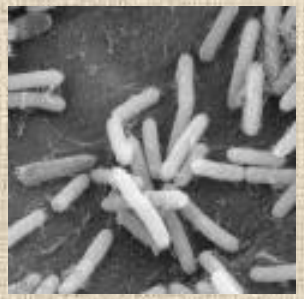
The University of Arkansas
Office of Environmental
Health and Safety

Presents:

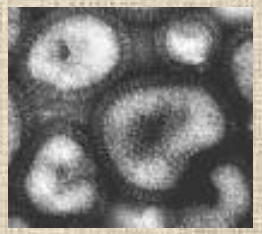
Principles of Biosafety

Why Biosafety?

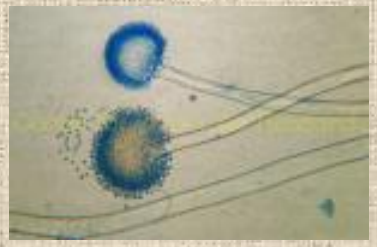
Bacteria, viruses, and other microorganisms have the potential to cause illness in a number of exposure situations if the proper precautions and procedures are not in place.



Leptospira



Influenza B



Aspergillus niger

What is a Biohazard?

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

The Importance of Biosafety

1941 - Meyer and Eddie

74 lab associated brucellosis infections in US

1949 - Sulkin and Pike

222 viral infections (21 fatal)

Only 27% related to known accidents

1951, 1965, 1976 - Sulkin and Pike

Surveys for lab-associated infections in more than 5,000 labs

Total of 3,921 cumulative cases cited.

Most commonly reported:

- **Hepatitis**
- **Typhoid**
- **Brucellosis**
- **Tuberculosis**
- **Venezuelan Equine Encephalitis**
- **Tularemia**

Fewer than 20% of cases were associated with known accidents.

Exposure to infectious aerosols are plausible (but unconfirmed) for >80% of reported cases.

Why Biosafety Principles

To Provide Protection for:

- **workers**
- **co-workers**
- **lab support personnel**
- **products**
- **environment**

Institutional Biosafety Committee

Research with a variety of biological materials presents potential hazards of exposure that need to be carefully considered. Guidelines for safe use of rDNA, viruses, bacteria, select agents and toxins, blood and human tissue, and other biological materials require that safe practices and procedures be in place to reduce or eliminate these exposure risks.

All research with biological materials also requires review and approval through the University's Institutional Biosafety Committee (IBC).

Institutional Biosafety Committee

Reviews and approves protocols for work with:

- Recombinant DNA
- Pathogens
  - (Human, Animal, Plant, or Opportunistic)

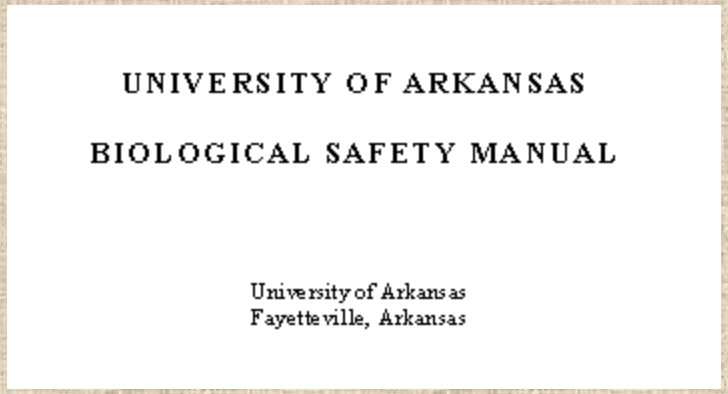
- Biotoxins
- Human and Nonhuman Primate Materials

- Animals
- Transgenic Plants

- Select Agents

Biosafety Manual

The Biosafety Manual provides information and references regarding all laboratory activities that may involve exposure to biohazardous agents or materials that come under the purview of the Institutional Biosafety Committee (IBC).



UNIVERSITY OF ARKANSAS

BIOLOGICAL SAFETY MANUAL

University of Arkansas
Fayetteville, Arkansas

Containment

Containment includes safe methods for managing infectious materials in the laboratory environment where they are being handled or maintained.

Purpose: to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Primary Containment

Protects personnel and the immediate laboratory environment from exposure to infectious agents

Provided by: good microbiological technique, appropriate safety equipment (including personal protective equipment), and vaccines (where applicable)

Secondary Containment

Protection of the environment external to the laboratory from exposure to infectious materials

Provided by: combination of facility design and operational practices

Three Elements of Containment

- Laboratory practice and technique
- Safety equipment (Primary barriers)
- Facility Design (Secondary barriers)

The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practice and Techniques

- **Be aware** of potential hazards
- **Be trained** and proficient in practices and techniques to handle material safely
  - A trained scientist must be responsible for work conducted with infectious agents.
  - The person in charge of the laboratory (i.e. PI) is responsible for providing or arranging the appropriate training of personnel.
- **Adopt a biosafety manual** that identifies the hazards that will or may be encountered and ways to minimize or eliminate these hazards

Safety Equipment (Primary Barriers)

These are designed to remove or minimize exposures to hazardous biological materials.

Examples: Biological Safety Cabinets, safety centrifuge cup, personal protective equipment (PPE), such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles

NOTE: If PPE becomes torn or otherwise defective, it should be changed immediately.

