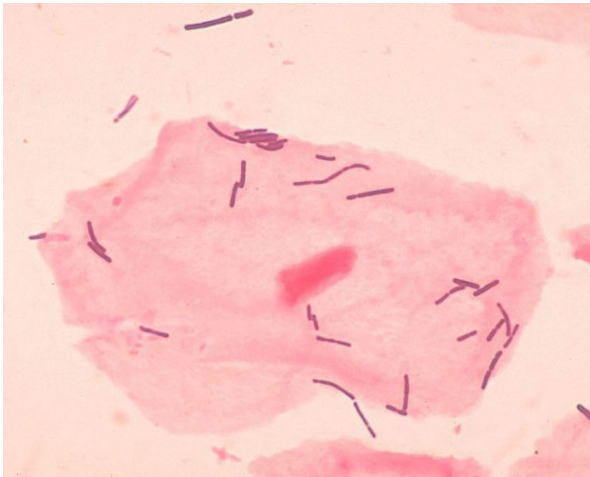


# Risk Group/Biosafety Level

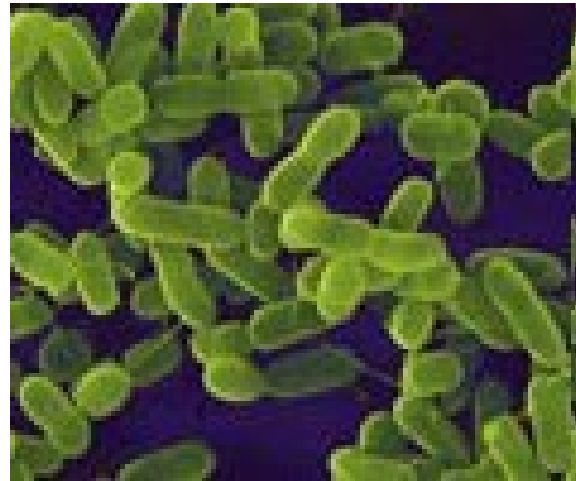
- Risk Group corresponds to the level of hazard associated with the agent
  - NIH Guidelines
  - American Biological Safety Association webpage
- Biosafety Level corresponds to the facility design, PPE and practices required to handle the agent.
- As described in CDC/NIH, "Biosafety in Microbiological and Biomedical Laboratories (BMBL) there are four biosafety levels (BSL) 1-4.

# Biosafety Level 1

- **Agents handled in BSL1**
  - Not known to cause disease in healthy adult humans
  - e.g., non-pathogenic *E. coli*, Lactobacillus



Lactobacillus



*E. coli*

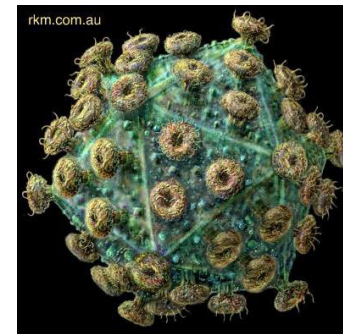
# Biosafety Level 2

- **Agents handled in BSL2;**

- Are associated with human diseases
- e.g. *Salmonella typhimurium*, *Cryptococcus neoformans*, HIV and HCV clinical samples
- Human products (blood, tissue, human cell lines)



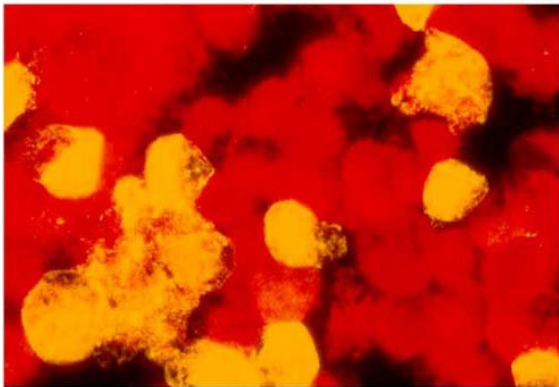
Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells



