Biosafety
for
Clinical Laboratories

Biosafety:
Preventing Lab Acquired Infections

- July 15, 2000. Alabama microbiologist, 35 y/o was evaluated in the ER for malaise, fever, myalgia.
- July 16, tech became tachycardic and hypotensive, died within hours.
- Cause???...

Biosafety:
Preventing Lab Acquired Infections

Neisseria meningitidis

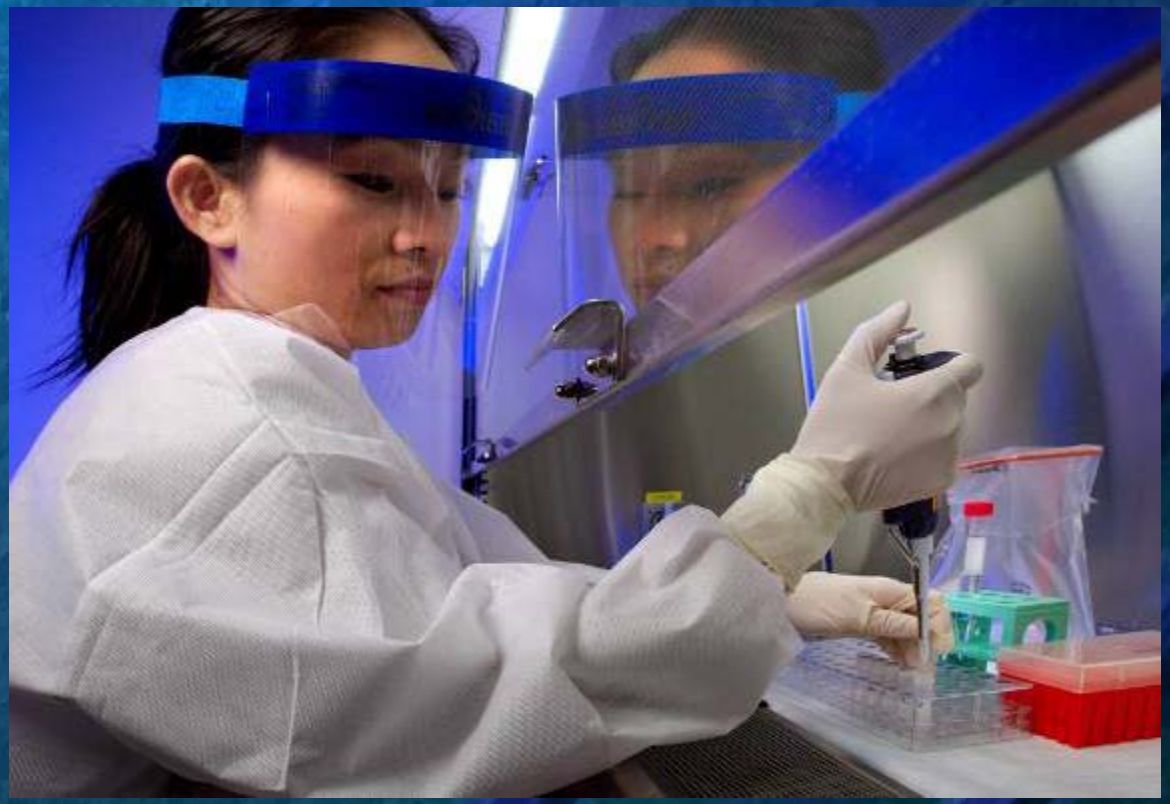
Possible exposure to *N. meningitidis*

- Prepared a Gram stain from the blood culture of a patient.
- Aspiration from blood cultures was performed on the open bench; biosafety cabinets, eye protection, or masks were not used routinely for this procedure.
- Patient subsequently shown to have meningococcal disease.

Biosafety in Clinical Laboratories

Why is it important?

Because of where you work and what you do…



Objectives

- Discuss the importance of biosafety in the clinical laboratory
- Describe recommendations for preventing Laboratory Acquired Infections.
- Describe the four biosafety levels
- List Standard Microbiological Practices in a BSL-2 and BSL-3 laboratories
  - *Describe special practices in BSL-2 and BSL-3 laboratories*
- Understand how to work safely in a biological safety cabinet (BSC)

What is Biosafety?

The combination of work practices, primary containment devices, and laboratory design to reduce the risk of laboratory infection or release of a microbe to the environment.

Principles of Biosafety

Good biosafety practices prevent occupationally acquired infections by:

Intercepting microorganisms before they can infect you (block routes of transmission) and breaking the "chain of infection"

Chain of Infection

Reservoir of pathogen

X

Portal of escape

X

Transmission

X

Route of entry/infectious dose

X

Susceptible host

X

Incubation period

X

Infection

Proper Work Practices

Protective Equipment

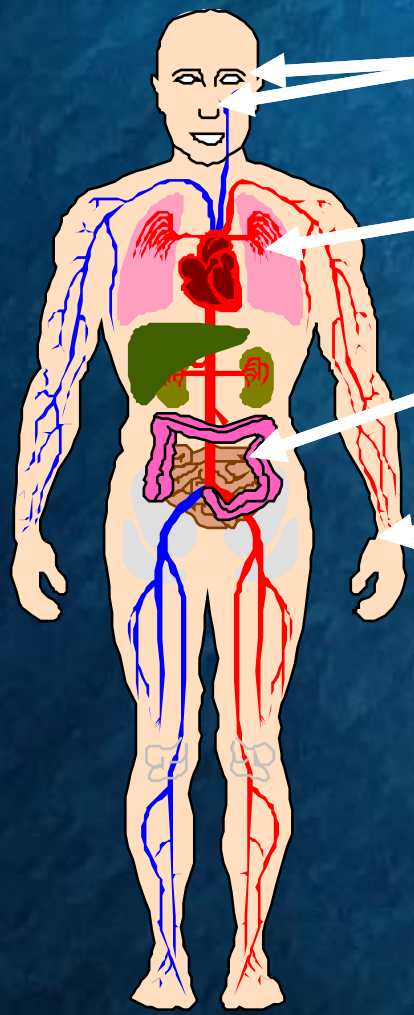
Immunization

Treatment

Surveillance

Risk Assessment

PORTALS OF ENTRY OF BIOLOGICAL AGENTS



MUCOUS MEMBRANES

RESPIRATORY TRACT (LUNGS)

GASTROINTESTINAL TRACT

SKIN

Standard (Universal) Precautions

- Trained on precautionary methods if in direct contact with body fluids
- Epidemiology and modes of Transmission and Prevention of:
  - *HIV*
  - *Hep C*
  - *HCV*
  - *Hep B*
- OSHA Bloodborne Pathogens Regulation

Biosafety Plan Key Elements

- Biosafety Manual and SOP's
- Responsibilities
- ID of Biohazards
- Entry Requirements and Signage
- Risk Assessment and Control of Biohazards
  - *Engineering Controls*
  - *Safety Equipment*
  - *Admin Controls*
- Gen Lab Safety Practices
- Waste Management
- Decontamination/Disinfection
- Emergency Procedures
  - *Spill Response*
- Training Programs
- Medical Surveillance
- Evaluation/Drills/Auditing Program and Documentation
- P&S Protocols

Laboratory Acquired Infections

Laboratory Acquired Infections

- 500,000 US laboratorians are exposed to infectious materials and cultures every day.
- Risks from biological hazards can be reduced by using:

  Appropriate microbiological procedures and techniques
  Containment devices/facilities
  Protective barriers

Challenges

- Workload and high stress
- Unfamiliar with agent (not endemic)
- Lack of time for training
- Limited staff
- Assumption that BSC and PPE are effective
- PPE usage not always enforced
- Worked on open bench before risk was known
- Not enough biosafety cabinet space

Any others?

