THE UNIVERSITY OF
ALABAMA IN HUNTSVILLE

Biological Safety

Biohazard Governing Agencies

National Institutes of Health

- http://www.nih.gov/

Centers for Disease Control

- http://www.cdc.gov/

UAH Biosafety Manual

Manuals and additional safety information are available by Selecting the above link to access the UAH Biological Safety Page on the OEHS website.

CDC Biohazard Definition

- An agent of biological origin that has the capacity to produce deleterious effects on humans

i.e. microorganisms, toxins and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.

Biosafety Hazards

- Between 1978 and 1998 were 1,267 overt infections attributed to biological laboratory work found in the literature
  - 663 more cases were subclinical
  - 22 deaths
  - 5 fetuses were aborted due to infection
- 80% of cases no specific accident was identified as being the cause
- Breathing of aerosols is most often attributed

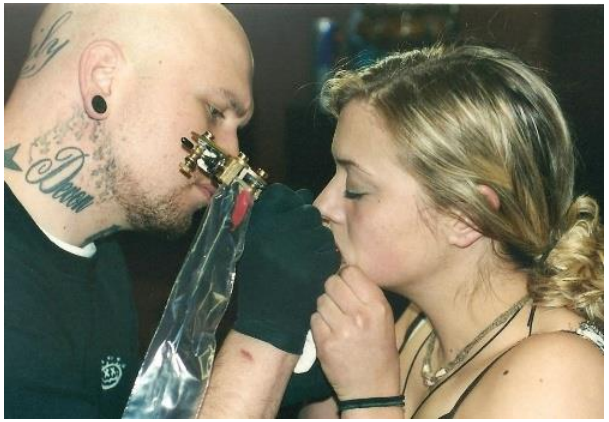
Goals of Biosafety

- Prevent biohazard from harming individuals
  - You (and fetus)
    - Correct PPE and microbiological procedures
  - Other lab workers
    - Promptly clean up spills
    - Minimize aerosols
  - Custodians
    - Proper waste removal
  - Researchers in other labs
    - Do not wear protective equipment outside of laboratory