Facility Design and Construction (Secondary Barriers)

- Contributes to the workers' protection
- Provides a barrier to protect persons outside the laboratory
- Protects persons or animals in the community from infectious agents which may be accidentally released from the laboratory

  Examples: specialized ventilation systems, air treatment systems, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory

- Lab management is responsible for providing facilities commensurate with the lab's function and the recommended biosafety level for the agents being manipulated

Biosafety Levels

Microorganisms are grouped into four biosafety levels (BSL):

- BSL 1
- BSL 2
- BSL 3
- BSL 4

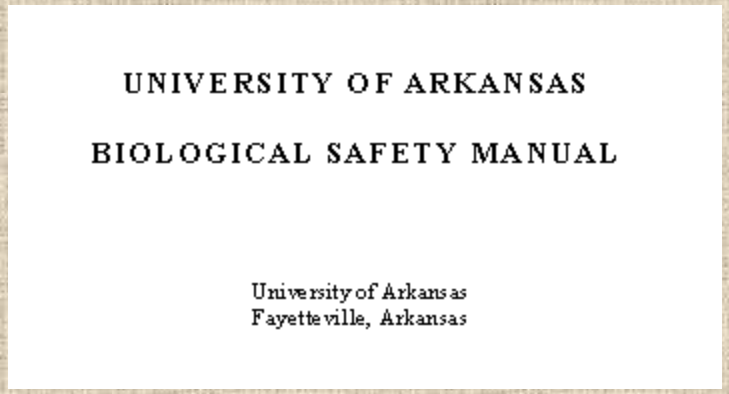
Work at BSL 3

Each level involves:

- Increasing levels of personnel and environmental protection
- Guidelines for working safely in microbiological and biomedical laboratories

All Biosafety Levels Require:

- A knowledgeable supervisor
- Personnel aware of potential hazards
- Personnel proficient in practices/techniques
- A biosafety manual **specific to the lab**


UNIVERSITY OF ARKANSAS

BIOLOGICAL SAFETY MANUAL

University of Arkansas
Fayetteville, Arkansas

Biosafety in the Microbiology and Biomedical Laboratory, 4th Edition

• Also known as BMBL 4—Describes the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) guidelines for each BSL

• Appropriate work for each BSL is described with combinations of the following :

- Laboratory Practices and Techniques
- Standard Practices
- Special Practices
- Safety Equipment (Primary Barriers)
- Laboratory Facilities (Secondary Barriers)

BSL 1

BSL 1 organisms are well-characterized agents not known to cause disease in healthy adult humans and are of minimal potential hazard to laboratory personnel and the environment

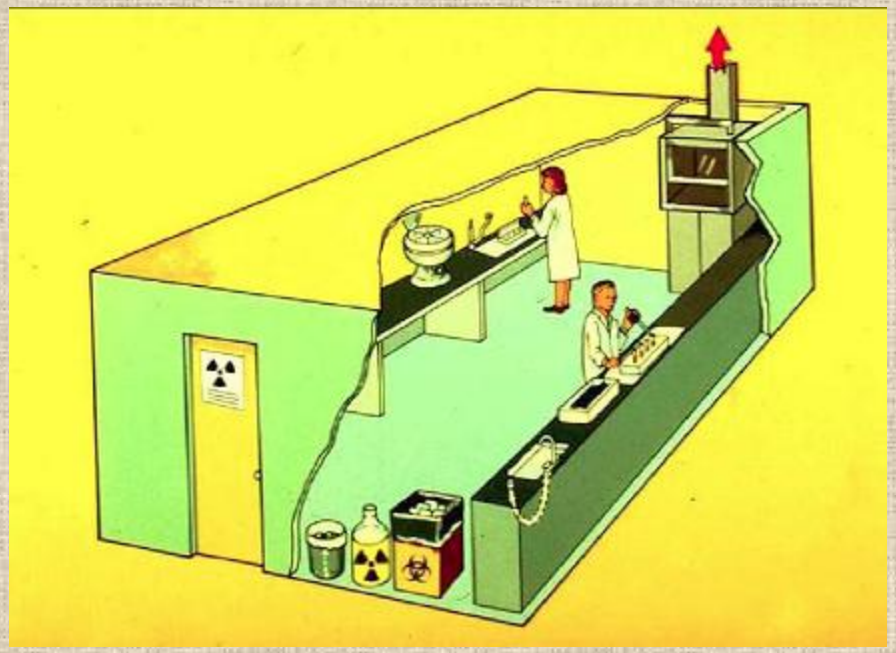
Examples: ***Bacillus subtilis, E. coli (certain strains), Lactobacillus acidophilus***

B. subtilis

E. coli

L. acidophilus

BSL 1 Lab Set-up



BSL1 Standard Microbiological Practices

- Use mechanical pipetting devices (NO mouth pipetting)
- Wash hands frequently, but at least after removing gloves and before leaving the laboratory

- Restricted or limited access to the lab space when working
- Eating, drinking, smoking, and application of cosmetics (including lip balm) in the laboratory are prohibited

- Minimize splashes and aerosols
- Decontaminate work surfaces daily

- Decontaminate wastes
- Maintain an insect and rodent control program

BSL 1 Personal Protective Equipment (PPE)

- Lab coat
- Gloves
- Eye protection (safety glasses)

Additional PPE may be needed, such as increased face protection (splash shield), depending on the activity.

If PPE becomes defective, remove it and put on fresh PPE.

