Massachusetts Department of Public Health
Bureau of Infectious Disease and Laboratory Sciences

Overview of Safety in the Laboratory

Shoolah H. Escott, MS, MT(ASCP)
Biosafety Manager
Massachusetts State Public Health Laboratory

Objectives

- Discuss the principles of safety in the clinical laboratory
- Describe recommendations for preventing Laboratory Acquired Infections (LAIs)
- Define the biosafety levels
- Describe how to apply BSL-3 practices in a BSL-2 laboratory
- Explain how to work safely in a biological safety cabinet (BSC)

Outline

- Principles of Safety
  - Risk assessment and containment
- Laboratory Acquired Infections
- Biosafety Levels
  - BSL-2 Standard and Special Practices
    - Sharps, Aerosols, Waste Disposal and Personal Protective Equipment (PPE)
  - BSL-3 Practices in a BSL-2 Laboratory
    - Respirators
- Class II Biosafety Cabinets (BSCs)
- Chemical Fume Hoods (CFHs)

What is Laboratory Safety?

The discipline addressing the safe handling, containment and practices to prevent exposure to potential hazards including chemical, biological, physical and radioactive hazards, as well as musculoskeletal stresses.

- **Risk Assessment**
- **Containment**

Principles of Safety Risk Assessment (RA)

***Process* to**:

- identify the hazards (agent, non-bio hazards, procedures and staff)
- evaluate the risks
- determine controls (mitigate)
- implement controls
- review and adjust

Risk assessment is the basis

of a safety program

Principles of Safety Containment

Risks can be reduced by using a combination of:

- Appropriate microbiological and laboratory practices
- Safety equipment and PPE
- Facility design

The term biosafety level is used often to describe containment.

Principles of Safety

Risk assessment and containment can reduce occurrence of lab acquired infections (LAIs) by:

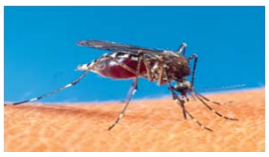
Reducing or minimizing exposure to microorganisms by breaking the "chain of infection"

- block routes of transmission
- protect the portals of entry

But….the risk is never zero!

Portal of Entry	Route of Transmission
Respiratory tract (lungs).....	Inhalation of aerosols or chemical fumes
Gastrointestinal tract (mouth)….	Ingestion
Mucous membranes (eyes, nose, mouth).....	Direct contact (splash, spill)
Non-intact skin……..	Percutaneous(sharps, bites, vectors)
Genitourinary tract……	Sexual
Various……..	Indirect (fomites*)





***Fomite - an inanimate object (as a computer, doorknob, phone or work surface) that may be contaminated with infectious organisms and serve in their transmission**

Chemical Hazards

- Laboratory chemicals may pose health or a physical hazards.
  - Health Hazard
  - Physical Hazard
- Detailed descriptions of chemical hazards are contained in the Hazard Communication training. These include information sources (Safety Data Sheets and labelling).

Chemical Health Hazards

Some examples:

- carcinogens
- toxic or highly toxic agents
- reproductive toxins
- irritants
- corrosives
- sensitizers
- hepatotoxins
- nephrotoxins
- neurotoxins
- hematopoietic damaging agents
- anything that damages
  - lungs, skin, eyes or mucous membranes

10

Chemical Physical Hazards

"Scientifically valid evidence" it is:

- combustible liquid
- compressed gas
- explosive
- flammable
- organic peroxide
- oxidizer
- pyrophoric
- unstable (reactive)
- water-reactive

11

Physical Hazards

Include, but are not limited to:

- Compressed gases
- Electrical equipment
- Lasers
- Radiation
- Thermal hazards (extreme heat and cold)
- Use of equipment
- Ergonomics
- Noise

Challenges in the Clinical Lab

- High stress
  - Critical nature of work
  - High workload and fast pace
- Limited staff and resources (less $$) leads to more stress
  - High workload, insufficient BSCs or CFHs or space, facility/infrastructure issues/breakdown
- Lack of time or $$ for training (limited staff)
  - Unsafe practices
  - Assumption that BSC/CFH and PPE are effective
- Unfamiliar with agent (rare) or chemical
  - Work conducted on open bench before risk is known
- Lack of management support
  - PPE usage not always enforced
  - Lack of training
  - Not enforcing good practices

Worker is pivotal in controlling the safe outcome of any operation!

Routine can become the enemy!

Everyone has different perceptions and risk tolerance

Laboratory Acquired Infections (LAIs)

Organisms Associated with LAIs (1979-99)

- *Mycobacterium tuberculosis*
- *Coxiella burnetii*
- Hantavirus
- Arboviruses
- HBV
- *Brucella spp.*
- *Salmonella spp.*
- *Shigella spp.*
- Hepatitis C virus
- *N. meningitidis*

Of the 1267 cases, *22 deaths resulted*

Study by Harding and Byers, 2006

LAI Surveys

- Only 16% of the cases were associated with a documented accident.

* Most related to mouth pipetting and the use of needles.