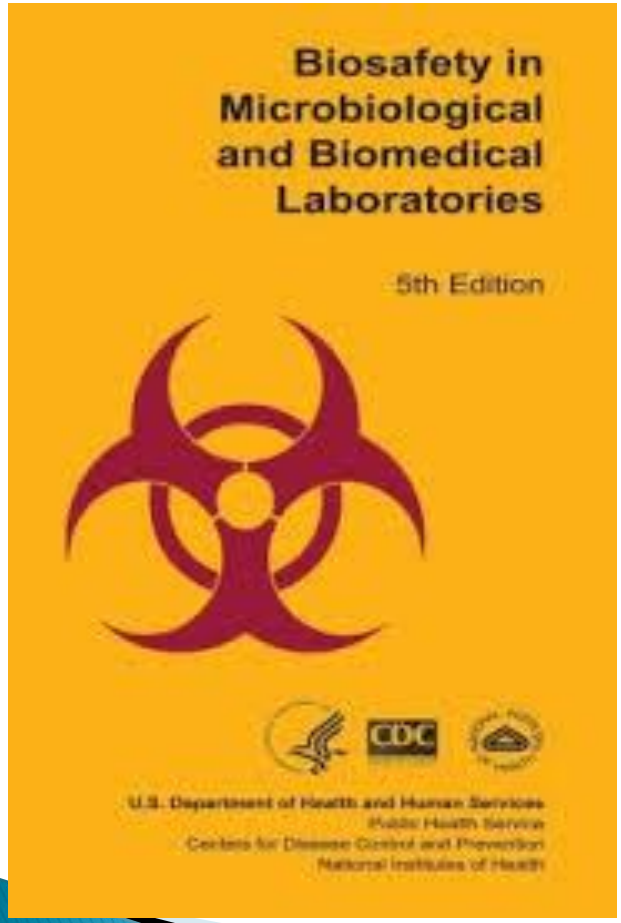
Biohazards in the Laboratory

- Infectious agents and pathogens:
  - Bacteria, virus, parasites, fungi
  - Human-derived tissues, cells, body fluids
  - Non-human primate tissues, cells, body fluids
  - Animals – wild, trapped or lab stock
- Biological toxins:
  - Botulinum, tetrodotoxin, ricin, etc.
- Recombinant DNA, RNA:
  - Plasmids, linear naked DNA, synthesized oligos, etc.
- Viral vectors:
  - Adenovirus, MuLV, lentivirus, etc.
    All are designed to express transgenes. Many insert in the genome. Modifications like VSV-G can increase your risk.

Biohazards in Real World



Resources



- CDC/NIH guide on Biohazards is the Biosafety in Microbiological and Biomedical Laboratories or BMBL

- Free PDF download at http://www.cdc.gov/biosafety/publications/bmbl5/

Biosafety Resources

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
  - http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

- Approval process for the use of rDNA in research is through the UAH Institutional Biosafety Committee
  - Contact greenm@uah.edu

Resources

- Biological Safety at UAH begins with the PI contacting the Office of Environmental Health & Safety
- The OEHS performs a preliminary review of

1. New Lab Start Up information
2. Project Registration requested by the Office of Sponsored Programs

- The OEHS brings information requiring further review to the attention of the Institutional Biosafety Committee.

Contact OEHS

256-824-6053

OEHS Website

Bloodborne Pathogens

- Work with human or primate blood and tissue have risk of transmitting disease
  - HIV, Hepatitis B,C
  - Immunization available for Hep B through the Faculty and Staff Clinic
- Free online training available to researchers
- Most diseases are prevented by preventing direct access to mucous membrane or into the blood stream

Prevent Accidental Exposure

- Analyze agents for potential harm
- Analyze routes of exposure and determine appropriate personal protection