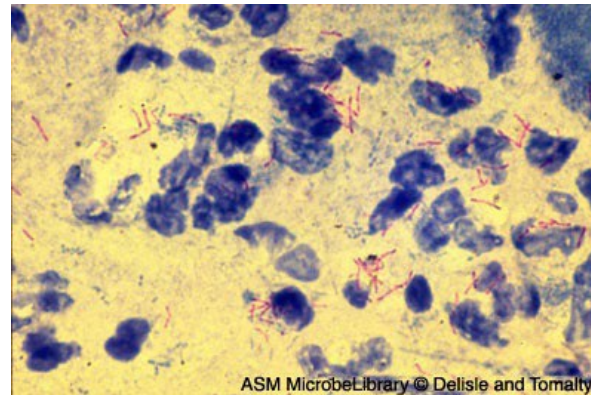
*Model of HIV virion*

# Biosafety Level 3

- **Agents handled in BSL3;**
  - > Indigenous/exotic agents associated with human disease
  - > Potential for aerosol transmission in the lab
  - > e.g. *M. tuberculosis*, Lyme disease, West Nile virus



West Nile Virus



Acid Fast Stain of *M. tb*

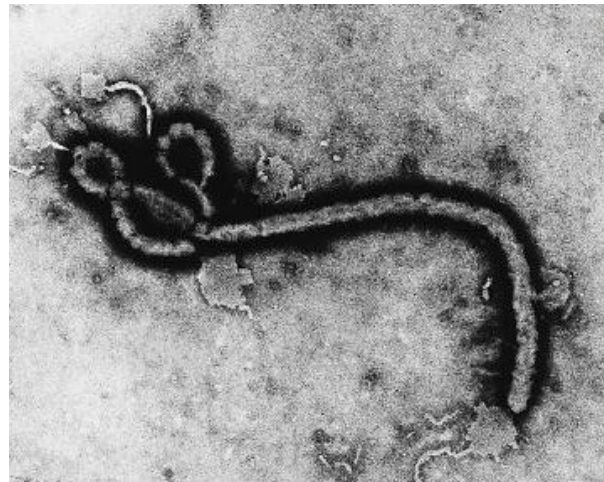
# Biosafety Level 4

- **Agents handled in BSL4**
  - > Most dangerous/exotic agents of life threatening nature
  - > e.g., Ebola virus, Marburg virus, Lassa virus

Lassa Virus



Image source: C.S. Goldsmith and M. Bowen (CDC)



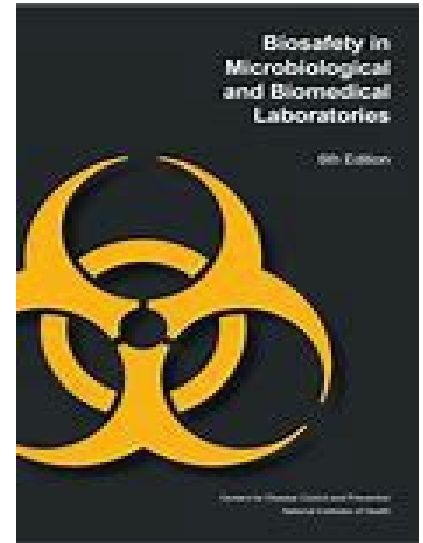
Ebola virus



BSL4 Lab Suit

# Facility Design (BMBL)

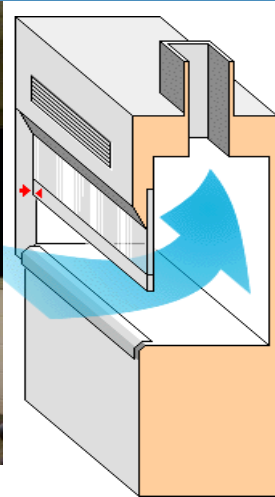
- BSL-1
  - Basic lab with sink for handwashing
- BSL-2
  - Sink for handwashing, biosafety cabinet, door signage, non-recirculated air, etc.
- BSL-3
  - Hand free sink, biosafety cabinet, door signage, ventilation negative pressure for containment, monolithic, sealed, 2X door entry, security, alarms, integral autoclave, etc.



# How Do You Protect Yourself?

- Engineering controls
  - Biological Safety Cabinets, sharps containers, safe sharps, centrifuge safety cups, etc.
- Work practices
  - Handwashing, aerosol avoidance, decontaminating work surfaces, etc.
- Personal protective equipment (PPE)
  - Lab coat, gloves, safety glasses, respirator

# Know Your “Hoods”!



## Chemical Fume Hood

- Closes completely
- Not meant for sitting
- Negative pressure
- May have chemical storage underneath



## Biological Safety Cabinet (BSC)

- Fixed sash opening (8 in.) (alarmed)
- Designed for seated work
- Negative pressure
- Check manufacturer label for type of cabinet