- However, in *80% of cases*, exposure to aerosols was a plausible but unconfirmed source of infection

Greatest risk to microbiologists:
Brucella* spp. and *N. meningitidis

Neisseria meningitidis
Special Risk to Laboratorians

Each year approximately 3000 isolates of invasive *N. meningitidis* are handled resulting in an estimated 9000 clinical microbiologists potentially exposed

From 2 studies covering 1979 – 2004:
- 31 cases total
- ***11 fatalities (>35% mortality)***

Potentially risky procedures:
Inoculating biochemicals, preparing suspensions, performing tests such as catalase on open bench

No BSC was used in 94% of the documented exposures

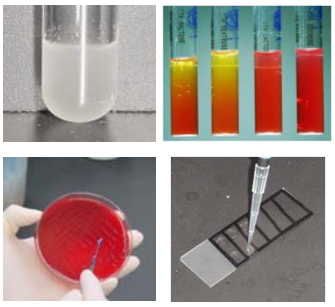
Laboratory Employees Face Greater Risks

Organism	Risk/100,000 microbiologists	Risk/100,000 general population
Brucella	641	0.08
Coccidioides	13.7	12
C.difficile	0.2	8
E.coli O157:H7	8.3	0.96
N.meningitidis	25.3	0.62
Salmonella	1.5	17.9
Shigella	6.6	6.6

From: *Baron EJ, Miller JM. 2008.* Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. Diagnostic Microbiology and Infectious Disease; 60 (3): 241-6.

High Risk Activities Identified

- Sniffing plates??
- Generating aerosols- (more on this later)
- Centrifuging /vortexing
- Making slides
- Inoculating biochemicals
- Not using or improper use of BSC



Trigger Points

- A trigger point is a recognized combination of diagnostic findings that can be *used to determine when to heighten the precautions* or conditions that a sample or culture is handled under.
- For example a trigger point would be used to determine when to begin working with an organism in a biological safety cabinet.

Some Trigger Points

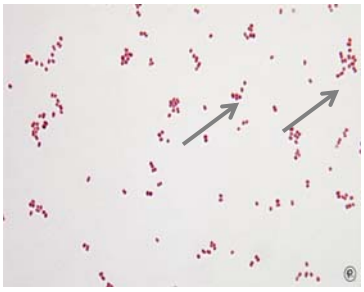
- Cultures from sterile body sites, e.g., CSF
- Slowly growing, tiny colonies at 24–48 hours with Gram stain showing Gram-negative rods or Gram-negative coccobacilli
- Slow growth in blood culture bottles (i.e., positive at ≥48 hours), with Gram stain showing small Gram-negative rods or Gram-negative coccobacilli

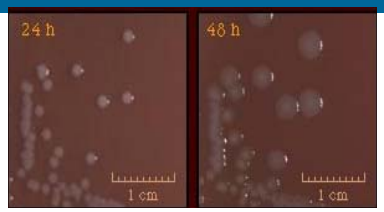
Some Trigger Points

- Growth only on chocolate agar
- Rapid growth of flat, nonpigmented, irregular colonies with comma projections and ground-glass appearance
- Gram stain showing boxcar-shaped, Gram-positive rods with or without spores

Where is your trigger point?

Gram negative cocci or Gram negative diplococci from blood or CSF





"round, smooth, moist, glistening, and convex" colony morphology on choc agar at 24 / 48 hr or Slow growth on BAP, No growth on MAC

Positive oxidase test

In the Event of an Exposure

Immediate first aid (i.e., an effective and timely cleansing response to a known wound) *may be the most critical determinant in preventing infection*

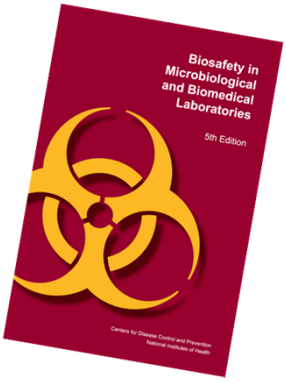
Exposure Response

- Have plan and SOPs in place
  - Available and accessible immediately and 24/7
  - Simple, easy to follow guidance
  - ***Practice*** emergency procedures with simulation drills
  - Review gaps and adjust
- Use Medical Alert Cards?
- Are first aid kit contents checked regularly?
- Are exposures linked to further assessment and reporting?

Biosafety Levels (BSLs)

Biosafety Levels

Biosafety (containment) levels are described in "Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2009"



Biosafety Levels

Combination of:

- Laboratory Practice and Technique
  - Standard Practices
  - Special Practices
- Safety Equipment (Primary Barriers and PPE)
- Facility Design and Construction (Secondary Barriers)

Biosafety Levels (BSL) 1- 4

2 Types of Containment are:

- **Primary** - Protects worker and the immediate lab environment
  - Strict adherence to standard microbiological practices and techniques
  - Safety Equipment and PPE
- **Secondary** – Protects worker and external environment
  - Facility design and construction

Biosafety Levels

Biosafety Level	Potential Hazard
BSL-4	HIGHEST
Specialty research labs – CDC	
BSL-3	
Public health/research/some clinical laboratories	
BSL-2	
Clinical laboratories	
BSL-1	
College/ teaching laboratories	LOWEST

Standard Microbiological Practices for Biosafety Level 1- 4

1. Limited access to lab while work is in progress
2. Avoid mouth and eye contact with potentially infectious materials
3. Wash hands
4. Safe sharps handling
5. Limit or contain aerosols
6. Training in procedures and biosafety
7. No eating, drinking, handling contact lenses, applying cosmetics or storing food
8. Decontaminate work surfaces after spill or completion of work
9. Decontaminate potentially infectious materials before final disposal
10. Universal biohazard symbol at lab entry
11. Pest management program