- Follow standard microbiological safety procedures
- Use engineering controls when necessary
- Decontaminate and disinfect waste

Risk Assessment

- Biological agents are classified by risk group
- Laboratories are classified into Biological Safety Levels on ability to contain biohazards
  - BSL 1 – No known human hazards
    - E. Coli and S. cerevisiae
  - BSL 2 – Moderate risk to people
    - Blood or body tissue
    - Bacteria
    - Human cells
  - BSL 3 – Severe risk to people
    - Tuberculosis
    - Airborne Anthrax
  - BSL 4 – Severe risk to people and greater society
    - Ebola

BSL 1 Laboratories Safety Standards

- Follow general microbiological laboratory practices
- Personal Protective Equipment
  - Wear gloves when handling material
  - Eye glasses with splash hazard
  - Recommend wearing lab coat
- Decontaminate surfaces
- Decontaminate cultures
- Wash hands after removing gloves

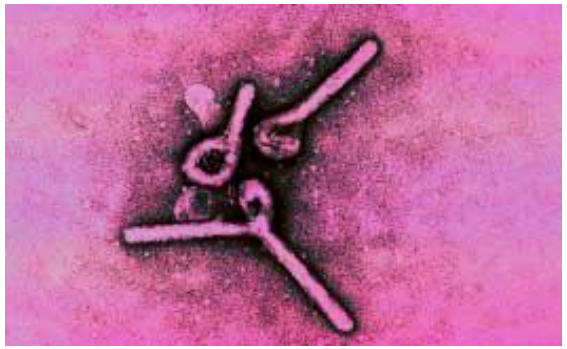
BSL 2 Laboratories

- Everything in BSL 1 plus
  - Limited lab access
  - Specific training provided by PI
  - Immunizations for laboratory risks
    - Report all exposures to hazardous materials
  - All splash/aerosol formation processes performed in biological safety cabinet
  - Gloves, eyewear, lab coat required
  - Lab manual outlining standard operating procedures

BSL 3 and 4 Laboratories

BSL4 facility at National Microbiology Laboratory in Canada (Winnipeg)

- Respiration hazards
- Requires significant training



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 Cabinet

Biological Safety Practices

- Leave the bio in the lab
  - Wash hands after removing gloves and before leaving
  - Don’t wear lab coat and gloves outside of the lab

Biological Safety Practices

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

Biological Safety Practices

- Reduce Aerosols
  - Gently expel fluids against the walls of tubes or flasks
  - Place and use a contaminated container in the BSC to reduce drips to the biohazard bag.

