Laboratory Safety and General Lab Practices
**Biosafety:** Containment principles, technologies and practices which are implemented to prevent unintentional exposure to pathogens and toxins – or their accidental release (World Health Organization- WHO)

<table>
<thead>
<tr>
<th>Four Primary controls of Biosafety</th>
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<tbody>
<tr>
<td><strong>Engineering</strong></td>
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<tr>
<td>Personal Protective Equipment (PPE)</td>
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<tr>
<td>Standard Operating Procedures (SOPs)</td>
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<tr>
<td>Administrative</td>
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<tr>
<td>Locks on doors</td>
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<tr>
<td>Directional Airflow</td>
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<tr>
<td>Interlocked doors</td>
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<tr>
<td>Biosafety Cabinets</td>
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<tr>
<td>Autoclaves</td>
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<tr>
<td>HEPA Filters</td>
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</tbody>
</table>
Biosafety Levels

- Range from BSL-1 to BSL-4.
  - Each biosafety level builds on the controls of the level before it.
- Every microbiology laboratory, regardless of biosafety level, follows standard microbiological practices.
Standard Microbiological Practices

- Lab personnel wash hands after handling viable materials, removing gloves, or leaving lab
- No eating, drinking, smoking, handling contact lenses, applying cosmetics, or storing human food in lab
- Contact lens users wear safety glasses, goggles or face shields
- Food stored outside lab in designated cabinets/refrigerators
- Mechanical pipetting devices are used (i.e., no mouth pipetting)
- Sharps handling policies/practices in place
- Procedures minimize splashes/aerosols
- Work surfaces are decontaminated at least daily and/or at completion of work
- Work surfaces are decontaminated after any spill/splash of viable material
- Disinfectants are labeled for agents being used
- Cultures/stocks/regulated wastes are decontaminated by approved method (e.g., autoclaving) before disposal
- Materials decontaminated outside of lab are transported in durable, leak-proof, closed containers
- Biohazard signage posted at lab entrance when infectious agents are present (signage lists agents and PI name/phone)
- Insect/rodent control program in effect
Biosafety Level 1

• Suitable for work with well-characterized agents not known to consistently cause disease in immuno-competent adult humans, and present minimal potential hazard to laboratory personnel and the environment.
• BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building.
• Work is typically conducted on open bench tops.
• Doors and hand-washing sinks, screened windows, slip resistant floors, adequate illumination, ample storage, non-porous cleanable surfaces
Biosafety Level 2

- Suitable for work involving agents that pose moderate hazards to personnel and the environment.

Lab design: Everything in BSL 1, plus: eye wash station, lockable doors, biosafety cabinets (if needed), directional (inward) airflow.
Biosafety Level 3

• Applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.

• Lab Design: Everything in BSL 2, plus: double entry door, directional (inward) airflow, access to an autoclave, biosafety cabinets, self-closing doors, hands-free sinks, sealable to allow decontamination.

• Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

• All procedures involving the manipulation of infectious material must be conducted within BSC or other physical containment device
Biosafety Level 4

- Required for work with Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).
- Requires specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics.
- Lab Design: Everything in BSL 3, plus: Interlocked doors, Double HEPA filtered exhaust facility, Double door autoclave (pass-through), Liquid effluent treatment, sealed lab, multi-level redundancy on all containment systems.
The Biological Safety Cabinet
Function of the BSC

• Several Classes of BSC exist
• Each provides different levels of protection

User
Product
Environment
User Protection

Directional airflow into the BSC
Any air entering the BSC through the sash opening is drawn inward by negative air pressure inside the BSC
Product Protection

• Incoming air is drawn into the front grill (not over product)

• HEPA filtered, vertical laminar airflow in cabinet. provides sterile flow of air over product and reduces swirling air currents.
  – Prevents aerosols from spreading to other materials in cabinet.
Environment Protection

- Air leaving the BSC passes through a HEPA filter.
- Inward airflow at sash
# Class I BSC

How does a Class I BSC protect the person and environment?