Organisms Associated with LAIs (1979-99)

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

Of the 1,267 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% exposure to aerosols is a plausible but unconfirmed source of infection

Greatest risk to microbiologists:

Brucella* spp. and *N. meningitidis

Neisseria meningitidis Special Risk to Laboratorians

Each year clinical microbiologists are potentially exposed to 3000 *N. meningitidis* isolates

From 2 studies covering 1979 – 2004:

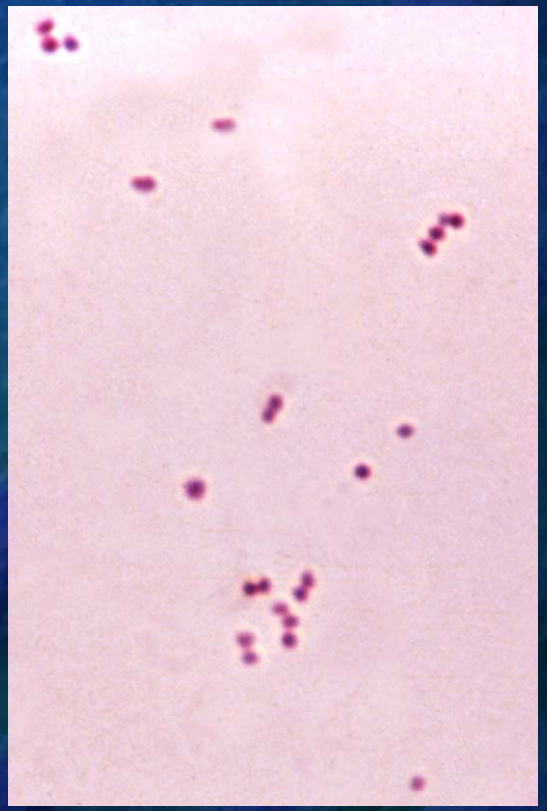
- 31 cases total
- 11 fatalities (>35% mortality)

Risky procedures:

subculturing, preparing suspensions

performing catalase on open bench

No BSC (94%)



Exposures Due to RB51 LPS Sample Based on Voluntary Lab Reporting

- 36 states, 2 cities, 1 county, DC
- Potential exposures:
  - *916 people in 254 laboratories*
    - 679 (74%) high-risk exposures
    - 237 (26%) low-risk exposures
- No cases of brucellosis reported

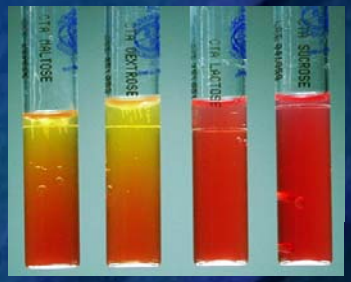
Laboratory Employees Face Greater Risks

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: Ellen Jo Baron and J. Michael Miller "Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks "Diagnostic Microbiology and Infectious Disease March 200860(3):241-6"

High Risk Activities Identified

- Sniffing plates
- Generating aerosols- anything that imparts energy to a suspension (catalase)
- Subculturing, picking colonies
- Making slides
- Inoculating biochemicals
- Improper use of BSC

Recommendations

- Establish procedures for handling phones, keyboards, microscopes, etc.
- Immunize for Hepatitis B and *N. meningitidis*
- Use engineering controls: automatic faucets, plastic shields, incinerator burners, alcohol hand gel, etc.
- Remind clinicians to notify lab if dangerous organism is suspected
- Do not use or trust automated instruments or kits with unusual, slower-growing isolates- call your PHL

Importance of First Aid

An effective and timely response to wound cleansing after an occupational exposure occurs *may be the most critical determinant in preventing infection*