There are no special practices beyond good microbiological technique necessary in a BSL 1 laboratory.

BSL 1 Laboratory Facilities (Secondary Barriers)

- Sink for handwashing
- Work surfaces that are easily cleaned
- Bench tops
- Sturdy furniture
- Windows fitted with fly screens
- Lab must be easily cleaned and decontaminated

BSL1 Training and Supervision

For **BSL 1** work, specialized training is minimal:

- Supervision—Scientist with general training in microbiology or related science
- Lab Personnel—Specific training in lab procedures

BSL 2

BSL 2 organisms are agents of moderate potential hazard to personnel and environment.

Characteristics:

- Immunization or antibiotic treatment is available
- Agents are not transmissible via the airborne route
- Extreme care is taken with contaminated needles or sharp instruments

Examples: Salmonellae, Hepatitis B virus, Bloodborne pathogens, Human body fluids—particularly when visibly contaminated with blood

BSL 2 Lab Set-Up

BSL 2 Standard Practices

The same as **BSL 1** with an emphasis on:

- Gloves
- Mechanical pipetting
- Attention to sharps

BSL 2 Special Practices

- Needles and Sharps Precautions
- Special policies and procedures for laboratory entry
- Post biohazard warning signs
- Use a biosafety manual specific to the lab
- Specific training provided to workers and must include annual updates
- Use leak-proof containers to transport infectious materials
- Immunize when possible
- Decontaminate work surfaces frequently
- Report to lab director spills and accidents that result in overt exposures of infectious materials

Needles and Sharps Precautions

- DO use sharps containers
- DON'T touch broken glass with hands
- DON'T break, bend, resheath or reuse syringes or needles
- DO use plastic ware whenever possible

BSL 2 Safety Equipment (Primary Barriers)

The same as in **BSL1** plus the use of a Biological Safety Cabinet (BSC) for work with infectious agents involving:

- Aerosols and splashes
- Large volumes
- High concentrations

BSL 2 Laboratory Facilities (Secondary Barriers)

The same as **BSL 1** plus an autoclave (preferably in the lab).

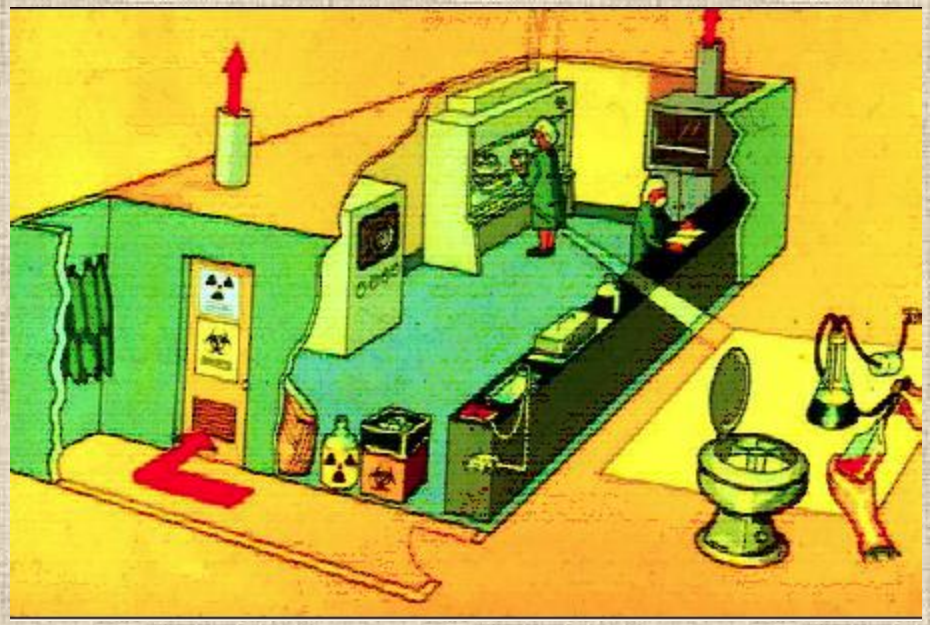
BSL 3

BSL 3 organisms are indigenous or exotic agents which may cause serious or potentially lethal disease and present the potential for aerosol transmission.

Laboratory personnel must have specific training in the handling of pathogenic and potentially lethal agents and be supervised by competent scientists who are experienced in working with these agents.

Examples: H5N1 Influenza Virus, *Bacillus anthracis*, *Yersinia pestis*, *Burkholderia*, *Francisella tularensis*, *Brucella*, *Clostridium botulinum*, *Mycobacterium tuberculosis*, *Coxiella burnetii*, Hantavirus, and West Nile virus.

BSL 3 Lab Set-Up



BSL 3 Standard Practices

Same as **BSL 2** plus potentially infectious waste should be decontaminated before removal for off-site disposal.

BSL 3 Special Practices

Same as **BSL 2** plus:

• Lab doors are closed when experiments are in progress.

• Lab director controls access to the lab and restricts access to persons required for program or support purposes. Persons at increased risk for infection are not allowed in BSL 3 rooms.

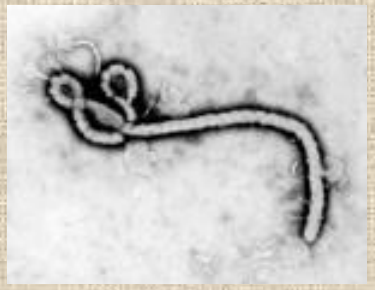
• All procedures with these agents must be performed in a BSC or other physical containment or by personnel wearing appropriate personal protective clothing and equipment.