Biosafety Level 2 (BSL-2)

Biosafety Level 2

Supervisor

- *Scientist competent in handling infectious agents and associated procedures*
  - Controls access to those that are trained and meet entry requirements
  - Restricts access to the laboratory when work is being conducted

Lab Personnel

- *Advised of potential hazards*
- *Trained and proficient in practices/techniques*

Staff Training Should Cover:

- Biohazards and hazard controls
- Risks of different types of exposures
- Available vaccinations and side effects
- Post-incident first aid and remediation
- Signs and symptoms of infection
- Emergency response procedures
- Incident reporting procedures

Staff Training

- Promote benefits of non-punitive reporting of exposures and near misses
- Use incident investigation in your training to accentuate the "**opportunity this presents**" not the "**failure it represents"**
  - Case studies of real incidents
  - CDC's MMWR (Morbidity and Mortality Weekly Report) www.cdc.gov/mmwr

Pathogen Safety Data Sheet (PSDS)

Agency of Canada publique du Canada

Public Health Agency of Canada
www.publichealth.gc.ca

Français | Home | Contact Us | Help | Search | canada.gc.ca

Home > Laboratory Biosafety and Biosecurity > Biosafety Programs and Resources > Pathogen Safety Data Sheets and Risk Assessment > Neisseria meningitidis

Agency Information
About the Agency
Diseases & Conditions
Infectious Diseases
Chronic Diseases
Health & Safety
Travel Health
Food Safety
Immunization & Vaccines
Emergency Preparedness & Response
Health Promotion
Injury Prevention
Lab Biosafety & Biosecurity
Research & Statistics
Surveillance
Information
Media Room
Reports & Publications
A-Z Index
Transparency
Completed Access to Information

+/- TEXT PRINT SHARE

NEISSERIA MENINGITIDIS

PATHOGEN SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Neisseria meningitidis*

SYNONYM OR CROSS REFERENCE: Meningococci [1], meningococcemia, meningococcal infection, meningococcal meningitis.

CHARACTERISTICS: *Neisseria meningitidis* belongs to the family Neisseriaceae [2]. It is a Gram-negative, non-spore forming, non-motile, encapsulated, and non acid-fast diplococci, which appears in kidney bean shape under the microscope [1-3]. It requires an aerobic environment with 5% CO_2 and enriched media containing blood for growth [1]. Medium-sized, smooth, transparent, non-pigmented, non-hemolytic, and convex colonies are produced on blood agar after overnight incubation at 35-37°C [1-3]. It is oxidase and catalase positive [1]. It has at least 12 serogroups, with serogroups A, B, C, W-135, and Y being the most commonly encountered serogroups from invasive disease cases [4-5].

SECTION II - HAZARD IDENTIFICATION

PATHOGENICITY/TOXICITY: *N. meningitidis* has a wide range of clinical manifestations, ranging from transient mild sore throat to fatal meningitis or meningococcal septicemia [3]. Meningitis and septicemia are the most common presentations of the disease [3].

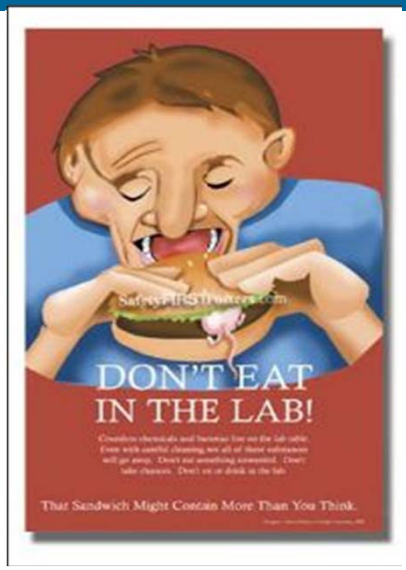
Transient meningococcemia: Patients present with mild flu-like symptoms such as fever, joint pain, and occasionally rash. The illness lasts for a few days or weeks [3].

Meningitis [1-3]: Most patients also present with signs of meningeal irritation, including, neck stiffness, bulging fontanelle (in infants), irritability, lying on one side away from light, and inability to extend the knee when hip is flexed in supine position (positive kernig's sign) [4-5]. Convulsions, declining level of consciousness, and coma may occur [3]. The petechial rash of meningococcemia may also occur [1].

Meningococcemia: Patients present with rapid onset of fever, vomiting, photophobia, convulsions, skin rash, lethargy, irritability, drowsiness, diarrhea, muscular pain, arthralgia,

http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

Fun Posters



Standard/Universal Precautions

- Standard (SP)/Universal Precautions (UP) are designed to reduce the risk of transmission of microorganisms **from both recognized and unrecognized sources** of infection
- SP are **more encompassing** and are not just for bloodborne pathogens**-handle all patient specimens as if they are infectious**
  - Hand hygiene
  - PPE
  - Safe sharps practices
  - Safe handling of contaminated equipment or surfaces
  - Respiratory hygiene

Standard Microbiological Practices

Use mechanical pipetting devices

Safe Handling, Minimizing & Disposal of Sharps






- Always use a proper leak proof and puncture resistant container to dispose of sharp materials
- Never fill sharps container to the top
- Use plastic vs. glass
- Use safer sharps, i.e., retractable/ shielded needles

Safe Handling, Minimizing & Disposal of Sharps

DON'T discard sharps in the regular trash
DON'T touch broken glass with bare hands