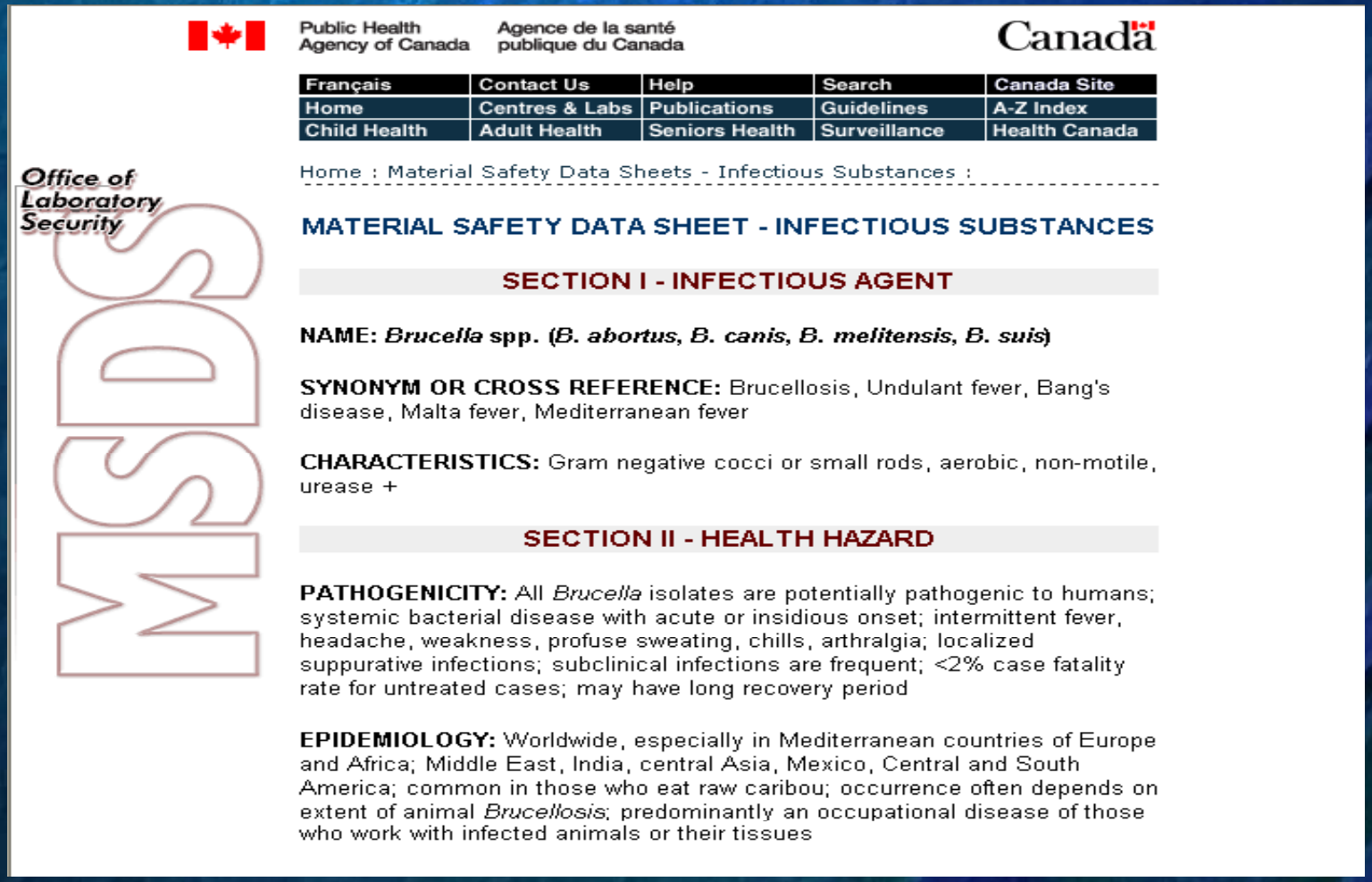
First Aid Response for Exposures

- *Have SOPs/Plan in place*
- *Available and accessible immediately and 24/7*
- *Simple, easy to follow guidance*
- *Widely known about and reviewed often (are kit contents checked regularly?)*
- *Linked to further assessment and reporting*
- *Practiced!!*

Creating an Environment of Safety

- Management sets the tone for safety culture
- Report exposures and near misses
  - *Promote benefits of reporting*
- 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

Material Safety Data Sheet (MSDS)



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Office of Laboratory Security

MSDS

Home : Material Safety Data Sheets - Infectious Substances :

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Brucella*** **spp. (*B. abortus, B. canis, B. melitensis, B. suis*)**

SYNONYM OR CROSS REFERENCE: Brucellosis, Undulant fever, Bang's disease, Malta fever, Mediterranean fever

CHARACTERISTICS: Gram negative cocci or small rods, aerobic, non-motile, urease +

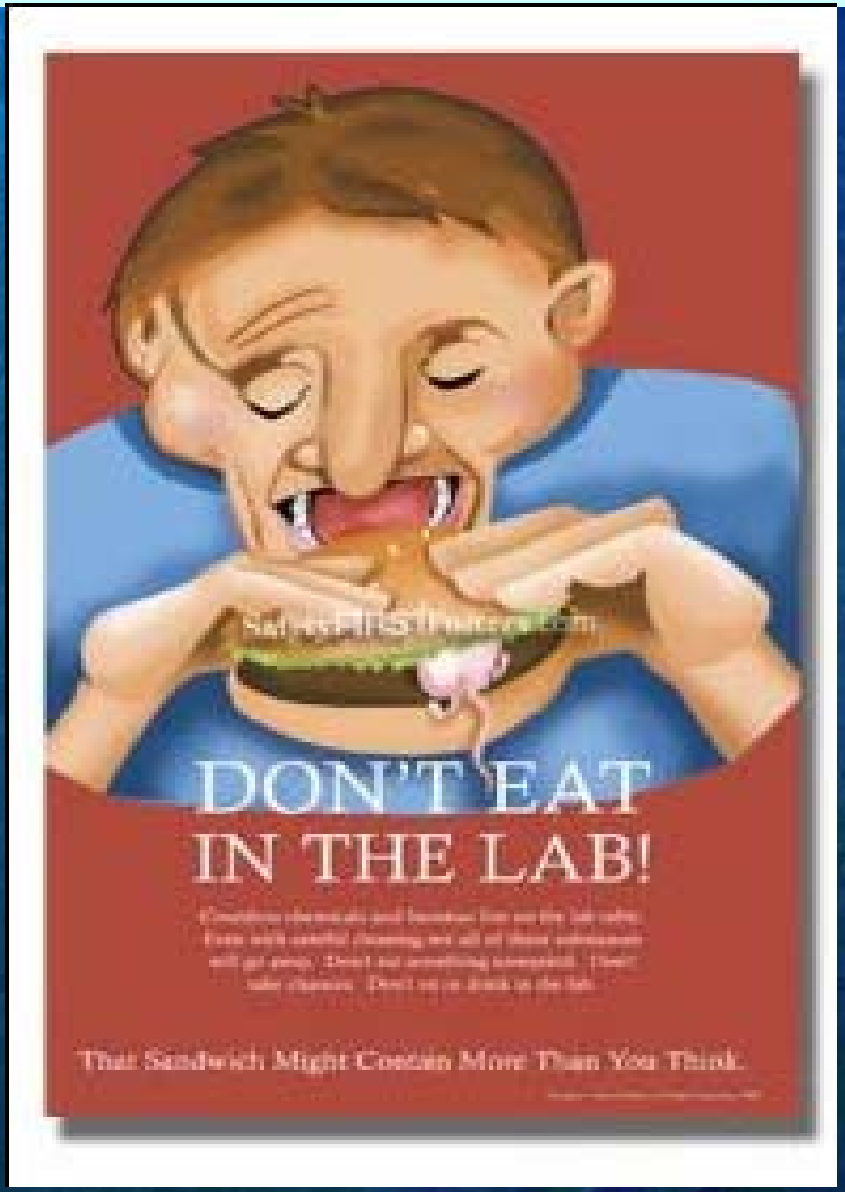
SECTION II - HEALTH HAZARD

PATHOGENICITY: All *Brucella* isolates are potentially pathogenic to humans; systemic bacterial disease with acute or insidious onset; intermittent fever, headache, weakness, profuse sweating, chills, arthralgia; localized suppurative infections; subclinical infections are frequent; <2% case fatality rate for untreated cases; may have long recovery period

EPIDEMIOLOGY: Worldwide, especially in Mediterranean countries of Europe and Africa; Middle East, India, central Asia, Mexico, Central and South America; common in those who eat raw caribou; occurrence often depends on extent of animal *Brucellosis*; predominantly an occupational disease of those who work with infected animals or their tissues

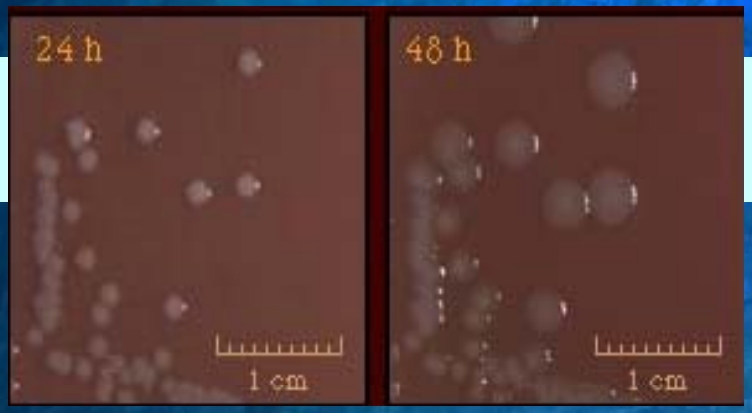
http://www.phac-aspc.gc.ca/msds-ftss/index.html#menu?