• Laboratory must have special engineering and design features (i.e. exhaust air from room is discharged outdoors, directional airflow into the room).

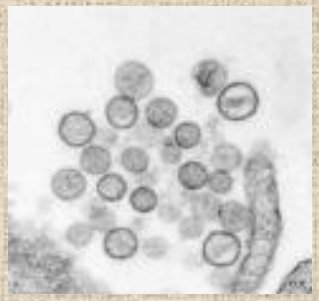
BSL 4

BSL 4 organisms are highly pathogenic and require handling in special laboratory facilities designed to contain them.

Examples: viral hemorrhagic fevers, such as Ebola, Lassa fever, Hantavirus pulmonary syndrome (HPS) and hemorrhagic fever with renal syndrome (HFRS)

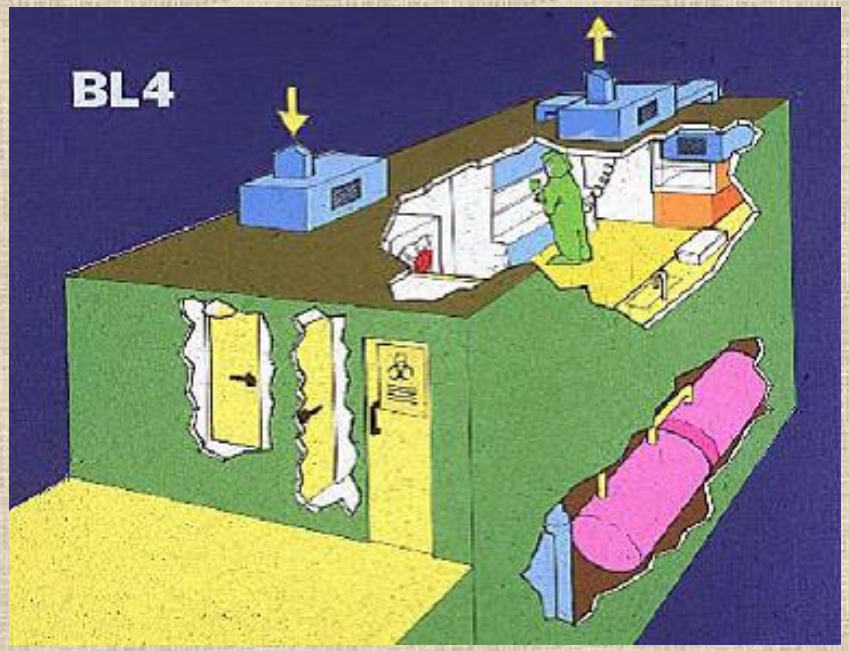


Ebola



Hantavirus

BSL 4 Lab Set-Up



University of Arkansas

There is currently no work at the University of Arkansas conducted at BSL-3 or BSL-4.

Biological Safety Cabinets

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed.

Three kinds of BSCs have been developed to meet varying research and clinical needs and are designated as Class I, II, and III.

The CDC has published “Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets.” A link to this publication is found on the EH&S website:

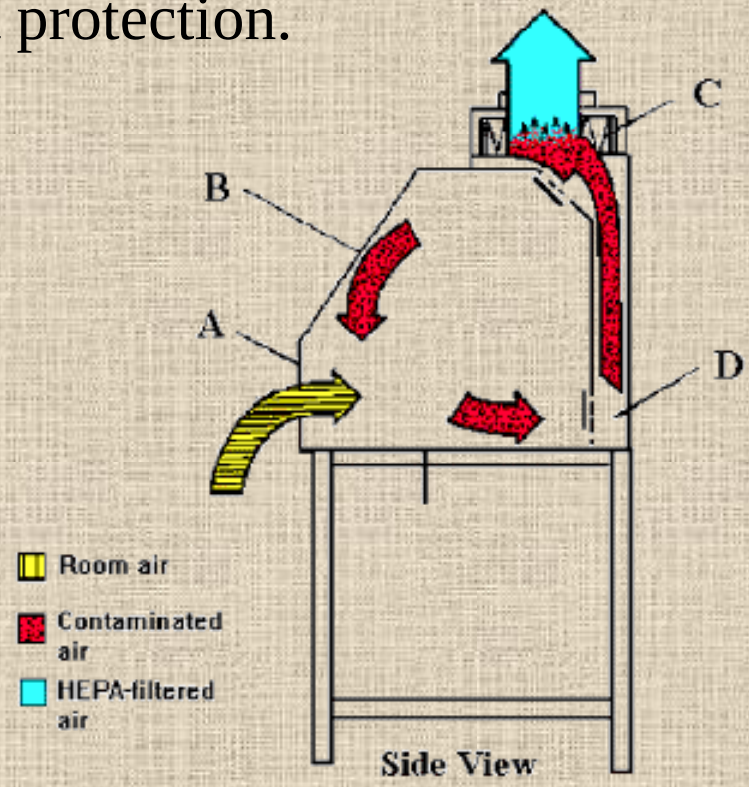
http://www.phpl.uark.edu/ehs/BiologicalSafetyCabinets.htm

Class I BSC

Class I BSCs are essentially the biological version of the traditional fume hood (chemical hood), and provide personnel and environmental protection, but no product protection.