Aerosols and Droplets

Aerosols and Droplets*

- Aerosol
  - Small particle (~<0.5 um) that can remain suspended in the air and can be inhaled deeply into the lung
- Droplet
  - Larger particle (~>0.5um) that can settle due to gravity
  - May contaminate surfaces and be picked up on hands

* the remainder of this presentation will refer to aerosols as defined above

Aerosols

Procedures that impart energy to a microbial suspension

Ubiquitous in laboratory procedures

Usually **undetected**

Extremely **pervasive**, putting all at risk

Likely to be the cause when other causes are ruled out and the person just "worked in the room" where the agent was

Aerosol Generating Procedures

Average Bacteria Recovered from Aerosol Generating Procedures

Procedures	Average Bacteria CFU recovered/ft from air during the procedure
Opening petri dish	0
Opening screw capped test tube	4
Picking colony from plate	0.005
Streaking on smooth agar plate	0.26
Pipette inoculating test tube	0.26
Syringe and needle withdrawal from rubber-cap bottle	16.0

Source: Kruse, R.H. et. Al. Biological Safety Cabinetry. Clinical Microbiology Reviews. 1991.4:207-241.

Procedures That May Generate Aerosols

- Performing catalase test
- Inoculating biochemicals
- Aspirating blood from blood culture bottles
- Pipetting
- Mixing
- Centrifugation
- Grinding
- Vortexing
- Pouring
- Opening lyophilized cultures
- Flaming loops
- Entering or opening vessels at non-ambient pressures, (e.g., fermenters, freezer vials)
- Using cell sorters
- Sonicating
- Loading syringes

Bottom line: most lab procedures can potentially generate aerosols

Minimize Aerosols

- Discharge liquid down side of container
- Deliver as close as possible to contents
- Use capped tubes when mixing or vortexing
- Use care with needles (gauze pad with alcohol on septum of blood culture bottle)
- Use pipette aids with filters
- Change procedures (i.e., perform catalase in tube or in a petri dish)

Minimize Aerosols

- Use incinerators
- Pour liquids carefully
- Work over absorbent pad
- Use centrifuge safety cups
- Use sealed rotors and open in BSC if possible




Decontaminate Workspace

Maintain a clean workspace and decontaminate daily with a disinfectant that is effective against the target organism

Sodium hypochlorite

Quaternary ammonium

Effectiveness of Disinfectants

There **is** ***no one universal disinfectant*** effective against all organisms-because of:

- Concentration of the disinfectant
- Concentration of the agent
- Type of agent
- Contact time
- Amount of organic material
- Environmental conditions - pH, temp, humidity

Generalized Order of Resistance to Disinfection

- Prions-**Most Difficult**
- Bacterial spores (*B. anthracis*)
- Mycobacteria (*M. tuberculosis*)
- Nonlipid (non-enveloped) viruses (Norovirus, Poliovirus)
- Fungi (*Aspergillus, Candida albicans*)
- Vegetative bacteria (*S. aureus*)
- Lipid (enveloped) viruses (Ebola, HIV) **Readily Killed**

Biological Waste Disposal

Biological waste containers should always be puncture resistant and labeled with a biohazard symbol

Biological Waste Disposal

- If this process is done on-site but remote from the laboratory, place the discarded items into durable, leakproof containers that are secured when they are moved.
- Decontamination may be done by a medical waste treatment contractor's facility if the waste is placed into medical waste shipping containers and packaged in accordance with applicable regulatory standards.

Biological Waste Disposal

Autoclaving:

- Personnel must be trained
- Validation of the autoclave cycles for effective decontamination of the projected loads is recommended in addition to a regular maintenance and QA program.
- Appropriate PPE (such as autoclave gloves) must be available and used properly

Personal Protective Equipment (PPE)

- ***Why?***
  - *Protect the worker*
  - *Protect product*
- ***What? Minimum of:***
  - *Lab coat-long sleeved and buttoned*
  - *Eye and face protection*
  - *Gloves*

PPE does not eliminate the hazard- Know the limitations!

Protect your eyes and mucous membranes against splashes and aerosols

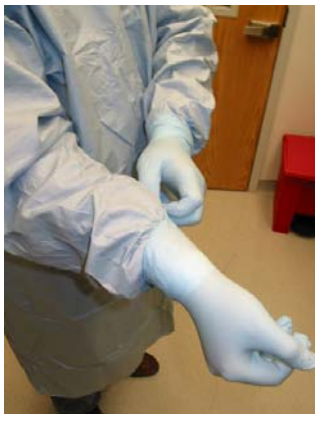
Safety glasses?

Face shields?

Benchtop Splash Shields?

Personal Protective Equipment: Gloves

- Not all gloves created equally; select best glove for the task
- Check integrity before use
- Do not wash or reuse
- Disinfectants or chemicals enhance permeation
- Change often - integrity decreases with use
- Do not touch “clean” surfaces



Gloves: Important Considerations

- Change gloves as soon as feasible after contamination or compromise
  - Jewelry and watches can puncture gloves
- Use utility gloves or double glove for spills
- *Latex allergies: Alternatives must be provided*
- Consider chemical hazards

59

When do you wear gloves in the general microbiology lab?

This is controversial!