SafetyFirstPosters.com

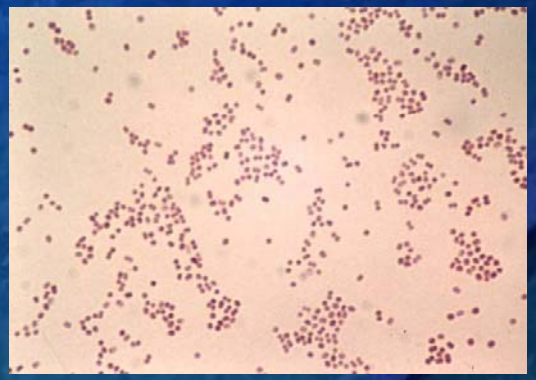


Trigger Points

- 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 a culture in a biological safety cabinet.



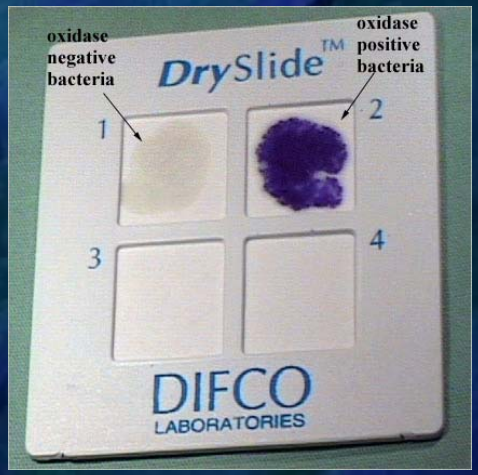
Gram – , or nonspecific staining, cocci or Gram-negative diplococci from blood or CSF



Colony morphology on choc agar 24 / 48 hr or
Slow growth on blood agar, no growth on MacConkey

Where is your trigger point?

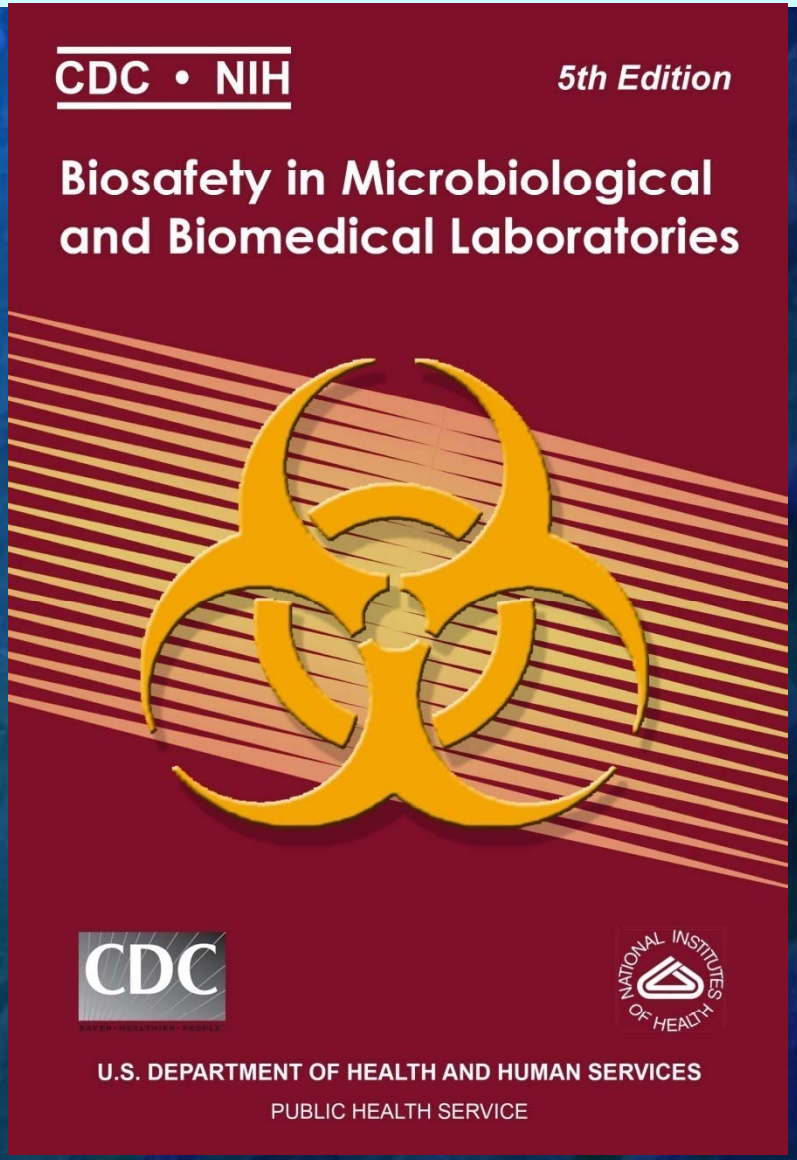
Positive oxidase test



Biosafety Levels

Biosafety Levels

Biosafety levels are described in "Biosafety in Microbiological and Biomedical Laboratories, 5th Edition"



Biosafety Levels

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

Principles of Biosafety

BIOSAFETY LEVEL	POTENTIAL HAZARD
BSL-4 Large research Labs – CDC, NIH	HIGHEST
BSL-3 University research laboratories	
BSL-2 Clinical laboratories	
BSL-1 High school laboratories	LOWEST

Biosafety Levels (BSL) 1 - 4

2 Types of Containment are:

- ***Primary - Protects worker and the immediate lab environment***
  - **Good Microbiological Techniques**
  - **Safety Equipment**
- ***Secondary – Protects worker and external environment***
  - **Facility Design**
  - **Operational Practices**

Standard Microbiological Practices for all biosafety levels

Standard Microbiological Practices for Biosafety Level 1- 4

1. Limited access
2. No mouth or eye contact
3. Wash hands
4. Sharps handling
5. Limit or contain aerosols
6. Training in procedures and biosafety