Unfiltered room air in drawn across the work surface and exhausted though a HEPA filter to protect the environment.

The inward flow of air provides personnel protection.



Class I BSC

Class I BSCs are used to enclose equipment (i.e. centrifuges, harvesting equipment, or small fermenters) or procedures (i.e. aerating cultures or homogenizing tissues) with a potential to generate aerosols that may flow back into the room.

The **Class I BSC** can be hard-ducted to the building exhaust system, thimble-connected, or recirculated back into the room depending on the use.

Consideration must be given to the chemicals used in a BSC with HEPA filters as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment.

Class II BSC

Class II BSCs have 4 different types: A1, A2, B2, and B2.

Class II BSCs provide personnel, environmental, and product protection.

Personnel protection: air is drawn around the operator into the front grill of the cabinet.

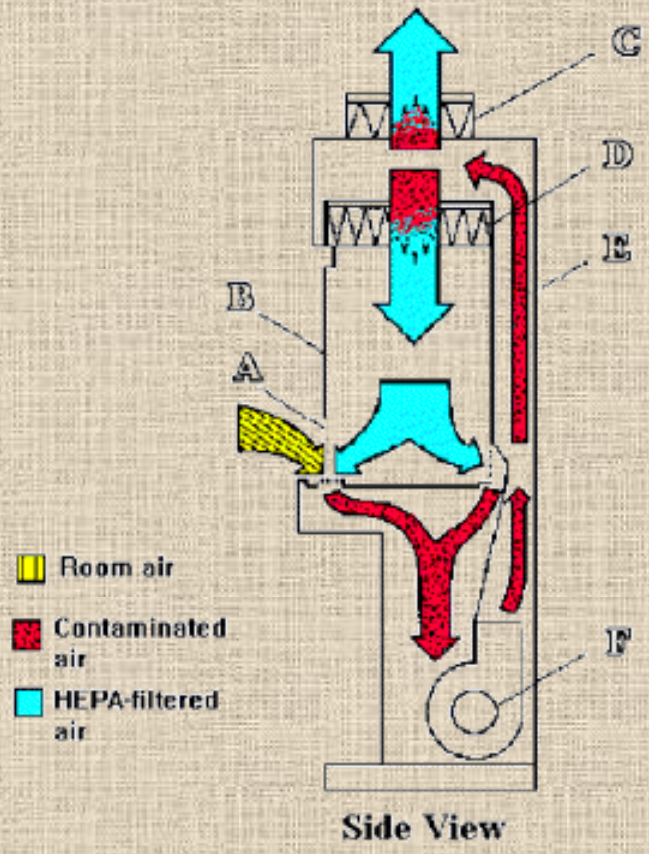
Product protection: the downward laminar flow of HEPA-filtered air minimized the chance of cross-contamination along the work surface of the cabinet.

Environmental protection: after cabinet air exhaust is passed through a certified exhaust filter, it may be recirculated into the laboratory (Type A) or exhausted out of the building (Type B).

Class II BSC

All Class II BSCs are designed for work involving microorganisms in BSL 1, 2, and 3.

Class II BSCs provide the microbe-free work environment necessary for cell culture propagation, and may also be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.



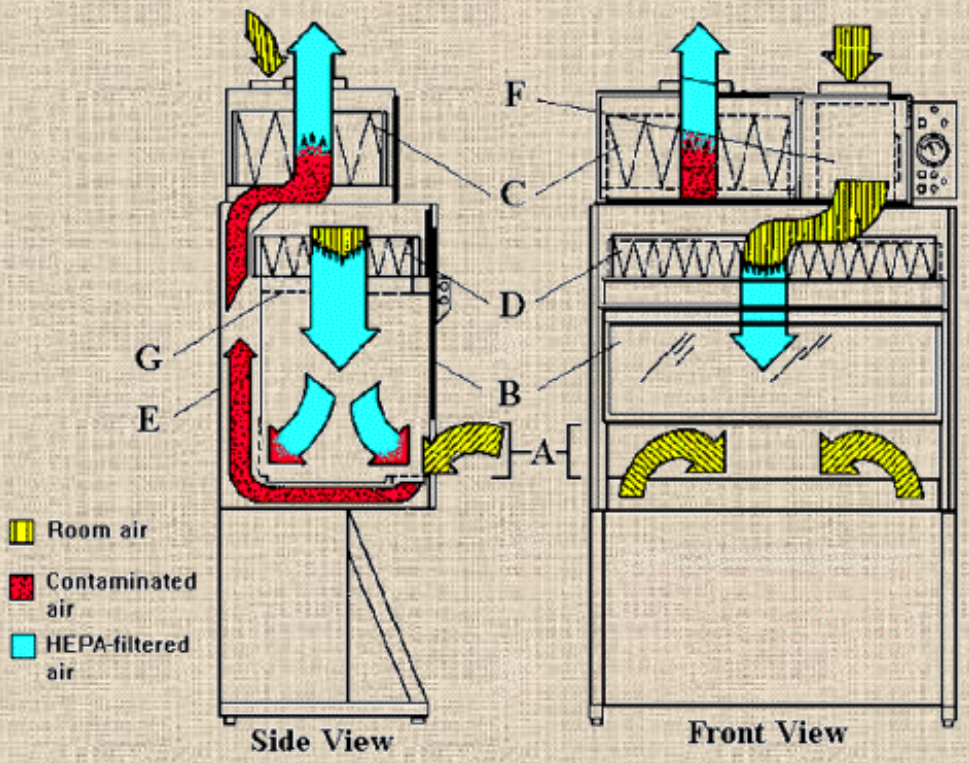
Class II, A1 BSC

HEPA Filters

HEPA filters are effective at trapping **particulates** and **infectious agents**, but not capturing volatile chemicals or gases.