- "Gloves should be worn at the specimen receiving and set-up areas, and in TB/virology labs, and when hands may contact potentially infectious material, contaminated surfaces or equipment." (CLSI M29-A3)
- "The wearing of gloves is therefore *encouraged* to augment good work practices while reading and subculturing plates." (CLSI GP17-A3 referencing OSHA letter on 5/19/2011)

When do you wear gloves in the general microbiology lab?

This is controversial!

- "Gloves must be worn to protect hands from exposure to hazardous materials" (BMBL 5th edition).
  - Based on a lab-specific risk assessment, laboratory management sets policy regarding when to wear gloves.
- "In the general microbiology laboratory, masks and disposable gloves ***are not required*** in the open laboratory but may be voluntarily used" (Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories, Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel, MMWR, January 6, 2012 / 61(01);1-101)

Biosafety Level 2: Special Practices

Policies and procedures for entry

Medical surveillance and immunizations as appropriate

Restricted access (doors closed) when work in progress

Biohazard signs on entry door

- Entry requirements-PPE, immunizations
- BSL
- Emergency contact info for responsible person

Site-specific safety manual

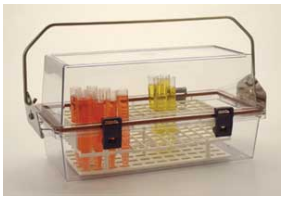
Biosafety Level 2: Special Practices

Use Class II BSC for work with infectious agents involving:

- Procedures that can generate aerosols/splashes
- Large volumes/high concentrations of organisms
- "Trigger Point" indicators
- Known routes of transmission via inhalation (airborne)
- High-risk pathogenic organisms that can cause severe or fatal infections

Biosafety Level 2: Special Practices

- Use leak-proof transport containers
- Report spills and accidents
- Baseline serum samples when indicated
- Appropriate medical evaluation and treatment are provided
- Written records are maintained



Chemical Decontamination Method for Select Agents

If a medical waste contractor is used for the facility, the cultures may be inactivated by completely immersing the open culture containers in a fresh 1:10 bleach solution overnight before discarding them into medical waste.

From *MMWR* / January 6, 2012 / *Vol*. *61*. 1. *Supplement*. Guidelines for Safe Work Practices in Human and Animal. Medical Diagnostic Laboratories

Additional Recommendations

- Establish policies for use of ***cell phones/personal electronic equipment*** in the lab
- Establish procedures for handling phones, keyboards, microscopes, etc.
- Remind clinicians to notify lab if high risk organism is suspected
- Consider the limitations of automated instruments and kits-especially with unusual, slower-growing isolates- ***call your PHL***

Biosafety Level-3 (BSL-3)

Additional Controls for BSL-3 vs. BSL-2

- ***MORE restricted*** access to the laboratory
- **Additional** PPE is worn
- Lab personnel must demonstrate proficiency **prior** to BSL-3 work
- **NO** work in open vessels is conducted on the bench-work in BSC or other containment equipment!
- Method for decontamination of all laboratory waste **before** final disposal

Additional Controls for BSL-3 vs. BSL-2

- Supervised by scientists experienced with specific agents
- Personnel have specific training to handle particular pathogens
- Supervisors evaluate effectiveness of training
- Personnel must be offered medical surveillance and immunizations

Additional Controls for BSL-3 vs. BSL-2

- Laboratory has **special engineering and design features**
  - Physical separation from access corridors
  - 100% Exhausted air - No recirculation
  - Directional airflow into laboratory (must be verified)
  - Entry through two self-closing doors that cannot be open at the same time (e.g., airlock or anteroom)
  - Hands free sinks – one near laboratory exit

What are BSL-3 practices?

- Restricted access to the laboratory
- Additional PPE (solid-front gown, gloves and eye protection as a minimum) are worn in the lab.
- Lab personnel must demonstrate proficiency prior to BSL-3 work.
- **NO** work in open vessels is conducted on the bench-work in BSC or other containment equipment!

What are BSL-3 practices?

All cultures, stocks and other potentially infectious materials are **decontaminated before (final) disposal b**y an approved and validated decontamination method, such as autoclaving……

Preferably within the Laboratory

When do you use BSL-3 practices in a BSL-2 lab?

- When working with known or suspected agents that are normally handled under BSL-3 conditions, and a BSL-3 laboratory is not available
  - *Brucella* spp., *Franciscella tularensis, Coccidioides*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, Mtb
  - **This is not for routine use!**
- When determined by the risk assessment
- When initially processing Proficiency Testing (PT) Survey specimens

Respirators

IF respirators are indicated by the risk assessment:

- Personnel must have medical clearance, be fit tested and trained annually (OSHA 29 CFR 1910.134)
- They must be maintained
- They REDUCE exposure, do NOT eliminate exposure-risk is never zero
- Remember:
  - Facial hair can interfere with N95 seal
  - ***Surgical masks are NOT respirators!***