Standard Microbiological Practices

Use Mechanical pipetting devices

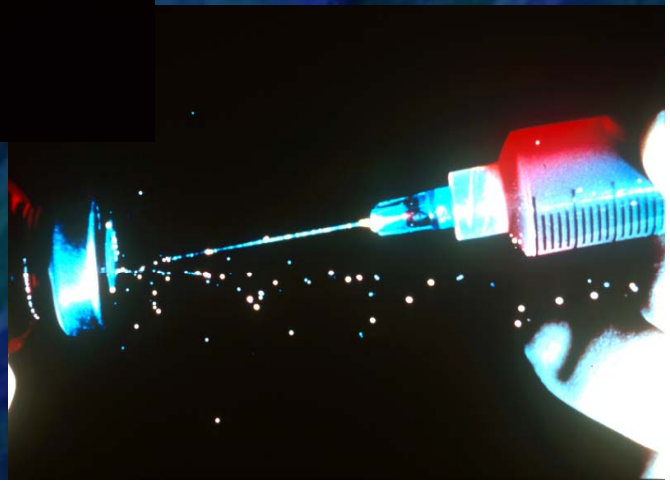
Safe Handling, Minimizing & Disposal of Sharps

- Always use a proper leak proof container to dispose of sharp materials
- Never fill sharps container to the top
- Use plastic vs. glass
- Use 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



Aerosols

Procedures that impart energy to a microbial suspension producing aerosols

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

Procedures That Emit Aerosols

- Catalase
- Inoculating biochemicals or blood culture bottles
- Pipetting
- Mixing
- Centrifugation
- Grinding
- Vortexing
- Pouring
- Loading syringes
- Lasers, cell sorters
- Splashes
- Opening lyophilized cultures
- Flaming loops
- Entering or opening vessels at non-ambient pressures, fermenters, freezer vials

Minimize Aerosols

Don't:

- *Use Bunsen burners*
- *Drop liquids onto hard surfaces*
- *Blow out last drop in pipette*
- *Mix by suction + expulsion*
- *Open centrifuge before it stops*

Minimize Aerosols

Do:

- ***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***

Minimize Aerosols

- *Use incinerators*
- *Pour liquids carefully*
- *Work over absorbent*
- *Use centrifuge safety cups*
- *Use sealed rotors*

Protect your eyes and mucous membranes against splashes and aerosols!

- *Safety glasses?*

- *Plastic shields?*

Standard Practice:

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

Sodium hypochlorite

70% Alcohol

Selected EPA-registered Disinfectants

- Listings of EPA's registered antimicrobial products effective against certain blood borne/body fluid pathogens, *Mycobacteria tuberculosis* (tubercle bacteria), human HIV-1 virus, Hepatitis B, Hepatitis C viruses, as well as products classified as sterilizers:

  http://www.epa.gov/oppad001/chemregindex.htm

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
- Time of contact
- Amount of organic material
- Environmental conditions
  - *pH, temp, humidity*

Generalized Order of Resistance to Disinfection

- Prions (MOST DIFFICULT)
- Protozoan cysts
- Bacterial spores
- Non-enveloped viruses
- Mycobacterium
- Fungal spores, fungi
- Vegetative bacteria
- Enveloped viruses (READILY KILLED)

Biological Waste Disposal

Biological waste containers should always be labeled with a biohazard symbol

Training

- *Biohazards*
- *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*

Biosafety Level 2

Biosafety Level 2

Supervision

- ***Supervisor is a competent scientist with increased responsibilities***
  - **Limits access if immunocompromised**
  - **Restricts access to immunized if necessary**

Lab Personnel

- ***Aware of potential hazards***
- ***Proficient in practices/techniques***

Personal Protective Equipment (PPE)

- *Why*
  - *Protect the worker*
  - *Protect product*

- *What*
  - *Lab coat-long sleeved and buttoned*
  - *Eye and face protection*
  - *Gloves*

When do you wear gloves in the general micro lab?

- "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)

- "Gloves must be worn to protect hands from exposure to hazardous materials" (BMBL 5th edition).

  - *Based on a lab-specific risk assessment, the Laboratory Director or supervisor determines laboratory hazards and when to wear gloves.*

Personal Protective Equipment: Gloves

- 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

Does not eliminate the hazard!

Biosafety Level 2: *Special Practices*

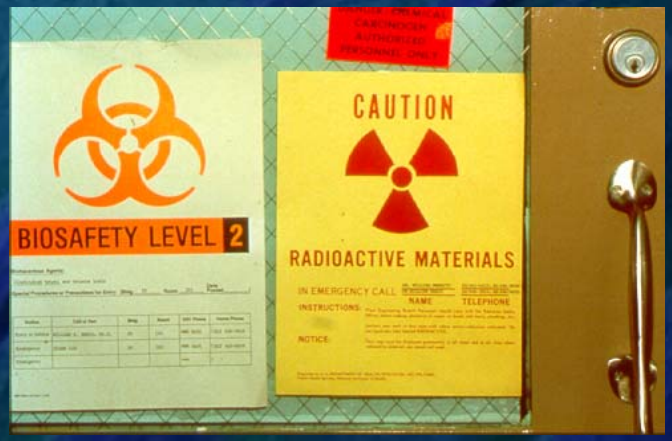
Policies and procedures for entry

Restricted access (doors closed) when work in progress

Biohazard signs on entry door

- ***Entry requirements-PPE, vaccinations***
- ***BSL***
- ***Emergency contact info***

Site-specific safety manual



Biosafety Level 2: *Special Practices*

Use biosafety cabinets (Class II) for work with infectious agents involving:

- ***Aerosols***
- ***Large volumes***
- ***High concentrations of organisms***
- ***Trigger Point Indicators***

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

Biosafety Level-3

Biosafety Level 3

Differs from BSL-2 in that:

- Personnel have specific training to handle particular pathogens
- Supervised by scientists experienced with these agents
- All manipulations of infectious material carried out in BSCs
- Laboratory has special engineering and design features
- Supervisors evaluate effectiveness of training
- Additional PPE

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

- When working with agents that are normally handled under BSL-3 conditions, and a BSL-3 laboratory is not available
- When determined by the laboratory director based on their risk assessment
- When specific high-risk pathogenic organisms are suspected (such as *Brucella* spp., *Coccidioides*, *Blastomyces dermatitidis*, *Franciscella tularensis*, *Histoplasma capsulatum*, Mtb, etc)