## Laminar flow Hood

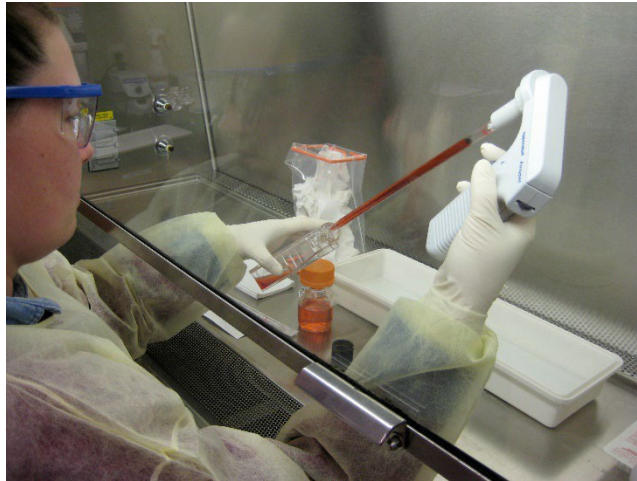
- HEPA filter visible in rear or top of unit
- Usually no sash
- Positive pressure – air blowing into face or breathing zone



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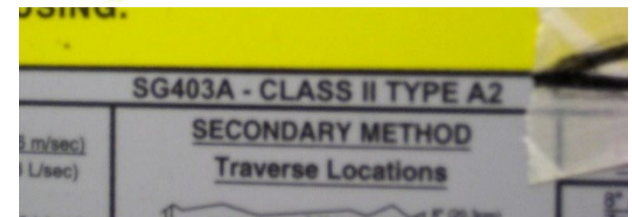
# Biological Safety Cabinet (BSC) *Primary Containment*

- Role:
  - To protect the user from the samples
  - To protect the environment from the samples
  - To protect the samples from external elements



# Biosafety Cabinets

- There are 3 classes of BSC
  - Class I: not often used
  - Class IIA and B: most often found in laboratories
  - Class III: primarily BSL3 or 4 labs
- Certification required:
  - When new and then annually
  - When hoods are moved
  - After repairs, filter changes
  - Vendor: TSS (Technical Safety Services)
    - ✓ Telephone: 866-536-5656



# Clean Bench (Laminar Flow Cabinet)

- Not to be confused with Class I BSC
- Inflow air is HEPA filtered
- Exhaust air is not filtered
- Used for Microbiology clean preparation (making agar plates) and molecular work (PCR)
- Air flow can be vertical or horizontal



# Class I BSC

- Is a negative-pressure ventilated cabinet
  - User and the environment is protected
- Open in the front with a sash
- Inward flow air is not filtered
  - Sample not protected
- Cabinet air HEPA filtered and 100% exhausted to the lab or outside
- Used with low risk agents
- Used to contain mixers, blenders, etc.



# Class II BSC

- Most commonly found BSC in laboratories
- HEPA-filtered vertical laminar air flow
  - User and sample protected
- HEPA-filtered exhausted air
  - User and environment protected
- There are 2 types and 2 subtypes (A1, A2 and B1, B2)



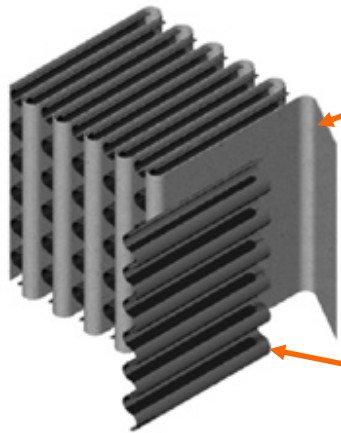
# Class III BSC

- Are totally enclosed
- Ventilated cabinet of gas tight construction
- Works under negative pressure
- Supply air is HEPA filtered
- Exhaust air is filtered by 2 HEPA filters or 1 HEPA filter 100% exhausted to the outside
- Offers the highest protection
- Work is done through integrated gloves
- All equipment is integrated in the BSC