Respirators

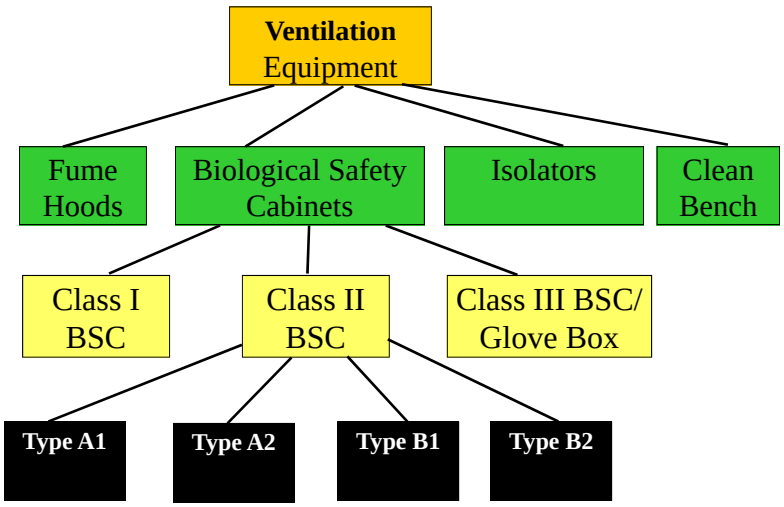
- N95
- PAPR

Laboratory Biosafety Level Criteria

Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	▪ No primary barriers required. ▪ PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
2	▪ Agents associated with human disease ▪ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: ▪ Limited access ▪ Biohazard warning signs ▪ "Sharps" precautions ▪ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ▪ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ▪ PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ▪ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: ▪ Controlled access ▪ Decontamination of all waste ▪ Decontamination of laboratory clothing before laundering	Primary barriers: ▪ BSCs or other physical containment devices used for all open manipulations of agents ▪ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: ▪ Physical separation from access corridors ▪ Self-closing, double-door access ▪ Exhausted air not recirculated ▪ Negative airflow into laboratory ▪ Entry through airlock or anteroom ▪ Hand washing sink near laboratory exit

Primary Containment Barriers



Primary Containment Barriers

	Personnel	Product	Environment
Chemical Fume Hoods	X		X
Laminar Flow Clean Benches		X	
Class I BSC	X		X
Class II BSC	X	X	X
Class III BSC	X	X	X

Fume Hoods ≠ Biosafety Cabinets

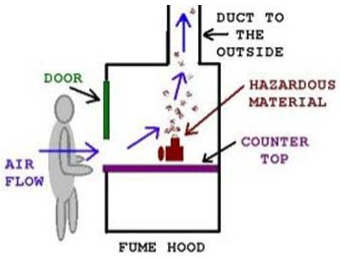
- BSCs do **NOT** filter out gases and vapors-
  - They only filter out particulates
- Fume hoods are used for volatile chemicals-not biohazardous materials

Chemical Fume Hood

Class II BSC Front Grill

Chemical Fume Hoods

- A device enclosed except for necessary exhaust purposes on three sides and top and bottom, designed to draw air inward by means of mechanical ventilation, operated with insertion of only the hands and arms of the user, and used to control exposure to hazardous substances.
- These devices are also known as laboratory fume hoods.



Chemical Fume Hoods

- Chemical fume hoods, when used properly, are one of the most reliable engineering controls in the laboratory. They protect workers by:
  - Containing vapors, dusts, gases, and fumes generated within the hood, and removing them as air flows into the hood and then out via the laboratory exhaust system
  - Contributing to laboratory ventilation as air flows through the hood
  - Shielding the worker with a clear sliding window, called a sash, that contains aerosols and prevents injury from splashes, fires, or minor explosions that may occur inside the hood

Chemical Fume Hoods

- Two kinds
  - **Constant air volume hoods:** The constant air volume (CAV) fume hood exhausts the same amount of air all the time, regardless of sash position. As the sash is lowered and raised, the velocity at the face of the hood changes.
  - **Variable air volume hoods:** Some newer models, called variable air volume (VAV) hoods, modulate air flow based on sash height and maintain 100 feet per minute face velocity at all sash heights.

Chemical Fume Hoods

- Videos on proper use of fume hood:
  - https://www.bing.com/videos/search?q=labconco+basic+fume+hood+operation&view=detail&mid=7A764B2CC5300D74D6887A764B2CC5300D74D688&FORM=VIRE (3:18 min), Labconco Corporation
  - https://ehs.berkeley.edu/fume-hoods (3:39 min.), EH&S UC Berkeley
- Should be inspected and tested at least annually.
- A certification sticker is placed on the front of each fume hood indicating the inspection results.

Chemical Fume Hoods

- Limitations:
  - **Biohazardous materials:** Fume hoods are not for use with biohazardous materials.
  - **Highly toxic materials:** In some cases, for highly toxic materials a glove box or another containment device is preferred over a chemical fume hood.

"Snorkel" or “Elephant Trunk"

- Are flexible arms with cones on the end that can be positioned directly over your work. Intended for small areas or machines, each snorkel exhaust has its own air damper.
- The effective range of snorkel exhaust is typically less than a foot (12 inches). Check manufacturer’s O&M Manual.

Class II Biosafety Cabinets (BSCs)

What is a Class II Biosafety Cabinet?

A ventilated cabinet for **personnel, product, and environmental protection.**

- open front with inward airflow for personnel protection,
- downward HEPA* filtered laminar airflow for product protection,
- HEPA filtered exhausted air for environmental protection (NSF 49, 2002)

*High Efficiency Particulate Air

What is a HEPA Filter?