<table>
<thead>
<tr>
<th>User</th>
<th>Product</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directional (inward) airflow</td>
<td>NONE</td>
<td>HEPA filtration of all exhaust</td>
</tr>
</tbody>
</table>

[Diagram showing airflow and filtration processes for User, Product, and Environment]
### Class II BSC

How does a Class II BSC protect the person, product and environment?

<table>
<thead>
<tr>
<th>User</th>
<th>Product</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directional (inward) airflow</td>
<td>All air coming into the BSC goes down into the grill - never over the product</td>
<td>HEPA filtration of all exhaust</td>
</tr>
<tr>
<td></td>
<td>All air coming down onto the product passes through a HEPA filter</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram](image-url)
Types of Class II BSC

A1
- HEPA filter
- Room Air
- Potentially contaminated air
- HEPA Filtered air
- Positive pressure
- Negative pressure

A2
- HEPA filter
- Room Air
- Potentially contaminated air
- HEPA Filtered air
- Positive pressure
- Negative pressure

B1
- HEPA filter
- Room Air
- Potentially contaminated air
- HEPA Filtered air
- Positive pressure
- Negative pressure

B2
- HEPA filter
- Room Air
- Potentially contaminated air
- HEPA Filtered air
- Positive pressure
- Negative pressure
### Class III BSC

**How does a Class III BSC protect the person and environment?**

<table>
<thead>
<tr>
<th>User</th>
<th>Product</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sealed box under negative pressure</td>
<td>NONE</td>
<td>All air entering the cabinet passes through a HEPA filter and all air leaving the cabinet passes through a HEPA filter</td>
</tr>
</tbody>
</table>
Arrange work to flow from clean to contaminated

- Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.
One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.
To reduce the risk of bacterial and fungal contaminants:
Spray and wipe surfaces of items with 70% ethanol before placing them in the BSC.
Disinfect all materials before removing from the BSC
• Infections waste must be disposed of in autoclave bags
• Sealed before removal from the BSC
• Placed in the infectious waste containers with Red liners.
Setup

• At startup, let run for 3-5 minutes to establish airflow and remove contaminants

• UV light off, fluorescent light on, and plenum valve closed.
• Disinfect interior walls and work surface with 70% ethanol.

• Place work materials in BSC and adjust sash to appropriate height.

• Wait ~ 1 minute to establish air flow over work surface.

• Wear appropriate PPE to protect yourself from potential exposure and prevent contamination of work.

• Do not operate if an alarm condition exists.
Working in the BSC
- Adjust seat to place your armpits at the level of the bottom of the sash.
- Work using slow deliberate movements
- Avoid repeated or rapid entry and exit.
- Discard all material in a waste container in the BSC.
- Keep all open work at least 10cm from the front grill.
- Use a plastic backed absorbent pad to catch spills and facilitate cleanup.
- Heavily contaminated materials or aerosol generating equipment should be placed as far back in the BSC as practical.
- **DO NOT** place any materials over the grill in the front or back of the BSC.
- Work from “Clean” to “Dirty”.
- No open flames….if unavoidable use one with a pilot light and keep as far back as possible.
- Place large/tall items to the sides.

Work in the middle of the BSC

**WHY?**
We will be working at Biosafety level -1

- Remove gloves before leaving the lab.
  - Discard in appropriate vessel
- Wash hands thoroughly before leaving the lab.
Removing Gloves

1. Begin by pulling the glove off the hand without touching the inner surface.
2. Fold the glove over the wrist to minimize contamination.
3. Continue removing the glove, keeping it folded over the wrist.
4. Ensure all parts of the glove are discarded properly.
Dispose of used gloves and other "medical" waste in the red bins

NOT in the Trash
Never Re-cap Needles
Dispose in an appropriate sharps container
Resources:


NIH Guidelines for Research involving Recombinant DNA Molecules (2013)

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition (2009)
http://www.cdc.gov/biosafety/publications/bmbl5/