Only BSCs that are exhausted to the outside should be used when working with volatile toxic chemical.

In certain cases a charcoal filter may be added to prevent release of toxic chemicals into the atmosphere.



Class II, B2 BSC

Class III BSC

Class III BSCs are designed for work with microbiological agents assigned to **BSL 4** and provides maximum protection to the environment and the worker.

It is gas-tight with a non-opening view window. Air is not recirculated. Instead, the exhaust air must pass through **two** HEPA filters or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a separate exhaust system exterior to the cabinet that keeps the cabinet under negative pressure.

Class III BSC

Arm-length, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet to allow for manipulation of the materials isolated inside.

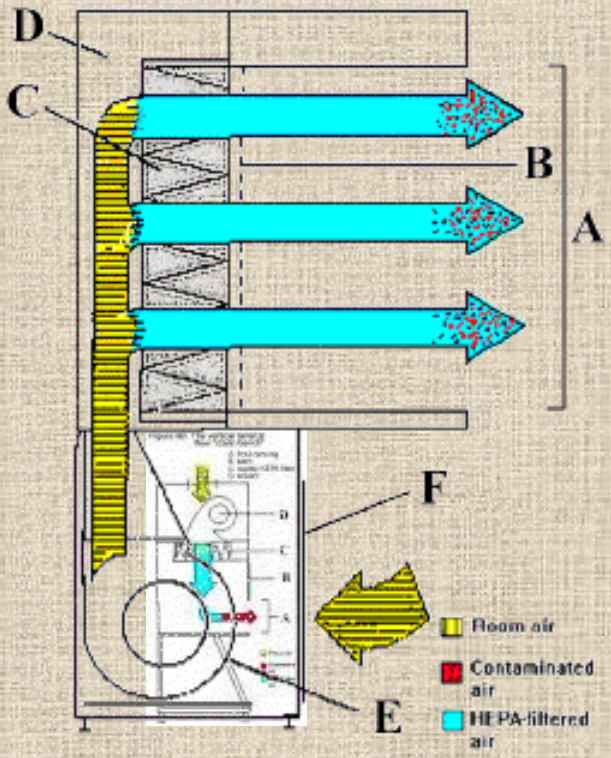
Dr. Kral with the Arkansas Center for Space and Planetary Sciences works in an anaerobic glove box, which has a similar glove attachment for working with organisms.

Clean Benches

Laminar flow "clean benches" are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. <u>These devices only provide product protection</u> (i.e. dust-free assembly of sterile equipment/electronics).

These benches should **NEVER** be used when handling cell culture materials or drug formulations, or when manipulating materials potentially infectious to humans.

<u>Clean benches must never be used as a substitute for a biological safety cabinet.</u>



Before Working in a Class II BSC

• Prepare a **written** checklist of materials needed for the activity and place them in the BSC

  •This minimizes the number of arm-movements that cause disruptions across the fragile air barrier of the BSC.

• When necessary, move arms in and out of the BSC slowly and perpendicular to the face opening to reduce the risk of disrupting the air curtain.

  **Other personnel activities in the room, such as rapid movement and opening/closing room doors, may also disrupt the air barrier.

PPE for working in a BSC

- Lab coats should be **BUTTONED** over street clothing

  **Note: a solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat.

- Appropriate gloves are worn to provide hand protection

  **Note: Gloves should be pulled over the knitted wrists of the sleeves rather than worn inside. Elasticized sleeves can also be worn to protect the investigator's wrists.

Before Working in a Class II BSC

- Adjust the stool height so that the investigator's face is above the front opening.
- After placing hands/arms inside the cabinet, **wait** approximately **one minute** before manipulating materials inside.
    - This allows the cabinet to stabilize and "air sweep" the hands and arms to remove surface microbial contaminants.
- **Do not block the front grill** with research notes, plastic wrappers, pipetting devices, or by resting arms flatly across the grill.
    - This may cause the room air to flow directly into the work area rather than being drawn through the front grill.

Working in a Class II BSC

- All operations should be performed at least **4 inches** from the front grill on the work surface.
- Materials placed inside the BSC may cause a disruption in airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment
    - Only the materials required for immediate work should be placed in the BSC.
    - Extra supplies should be stored outside the cabinet.

Reduce Contamination in the BSC

- Run the BSC 24/7; this also helps control dust and airborne particulates in the lab
- If it is run intermittently, start the BSC blowers **at least** five minutes before beginning work to purge the cabinet of any particulates.
    - Wipe down the work surface, interior walls (not including the supply filter diffuser), and the interior window
    - Use a 10% bleach solution or other disinfectant that will sterilize the agents used.
    - If bleach is used, wipe a second time with **sterile** water to remove residual chlorine and prevent the corrosion of stainless steel surfaces.

Reduce Contamination in the BSC

- Wipe the surfaces of materials placed in the BSC with a 10% bleach solution
  - Reduces the induction of mold spores
  - Minimizes contamination of cultures

**Periodic decontamination of incubators and refrigerators further reduces the microbial load on materials placed in BSCs.