Decontamination of Work Spaces

- Liquid Disinfectants
  - 70% Isopropyl alcohol or ethyl alcohol
    - Volatile and flammable
    - Acts 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

Decontamination of Waste

- Autoclave liquid waste
  - Solid waste can go directly into the biohazard box
- Autoclave safety
  - Follow SOP for sterilization
  - Wear heat resistant gloves, lab coat, goggles
  - Do not autoclave chemicals
  - Do not overload autoclave bags

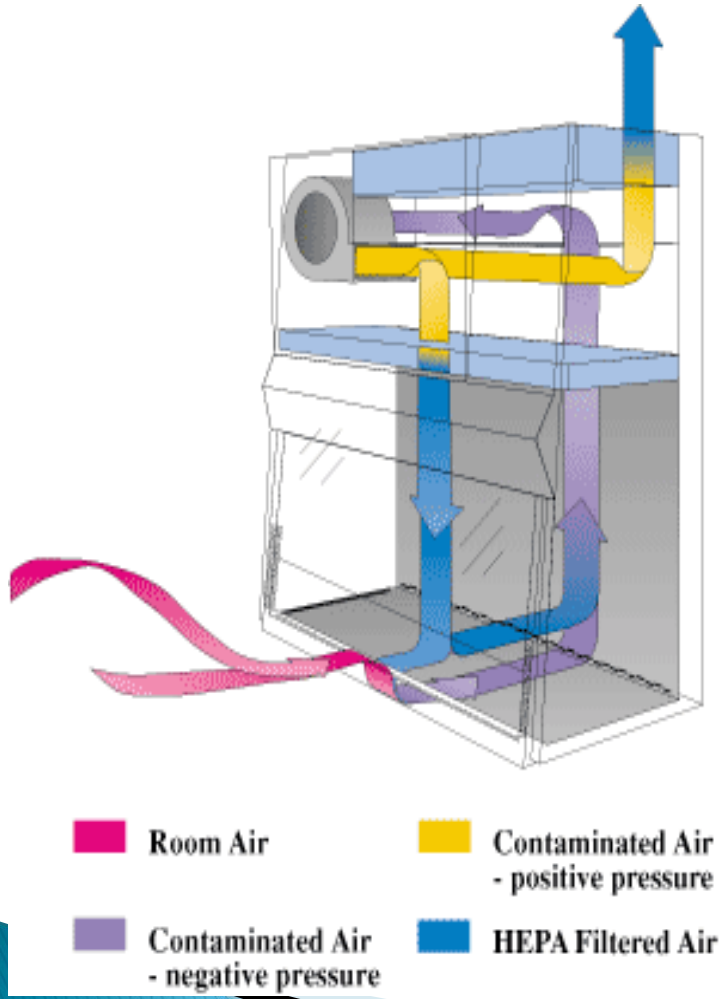
Aerosol formation

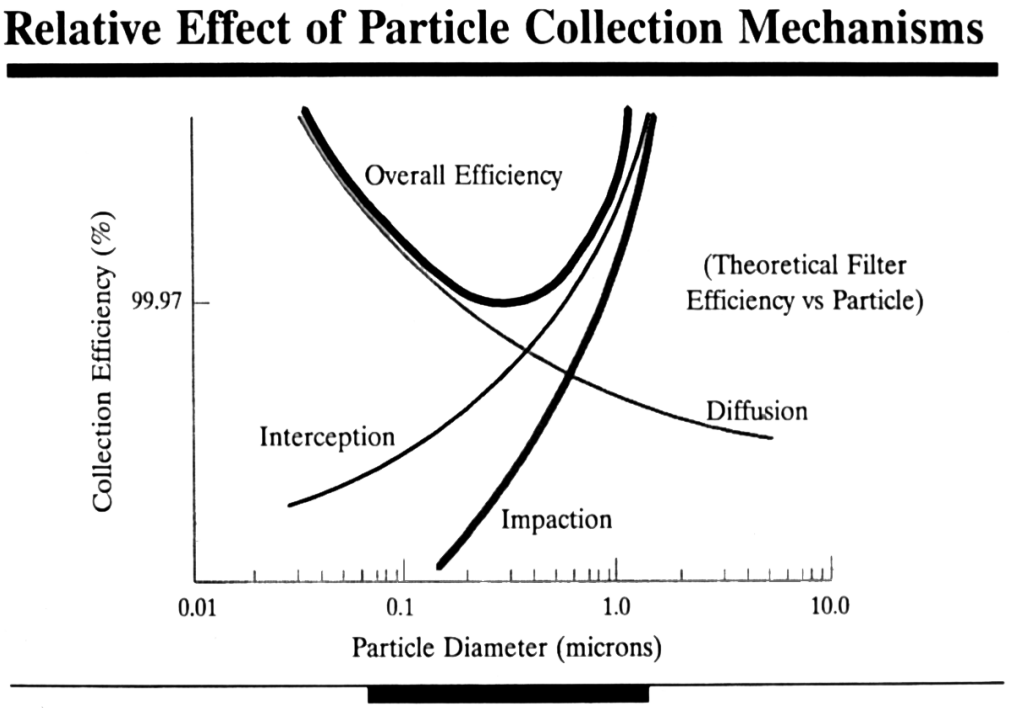
- Common operations that produce aerosols
  - Pipetting
  - Centrifuging
  - Grinding
  - Blending
  - Shaking
  - Mixing
  - Sonicating
  - Opening containers of hazardous materials
- Perform aerosol forming experiments in Biological Safety Cabinet