# HEPA Filters

## High-Efficiency Particulate Air filter

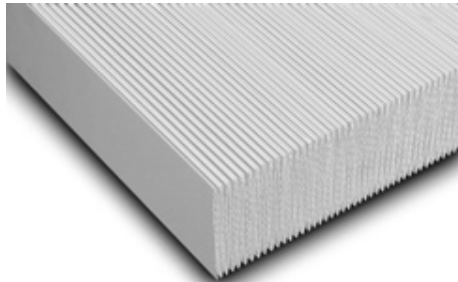


Borosilicate filter medium

Aluminum separator



Wooden frame



Continuous sheet of flat filter medium

Figure 3. Air Filtration Theory Particle Collection Mechanisms

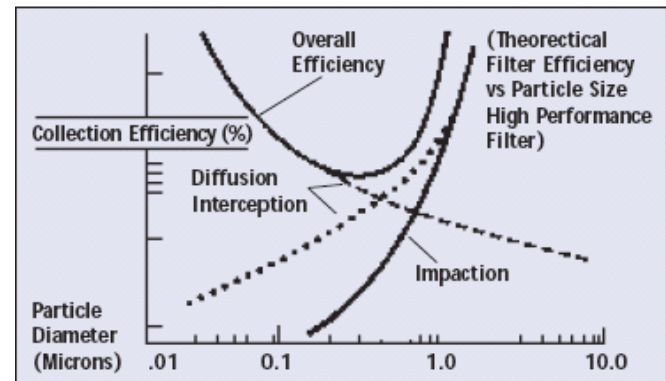


Figure 4. Relative Effect of Particle Collection Mechanism

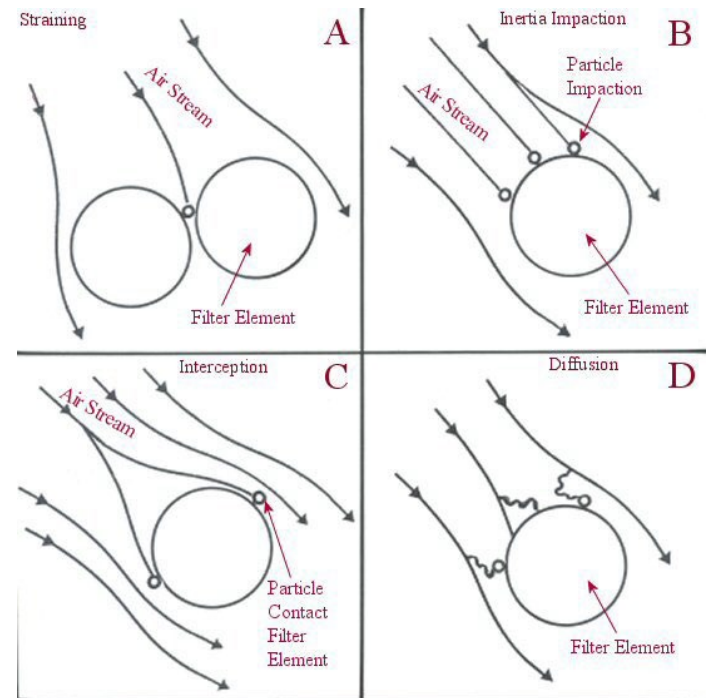
Labconco



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# Function of HEPA

- Particles removed by:
  - > **Inertia impaction**
  - > **Interception**
  - > **Diffusion**
  - > **Straining**
- Particles attach by electrostatic (Van der Waals) forces
  - > **difficult to dislodge**
    - (unlikely that a properly functioning HEPA is the cause of contamination)



# Particle Size Ranges

Typical aerosolized bacteria

Droplet nuclei

Fungi

Viruses

Bacteria

.01

.10

0.3

1.0

10

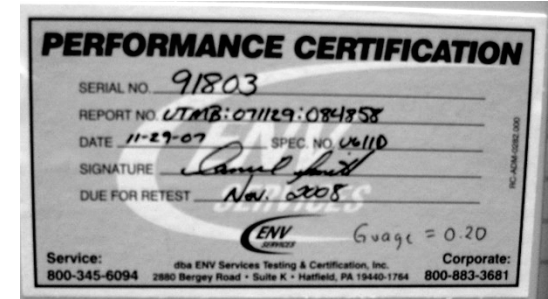
50

X

microns

# Proper Use of a BSC

- Before starting
  - If BSC off, turn on 20 min before using for infectious agents
    - Can set up work during this time
  - Check the monitors of the BSC



- Set up work area
  - Decontaminate all surfaces of the BSC
    - Walls, work surface, glass
  - Place the essential material needed for a specific experiment in the BSC after surface decontamination
  - Organize work place
  - Wait 5 min before starting work

# Completion of Work



- Surface decon and remove experimental cultures
- Discard infectious waste into the waste container
- Cover, and secure waste containers
- Surface decon all equipment and remove from BSC
- Surface decon and remove waste containers
- Surface decontaminate all surfaces of the BSC
  - Consider use of a dry “Swiffer” to reach all surfaces