Working Inside the BSC

• Plastic-backed absorbent toweling can be placed on the work surface, but not on the front or rear grill openings.

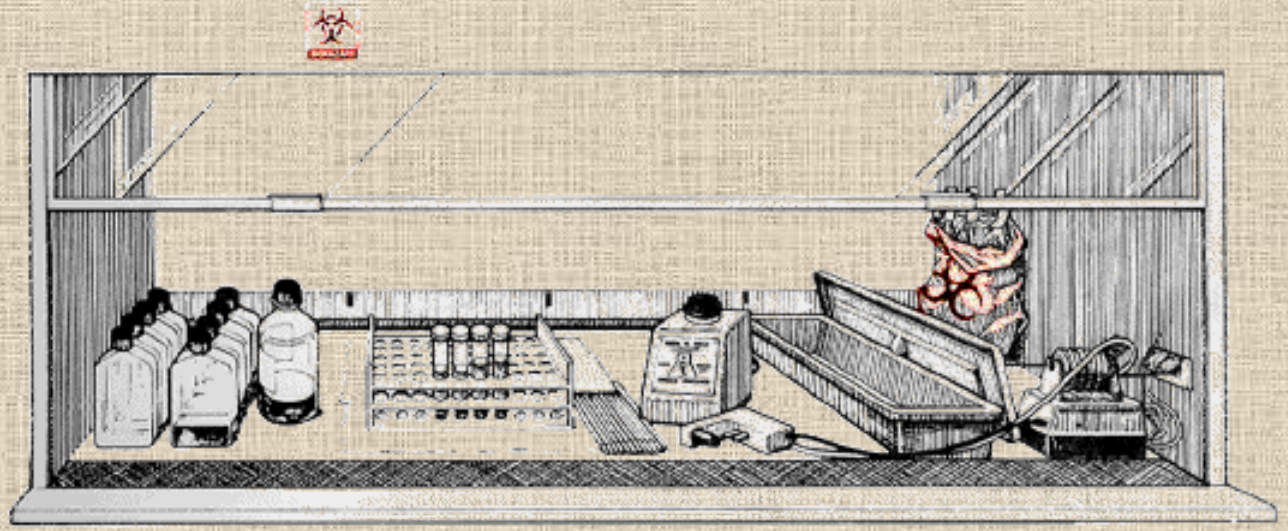
• Place all materials as far back in the cabinet as practical.

• Aerosol-generating equipment (i.e. vortex mixers and table top centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split.

• Active work should flow from “clean” to “contaminated” area across the work surface.

• Bulky items, such as biohazard bags and discard pipette trays, should be placed to one side of the interior of the cabinet.

Lay out of a BSC



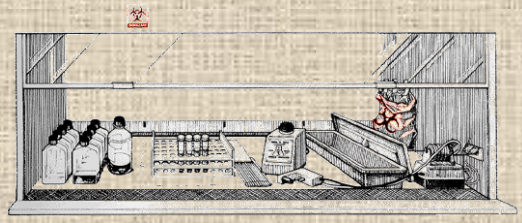
Work in the direction from “clean” to “contaminated”.

What NOT to do in a BSC

- **Do NOT** tape biohazard bags to the outside of the BSC.
- **Do NOT** use an **upright** pipette collection container in the BSC .
- **Do NOT** place a pipette collection container on the floor outside the cabinet.
    - Frequent movement in and out of the BSC disrupts the integrity of the cabinet air barrier and can compromise both personnel and product protection.
- **Do NOT** remove potentially contaminated materials out of the BSC until they have been surface decontaminated.

Use Good Microbiological Techniques

- Reduce splatter and aerosol generation
- Keep “clean” materials at least one foot away from aerosol-generating activities to minimize cross-contamination



- Opened bottles or tubes should not be held in a vertical position
- Hold lids above Petri dishes and tissue culture plates to minimize direct impaction of downward air.

- Bottle or tube caps should not be placed on the toweling/BSC surface.
- Items should be recapped or covered as soon as possible.

Open Flames in a BSC

- Not required or recommended in a BSC
  - Creates turbulence that disrupts the pattern of air supplied to the work surface

- Instead, use touch-plate microburners with a pilot light to provide a flame on demand.
  - Turn off burner when work is completed!
- Small electric “furnaces” are available to decontaminating bacteriological loops and needles
  - Preferable to an open flame in a BSC

- Disposable sterile loops can also be used.

Decontamination

• All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed.

• **At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass.**

• If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary.

****Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.****

Handling Spills in a BSC

- Remove contaminated toweling and place into biohazard bag
- Wipe up any splatters with a towel dampened with decontaminating solution
- Change gloves after work surface is decontaminated and before placing clean absorbent toweling in the cabinet
- Wash hands whenever gloves are changed or removed

Handling Spills in BSC (cont.)

Spills resulting in flowing liquid through front or rear grills require more extensive decontamination:

- Surface decontaminate and remove all items in the cabinet
- Ensure the drain valve is closed, then pour decontaminating solution onto the work surface and through the grill(s) into the drain pan
- Allow appropriate contact time for decontamination (20-30 min.: follow manufacture's directions)
- Wipe surface with paper towels and discard in biohazard bag
- Empty drain pan into container with disinfectant via a flexible tube submerged into the disinfectant to minimize aerosol generation
- Flush drain pan with water and remove drain tube

Handling Radioactive Spills

For spills involving radioactive materials, contact the Radiation Safety Officer, Maksudur Sarder, at **575-3379**.