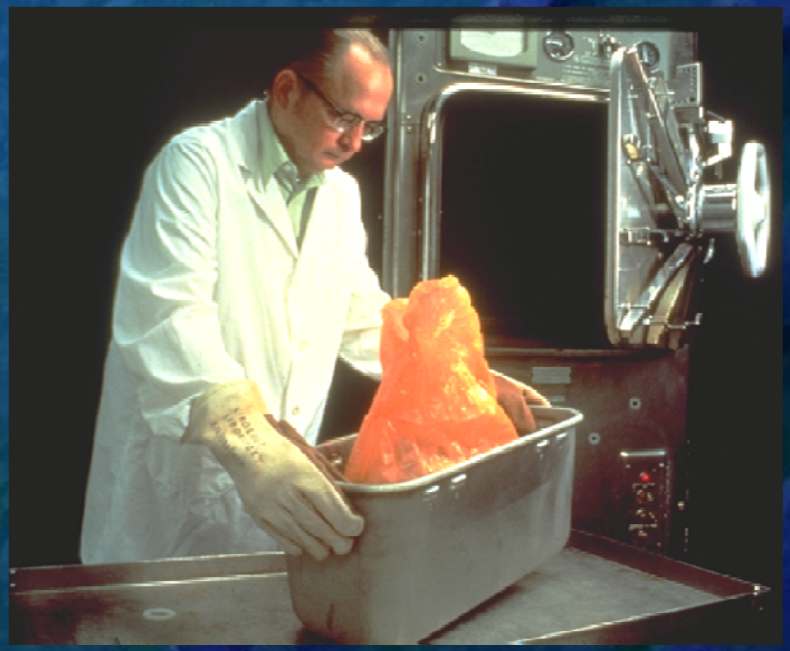
What are BSL-3 practices?

- 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 regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving……

Preferably within the Laboratory



Biosafety Level 3 Respirators

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

Biosafety Level 3 Respirators

- N95 Mask
- PAPR

Biosafety Cabinets

What is a Biosafety Cabinet (BSC)

A ventilated cabinet for personnel, product, and environmental protection. The biosafety cabinet has an open front with inward airflow for personnel protection, downward HEPA filtered laminar airflow for product protection, and HEPA filtered exhausted air for environmental protection (NSF 49, 2002)

HEPA Filters

- **High Efficiency Particulate Air Filters**
  - ***Rigid, throw-away, dry type fiber filter***
  - ***Particles at .3 microns are captured with an efficiency of 99.97%.***
  - ***Particles larger than .3 microns and particles smaller than .3 microns are captured with greater efficiency than 99.97%.***
- **Filter Loading**
  - ***Resistance Increases***
  - ***Efficiency Increases***