# BSC Reminders

- Use BSC for manipulating infectious agents or human materials
- Keep work areas free of unnecessary clutter including equipment and supplies.
  - Ignoring this may result in a loss of proper airflow and an increased risk of contamination
- Organize your work time to avoid rushing
- Keep amount of work to be done realistic
- If you feel yourself getting tired, take a break



# DO

- **ALWAYS** surface decontaminate all surfaces and material coming out of BSC
- **ALWAYS** change or decontaminate gloves before taking hands out of the biosafety cabinet
- Decontaminate any surface, glove, lab coat that may become contaminated by biological material
- Always use mechanical pipetting aids
- For centrifugation: place the rotor inside the BSC, then wiped down before removal from BSC



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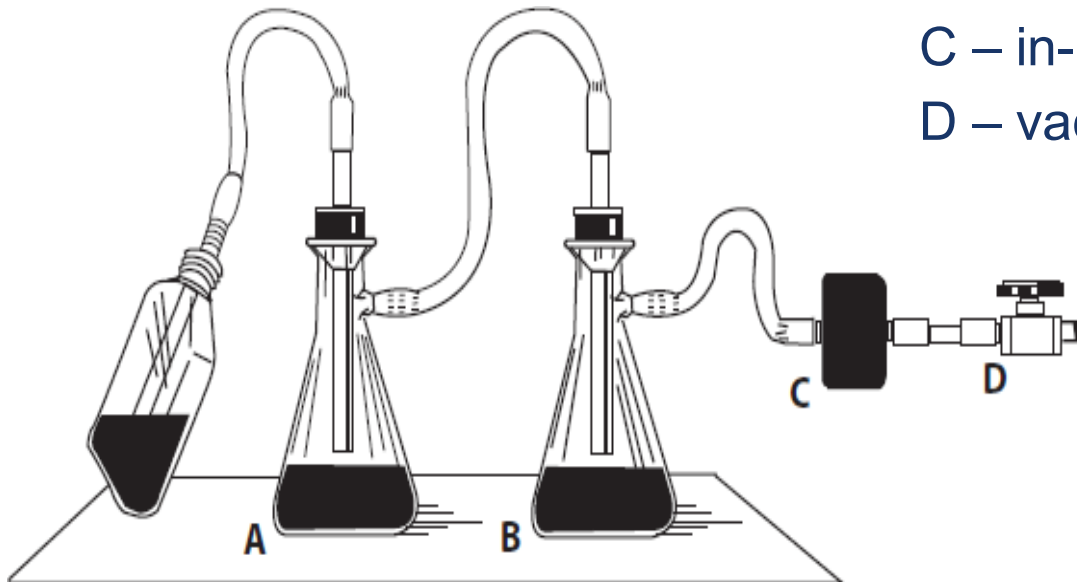
# Don't



- Discard materials outside BSC during experiment
- Cross hands while working in the BSC
- Block front and rear intake grills
- Scratch nose/eyes, get hair out of face with gloves on
- Make sudden swift movements of hands in the biosafety cabinet
- Over-fill waste containers
- Do not use Bunsen burners in BSCs
- Work in the BSC if alarm or warning light is on

# Vacuum Set-Up

- A – disinfectant containing flask
- B – overflow flask
- C – in-line HEPA filter
- D – vacuum system



# Aerosol-Producing Procedures

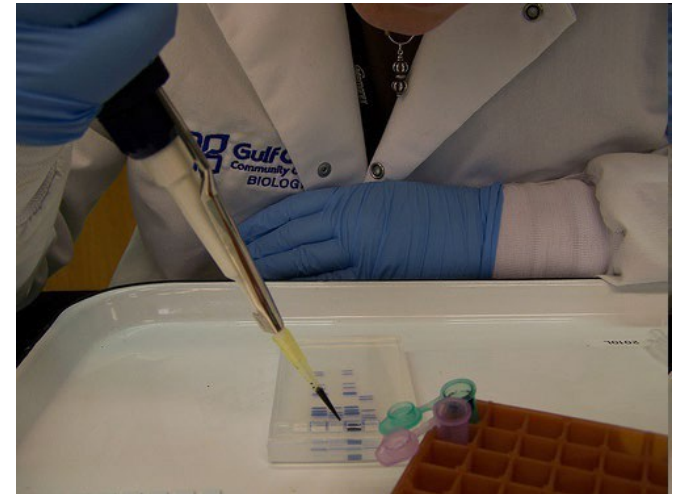
- Centrifuge
- Vortex
- Rocking/shaking cultures
- Lyophilization
- Sonication
- Tissues grinding
- Cell Sorting
- Pipetting
  - Opening tubes