Gas Decontamination of BSCs

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.

A risk assessment, which considers the agents manipulated within the BSC, must be done to determine the need for decontamination.

Common decontamination methods: formaldehyde gas and hydrogen peroxide vapor.

Ultraviolet Lamps in BSCs

UV lamps are not required or recommended in BSCs. If operated properly, BSCs do not need UV lights.

If installed UV lamps must be:

- Cleaned weekly to remove dirt and dust
  (they block germicidal effectiveness of UV light)
- Checked periodically to ensure the appropriate intensity of UV light is being emitted
- Turned off when the room is occupied to protect eyes and skin from UV exposure

**UV light can burn the cornea and cause skin cancer

Things to Remember about BSCs

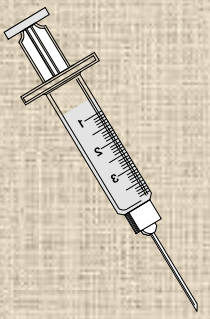
Biological safety cabinets were developed as work stations to provide personnel, product and environmental protection during the manipulation of infectious microorganisms.

However, to provide adequate protection to personnel, certain considerations must be met and practices be used to ensure maximum effectiveness of BSCs.

Biowaste Disposal

Regulated Biowaste:

- Liquid or semi-liquid blood or other potentially infectious materials;
- Items caked with blood;
- Sharps;
- Pathological or microbiological wastes;
- Other potentially infectious materials



Infectious or Physically Dangerous Medical and Biological Waste Picked Up by EH&S

1. Contaminated animal carcasses/body parts
2. Sharps

*Call EH&S at 575-5448 for pick up.

Sharps containers are available from EH&S.

Infectious or Physically Dangerous Medical and Biological Waste <u>Autoclaved</u> Prior to Disposal

1. Blood or blood products
2. Pathological waste (cell cultures, tissue samples, etc.)
3. Cultures and stocks of infectious agents and associated biologicals
4. Contaminated animal bedding
5. Biotechnological by-product effluents

*Must be properly treated or packaged prior to disposal

**** Ensure the autoclave is working properly****

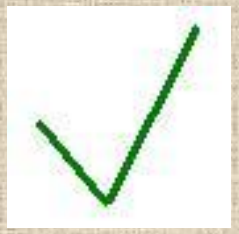
Do NOT Autoclave:

1. Corrosives (e.g., acids, bases, phenol)
2. Solvents or volatiles (e.g., ethanol, methanol, chloroform)
3. Flammables
4. Radioactive materials

After Autoclaving

For infectious waste that has been treated by steam sterilization:

- Pour liquids down the drain
- **DO NOT POUR LIQUID OR SOLID AGAR DOWN THE DRAIN!**
- Semi-solid and solid materials sterilized in an autoclave bag must be put into another dark plastic bag to be **disposed as regular lab waste**.
- All “Biohazard” labeling must be removed or covered.
- Glass must be in separate puncture-proof, leak-proof containers and labeled as “Glass.” Call EH&S for pickup.



For further information regarding Autoclave Safety, check out the online training located under "HOT TOPICS" on the EH&S Biosafety website.

http://www.phpl.uark.edu/ehs/HotTopics.htm

If you are occupationally exposed to blood or other potentially infectious materials (OPIM), you are required by the Occupational Safety and Health Administration (OSHA) to receive Bloodborne Pathogens Training. You may take an online course found on the EH&S website (http://www.phpl.uark.edu/ehs/Bloodborne.htm) or call EH&S to schedule in-person training.

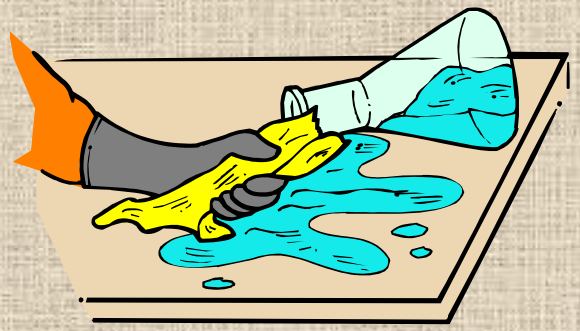
University of Arkansas
Office of Environmental Health & Safety

Presents

Bloodborne Pathogen Training

Spills

- When cleaning up surfaces use 10% bleach solution or approved disinfectant such as Hepacide Quat®. (Mix bleach solution fresh each time.)
- Spray and allow it to stand for at least **ten minutes** before wiping up up.
- Dispose of all wipes in biohazard containers.
- Decontaminate any materials used to clean up blood or OPIM (mops, sponges, buckets, etc.)
- PPE should be removed and disposed of in biohazard containers.
- Spill procedures found on EH&S website.



Laboratory Hygiene

DO NOT:

- Eat
- Drink
- Smoke
- Apply cosmetics (including lip balm)
- Handle contact lenses
- Store food or drink in lab refrigerators
- Wear open-toed shoes

Hand Washing

- Wash hands immediately after removing PPE
- Use a soft soap
- A hand sanitizer can be used but wash with soap and water as soon as possible.

Questions?

Office of Environmental Health and Safety

575-5448