- High Efficiency Particulate Air Filter
  - Rigid, folded cellulose/borosilicate media with very large surface area
  - Particles at 0.3 microns are captured with an efficiency of at least 99.97%.
  - Particles >0.3 microns (many bacteria) and particles <0.3 microns (viruses) are captured with efficiency ***greater than*** *99.97%.*

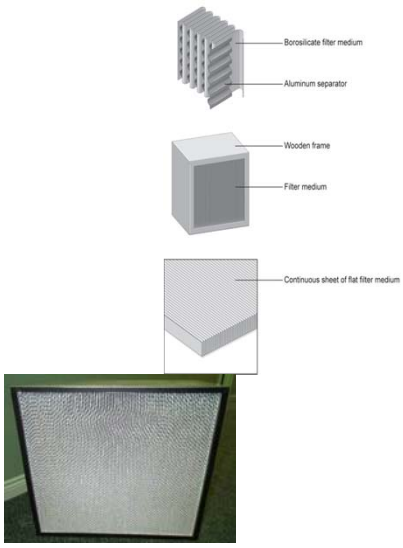
HEPA Filters

DO NOT

filter out

gases and vapors

They ***only*** filter out particulates



Class II BSC

- Most clinical labs use Types A1 or A2
  - Protects product, personnel and immediate lab environment
  - Reduces potential for exposures, does not eliminate (***risk is never zero!)***
  - Staff need to be trained
  - BSC needs to be certified annually and whenever moved

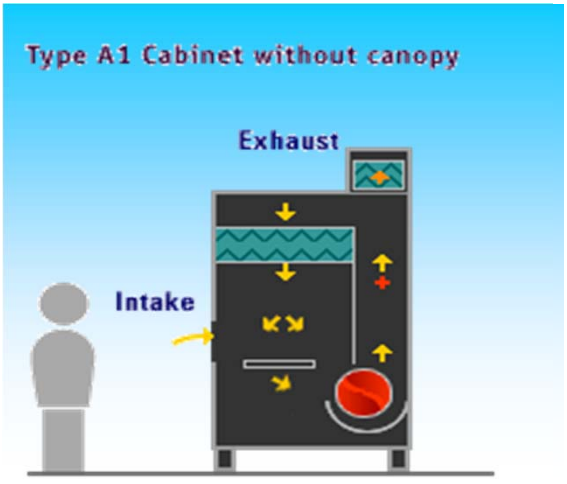
Class II Type A1 BSC

"Type **A1** cabinets are ***not suitable*** for work with volatile toxic chemicals and volatile radionuclides".

Why are A1 BSC not suitable for chemicals?

Because 30% of the HEPA filtered air is recirculated ***back into the lab***, and 70% of the HEPA filtered air is recirculated back into the cabinet, which can potentially concentrate the volatile chemicals *(NSF/ANSI Std. 49-02)*

Class II Type A1 Airflow



Class II Type A2 BSC

"Type A2 cabinets used for work with **minute quantities** of volatile toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies ***must be exhausted through properly functioning exhaust canopies."***

Why can minute quantities of chemicals be used?

Because the air is exhausted to the outside through the building exhaust system

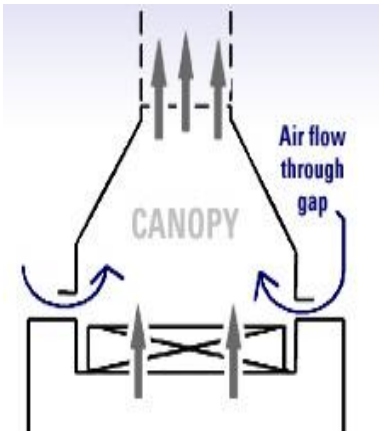
(NSF/ANSI Std. 49-02)

Class II Type A2 BSC

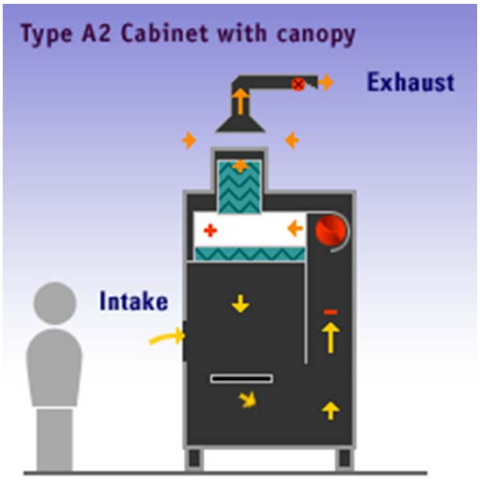
What is an Exhaust Canopy (or Thimble)?

- A small opening or air gap (usually 1 inch) between the top of the BSC and the ductwork for the building exhaust
- The air gap on the canopy allows for exhaust system fluctuation to occur and not affect the BSC containment performance
- Easier to maintain appropriate air balance than hard-ducted Type B1/2 BSCs

Exhaust Canopy (or "Thimble")



Class II A2 Airflow



Location, Location, Location

Preferred BSC operating location:

- Isolated from other work areas
- Removed from high traffic areas
- Away from lab HVAC exhaust and supply vents
- Away from laboratory entry doors
- 12-14” away from ceiling and walls

Air Curtain at Front Sash Can Be Compromised-Example

- Face velocity at the sash for A2 BSC is **100 fpm**
- 100ft/min X 60 min/hour X 1 mile/5280 ft= 1.14 mi/hr
- **At a walking rate of 1.14 mi/hr, you can *pull air out of the BSC***

Prior to BSC Operation

- Plan ahead
- Schedule uninterrupted work time when not in use by others (if possible)
- Keep doors closed
- Assemble all materials needed
- If not running continuously - turn BSC on and allow to run for 10 minutes (refer to manufacturer instructions).