Biological Safety Cabinet

- Requires yearly certification

Biological Safety Cabinet



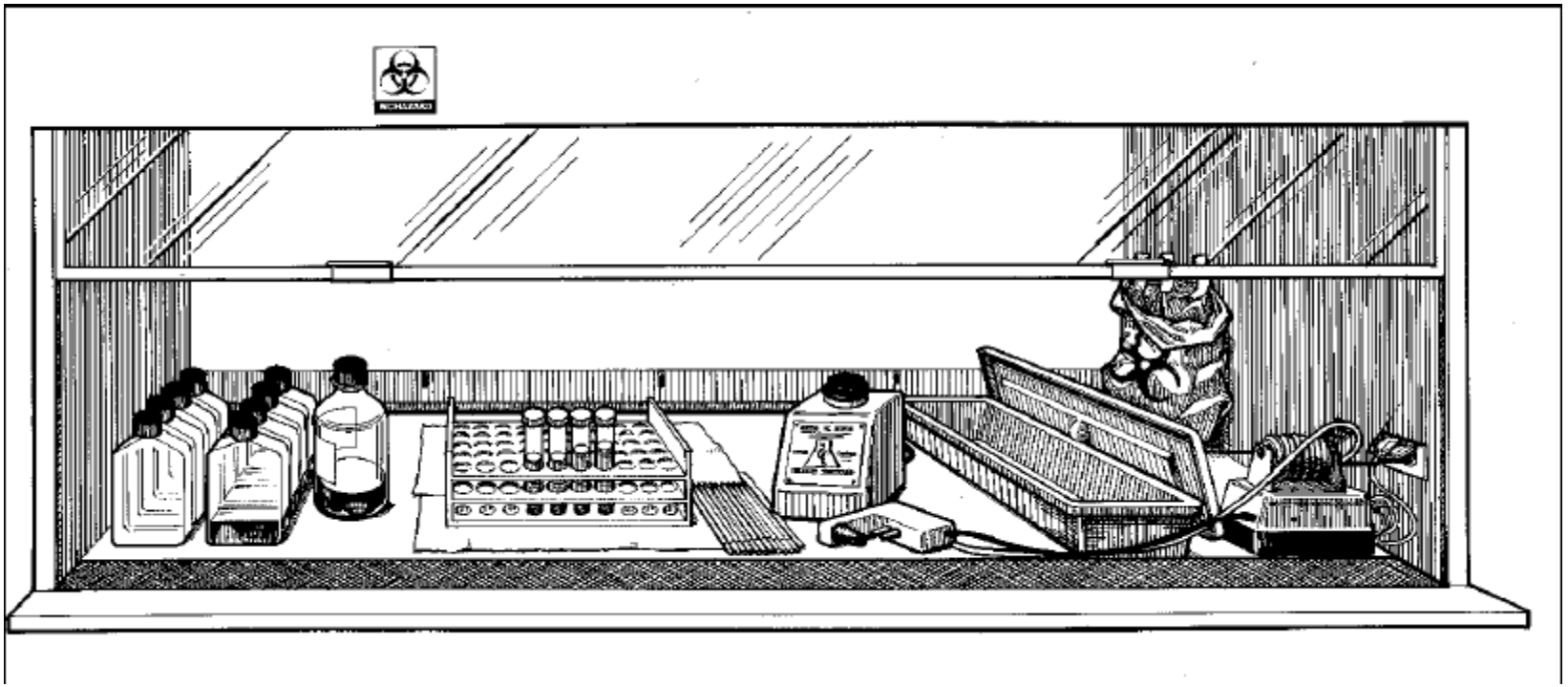


Biological Safety Cabinet

- 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

Biological Safety Cabinet Operation

Prevent Cross Contamination



Clean Area

Working Area

Contaminated Area

Biological Safety Cabinet

- BSC are not fume hoods

Protection of Vacuum Lines

- Protect the vacuum lines from aspiration flask by using filter

Work with Hazardous Chemicals

- Biological Safety Cabinets are not suitable for work with highly flammable or toxic material
  - Material is recycled into cabinet and not removed by HEPA filter
  - Material is recycled into the room
- Use chemical fume hood or specially designed BSC instead

Biological Waste

- Solids go into biohazard box
- Sharps containers go into box with lid fastened
- Autoclave or chemically deactivate liquids

Biohazard Box and 5 Gallon Biohazard Pail

Cleaning up Spills

- Small Spills
  1. Replace contaminated clothing and put on appropriate protection
  2. Cover spill in adsorbent material soaked 10% freshly made bleach solution
  3. Wait 20 minutes and then clean up
  4. Dispose of materials into biohazard bag and wash hands

YouTube video Cleaning a Bloodborne Pathogen Lab Spill

Cleaning up Spills

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

Exposure to Biohazards

1. Remove contaminated clothing
2. Wash area with soap and water
3. Call (256)824-6633 and tell them nature of exposure (Risk Mgt will coordinate an appt or advise otherwise)
   - Agent/organism
   - Route of Entry
   - Concentration
   - Amount
4. Notify lab supervisor
5. Go to ER after hours

Lab Coat Cleaning

- Decontaminate lab coat before using cleaner
  - Use autoclave or chemical disinfectant
  - Use appropriate commercial cleaning agency or contractor service

Sharps

- Put all used sharps into approved sharps container
- Do not overfill sharps container
- When filled, secure lid and contact the OEHS for a pick-up

Biosafety Conclusions

- Know hazards of agents
- Correctly use biosafety cabinet to reduce laboratory aerosols
- Correctly use sharps
- Correctly dispose of material
- Correctly clean up spills
- Treat and report exposures

Training Confirmation

Select the link below to acknowledge training. This is required for PI's and employees working in biological laboratories.

- http://goo.gl/forms/rcGYnGBV5n