HEPA Filters

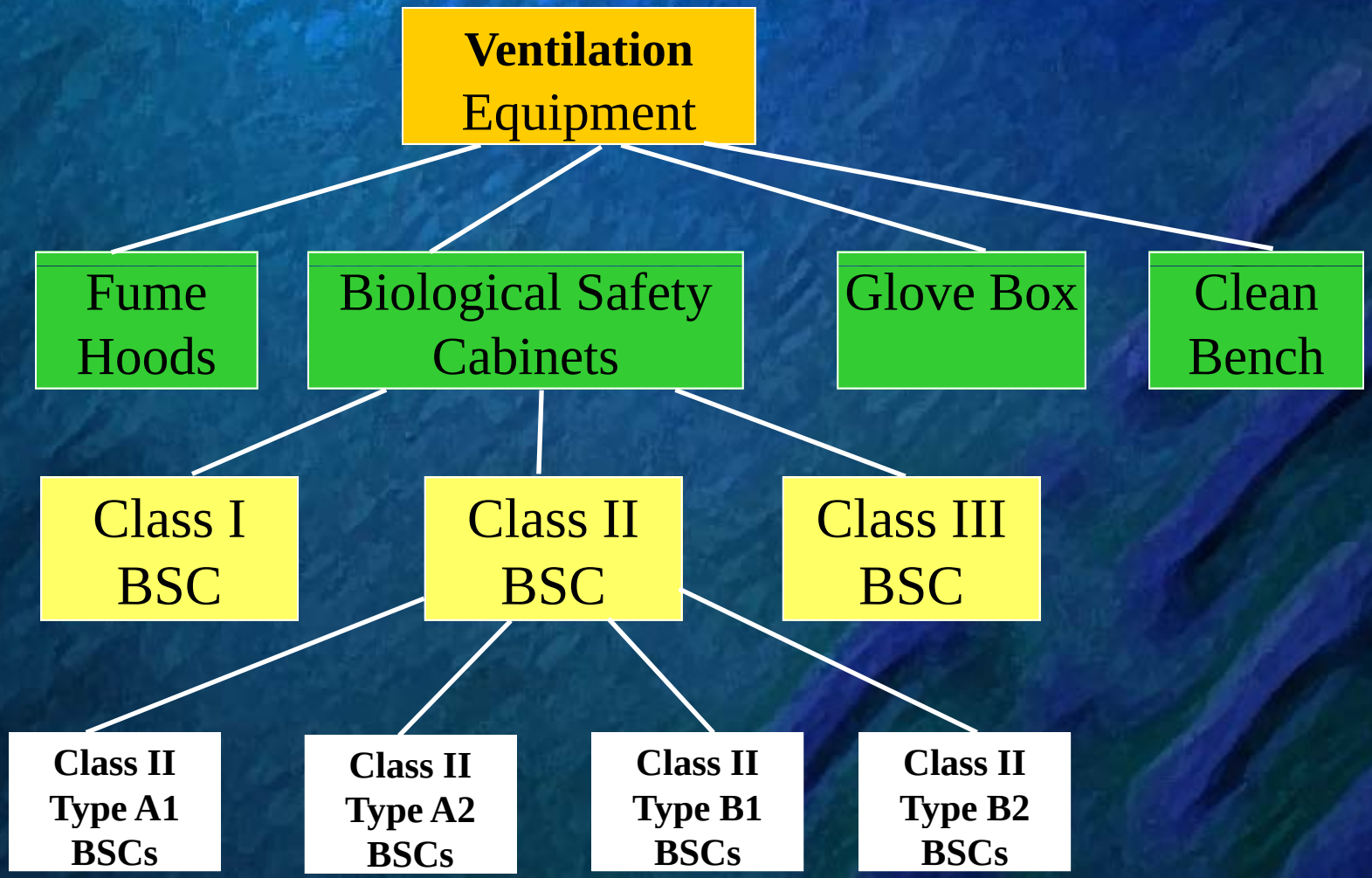
DO NOT

filter out

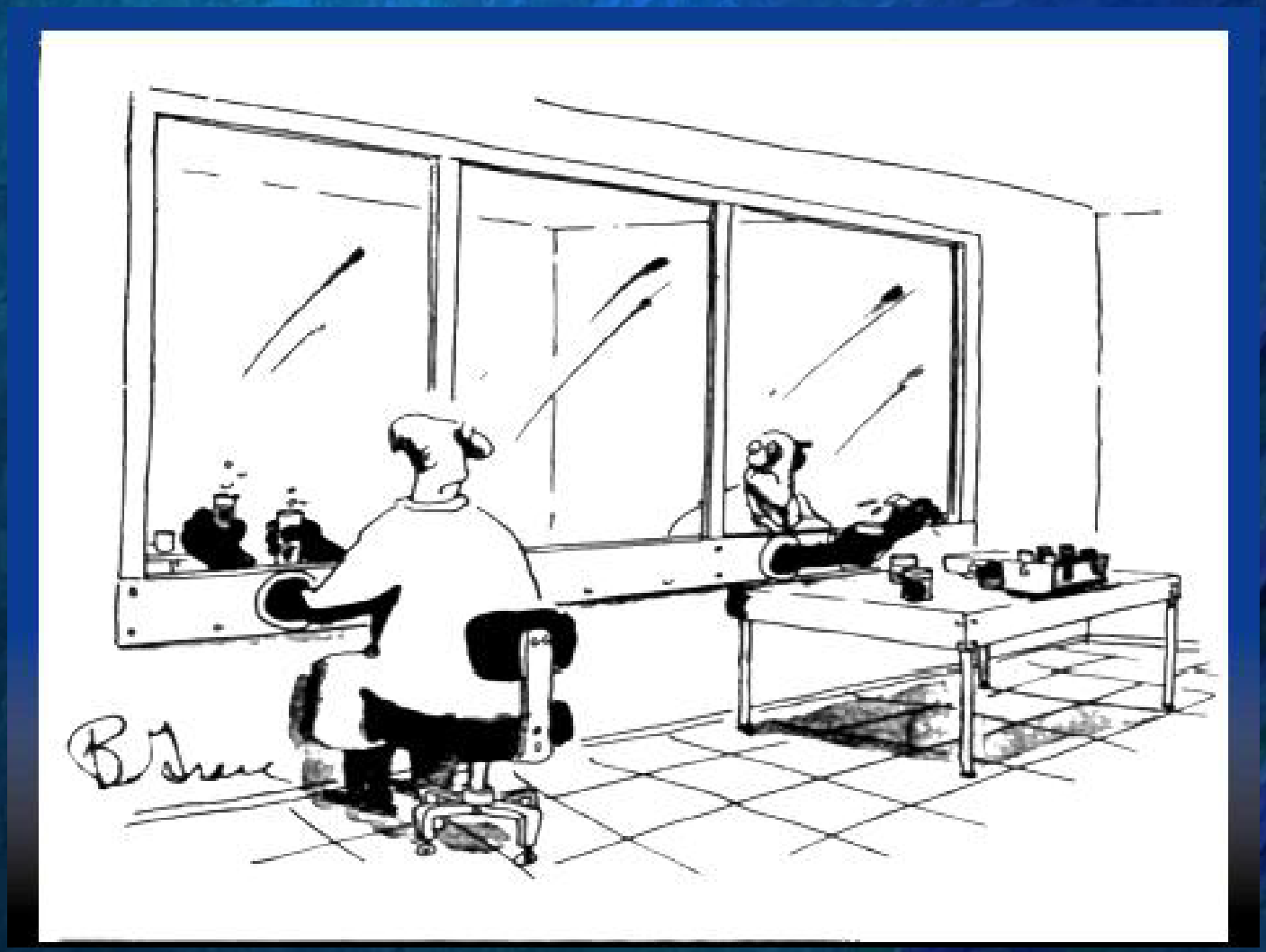
gases and vapors

They *only* filter out particulates

Primary Containment



Glove Box



Fume Hoods ≠ Biosafety Cabinets

- **Fume hoods are used for volatile chemicals- not biohazardous materials**

Chemical Fume Hood

Class II BSC Front Grille

Primary 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

Class II BSC

- **Most clinical labs use A1 or A2**
  - ***Protects personnel and immediate lab environment***
  - **Reduces** ***exposure, does not eliminate (risk is never zero)***

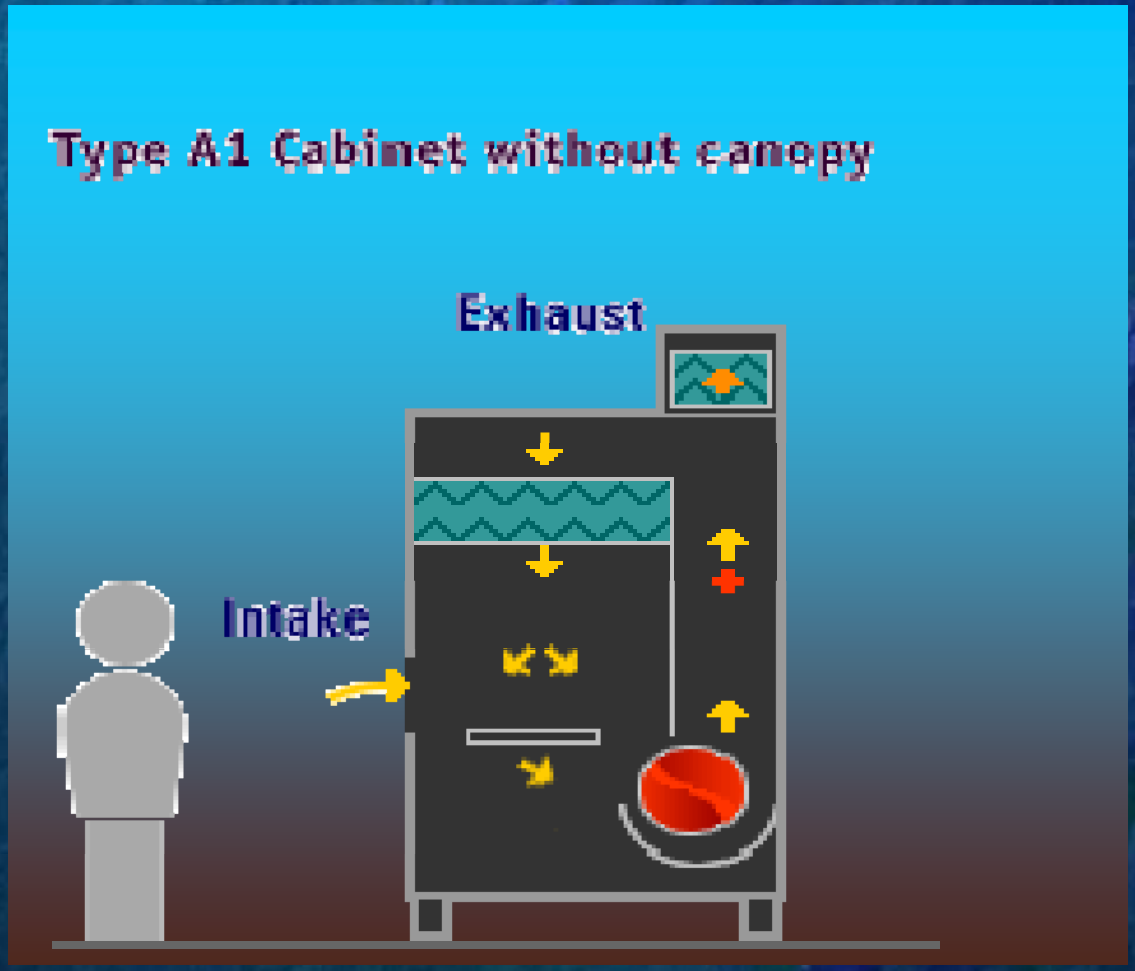
Class II Type A1 BSC

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

Because they recirculate 70% of the HEPA filtered air back into the lab

(NSF/ANSI Std. 49-02)