Prior to BSC Operation

- Check expiration date on certificate
- Must be certified when installed, whenever moved, and at least annually
- Usually conducted by safety office or outside vendor

Prior to BSC Operation

- Decontaminate work surface, rear wall, sides, inside front window
- Use an extendable mop or wand with removable pads “swiffer-like ” to reach the back wall - don’t put your head inside the BSC
- What disinfectant?
- Bleach will pit the stainless steel
- Rinse bleach off with water or alcohol

Prior to BSC Operation

- Check sash height, inward airflow (tape or Kimwipe),
- ***Make sure alarms are ON***
- Load BSC with all needed supplies before work.
- Before each use and after any power fluctuation, the pressure differential gauge should be checked and recorded
  - measures in units referred to as inches of water
  - an indication of the static pressure within one of the cabinet plenums

Pressure Differential Gauge

Look for change up or down from what was recorded on the certification sticker when last certified

- Gradual changes will generally occur over time due to filter loading
- Sudden change (> 10%) in pressure reading indicates that there is filter damage or other operational problem
- Under this scenario, the cabinet should not be used until it is checked by a service technician

Pressure Differential Gauge

- Perhaps most importantly, the reading should never be zero.
- *Sudden (vs. gradual) increase indicates the resistance is up and the filter is loaded or there may be a blockage*
- *Sudden decrease indicates the resistance is down and may be a hole or tear*
- Note: Changes in pressure/resistance can also be caused by problems with the cabinet's blower motor; either way the *cabinet should not be used until it is checked by a service technician*.

Working in a BSC

Both chemicals and flames can compromise the integrity of the filter

Do not:

- **Use NFPA 4 flammables***
  - BSC fans NOT spark proof
  - Chemical use may result in fire/ explosion
- **Use Bunsen burners (open flame)**
  - Fire hazard
  - Can damage HEPA
  - Interferes with proper airflow

NFPA 4 flammables

* NFPA stands for the National Fire Protection Association and the NFPA has developed a hazard rating system for common chemicals used in the lab

- A flammable rating of 4 means that it is a flammable gas or extremely flammable liquid that can rapidly vaporize at atmospheric pressure and normal ambient temperature or, when readily dispersed in air, can burn easily. Examples include acetylene, propane gas, and liquid hydrogen

Working in a BSC

Do not:

Go in and out of the BSC

Tape the biohazard bag to the outside

Overload cabinet

Block front or rear grilles

Work inside with 2-3 people. (If >1 person is necessary for a specific protocol, use a 6 ft BSC)

Any comments?

Ultraviolet Germicidal Lights

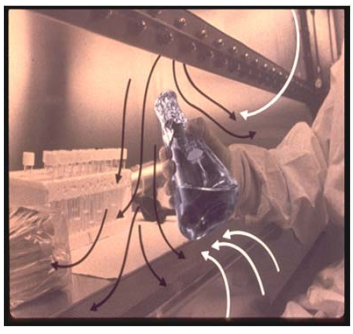
Not recommended as the sole method of decontamination because of limitations:
Intensity decreases with:
- Time - check with meter
- Dirt and dust - clean weekly
- Distance - from the lamp

UV has limited penetrating power - surface or air only
Can lead to a false sense of security

Working in a BSC

Do:

- Move arms in and out slowly and perpendicular to the BSC opening
- Work in center of work area (or at least 4 inches from the front grill)



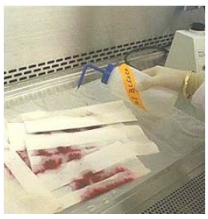
Working in a BSC

Do:

- Clean up spills promptly
- Separate clean from dirty
- Adjust chair height so that your face is above the front sash opening and the bottom of the glass screen is even with your underarms

Working in a BSC

- Leave BSC on -Why?
- Cover spill with absorbent towel
- Carefully apply effective disinfectant and allow appropriate contact time
- Flood catch basin if contaminated
- Wipe up towels and dispose of in biohazard waste container
- Repeat process with fresh absorbent towels and disinfectant
- Decontaminate objects within BSC if contaminated
- Allow BSC to run for 10 minutes before resuming work



After Work is Completed

- Disinfect materials before removal from BSC
- Close and remove and properly dispose of biohazardous waste
- Disinfect work surface, rear wall, sides, inside front window
- Leave cabinet running if possible (or close sash)

BSC Maintenance by Laboratory Staff

- Routine housekeeping-(don't forget front catch basin)
- Daily, weekly, monthly, semi-annually cleaning
- Don't store materials on top of BSC

A Few Ways to Make a BSC Fail

- Walk past it
- Open a door near it
- Overcrowd it
- Cover the front grill
- Move hands in a sweeping motion through the barrier

Videos

Grill

Walking

Arm Movement

BSC Safe Operation

- In general, *not* designed for chemical use
- *Volatile chemicals NOT retained by HEPA filter*- exposes personnel if not exhausted
- Both chemicals and flames can compromise the integrity of the filter
- *BSC fans NOT spark proof*- chemical use may result in fire/ explosion-never use NFPA 4 flammables

BSC Safe Operation

- The air curtain at the front opening can be easily compromised
- As with any piece of lab equipment, personnel must be **trained** in the proper use of it and what to do if the BSC fails (power outage, fan failure)
- If you have equipment, it must be **maintained**.