# Pipetting



- Open centrifuge tubes carefully
  - Cap openers prevent spills on thumbs
- Pipette in a slow and controlled motion
- Should not hear the “clicking sound” of your pipetter
- Filtered pipette tips do not replace good technique



# Centrifuges used for Potentially Infectious materials

- You need a sealed or gasketed rotor if run outside of BSC
  - For blood and infectious agents
- If no seals/gaskets
  - Order “safety cups”
  - Load, unload and run inside the BSC



Safety cups

# Proper Use of Centrifuges

- All personnel must be trained on the proper use of centrifuges.
  - Rotors and seals must be checked before and after each procedure.
  - Check tubes for cracks before and after use.
  - Balance tubes
    - Critical for ultracentrifuges
  - Wait 5 min before opening the centrifuge



# Disinfection

- Solutions most commonly used
  - 10% bleach (freshly made)
    - Allow sufficient contact time (10-15 minutes) to ensure decontamination.
  - Other EPA registered tuberculocidal disinfectant (Cavicide, Wescodyne, Vesphene, etc.)
  - 70% ethanol (kills environmental contamination -- for items going into BSC)



# Disinfection Procedure

- Centrifuges
  - Use damp cloth at the end of each procedure
  - Clean Rotors/bucket
    - Inside-at the end of each experiment
    - Outside-wipe each time it is taken out of the BSC
- Bench top
  - Wipe down
    - Before and after each experiment
    - End of the day
    - After a spill



# Spill Procedures In a BSC

- Alert your co-workers
- Change PPE
- Cover with absorbent material
- Flood area with appropriate disinfectant
- Allow 15 min for disinfectant to be effective
- Notify supervisor
- Decontaminate all material
  - Place cleaning waste into waste container in the BSC
  - Disinfect equipment, remove, and clean the BSC
- Place all waste in red biohazard bag
- Change PPE
- Resume work

# Spill Procedures in Centrifuge

- If centrifuge is in the BSC, use BSC spill procedure
- If the centrifuge is outside of BSC:
  - Close centrifuge and stop work immediately
  - Notify co-workers and place a note on centrifuge so no one uses it. Leave it closed for 60 min
  - Notify your supervisor
  - Don necessary PPE
  - Absorb spilled material with paper towel, pour disinfectant on spill, and wait 15min
  - Clean all potentially exposed surfaces
  - Remove rotor and decontaminate it in BSC

# Spill Procedure in the Laboratory

- Stop work immediately and notify co-workers
- Remove any contaminated clothing
- Wash any exposed skin, flood eyes, etc.
- Allow aerosols to settle for 30 min
- Notify supervisor
- Don PPE
- Contain spill with absorbent material
- Pour disinfectant on spill and wait 15 min
- Clean-up spill
  - Remove broken glass (no bare hands)
  - Soak contaminated area again with proper disinfectant and clean outside spill area
  - Remove waste into biohazard red bag
- Remove PPE
- Return to work



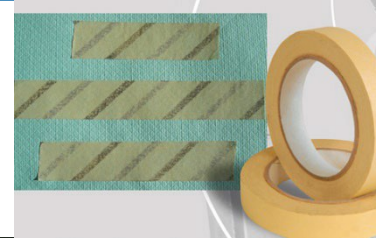
# Disinfection Procedure



- Biosafety cabinet
  - Surface decontaminated before and after each experiment
  - Complete decontamination including underneath the tray - monthly
- Incubators should be decontaminated monthly following the manufacturer's guidelines

# Autoclave Procedures

- Autoclave is used primarily for solid waste
- 3 ways to verify the autoclave was run
  - Autoclave tape (each run)
  - Biological/chemical indicator
  - Read printout (each run)
    - ✓ Indicates temperature, pressure, and time run
- Autoclave malfunction
  - Do not open door (post sign)
  - Notify administrator and supervisor for repair



# Emergency Procedures

- Remain Calm
- Wash any area that may have been exposed for 15 minutes.
- Protect yourself and your colleagues from further harm.
- Notify supervisor
- If needed, notify emergency services (911)
- Notify Security (X4111) and EH&S (X4150)
- Seek medical attention, if necessary

# Radiation

- If you handle, store or dispose of radioactive materials, you must take radiation safety training.
- Please contact EH&S at X2243 for training information.