Class II Type A1 Airflow

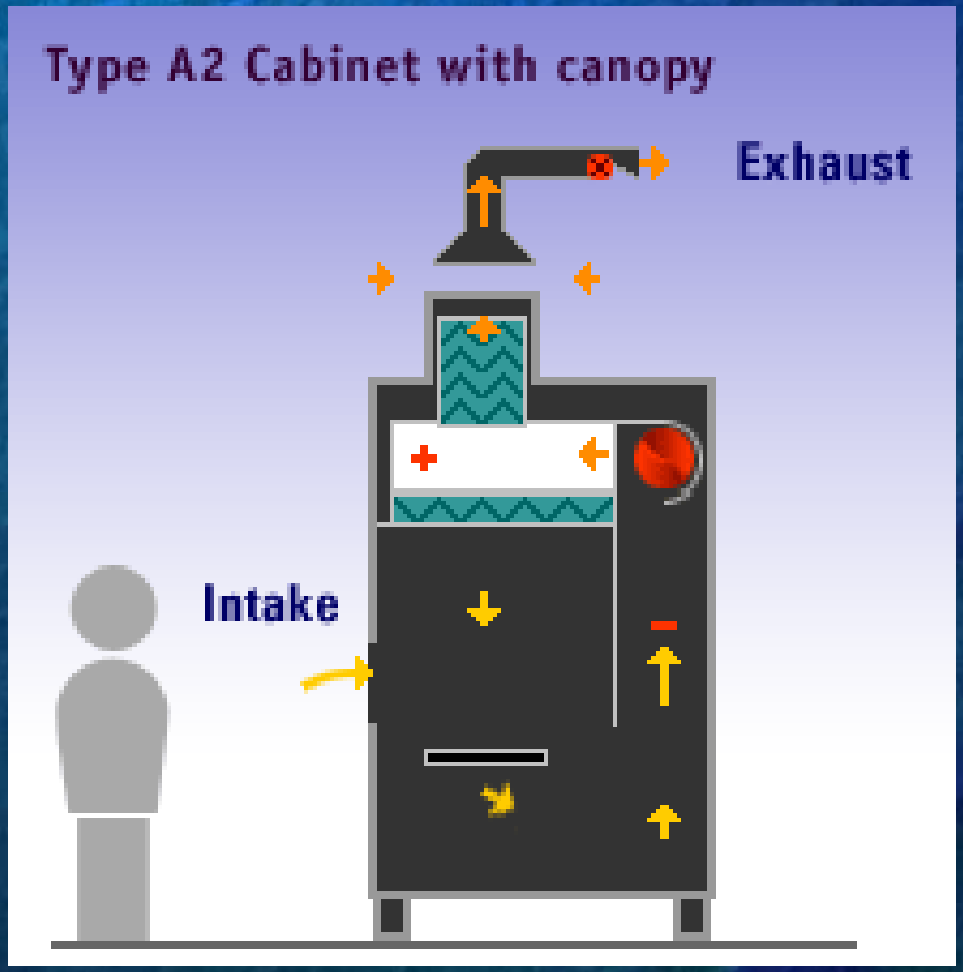


Class II Type A2 BSC

"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."*

(NSF/ANSI Std. 49-02)

Class II A2 Airflow



BSC Preferred Operating Location

- Isolated from other work areas
- Removed from high traffic areas
- Away from airflow ducts
- Away from laboratory entry doors
- 12-14” away from ceiling and walls

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

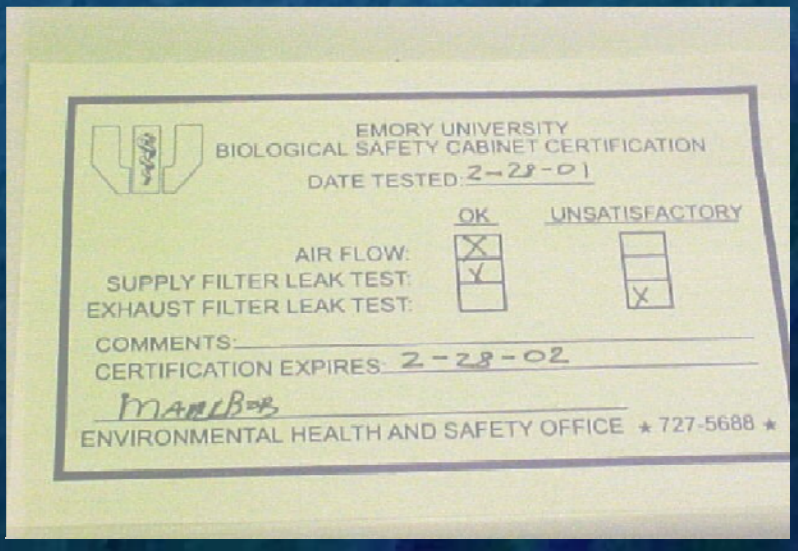
- At a walking rate of 1.14 mi/hr, you can pull air out of the BSC

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
- Turn BSC on and allow to run for 10 minutes (if not running continuously).

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 a "swiffer" 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), pressure gauge, and make sure alarms are ON
- Load BSC with all needed supplies before work.
- Before each use and after any power fluctuation this indicator should be observed

Pressure Differential Gauge

- Measures static pressure drop across HEPA filter
- Look for large change up or down from what was recorded on the certification sticker when last certified
- Increase in gauge when filter loads or blockage-resistance up
- Decrease in gauge when hole or tear in filter-resistance down

Working in a BSC

Both chemicals and flames can compromise the integrity of the filter

Working in a BSC

Do not

- **Use NFPA 4 flammables**
  - *Flash point below 23°C (73°F)*
  - *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*

Working in a BSC

Do not

- Go in and out
- Tape the biohazard bag to the outside
- Overload cabinet
- Block front or rear grilles
- Work inside with 2-3 people

Any comments?

Ultraviolet Germicidal (?) Lights

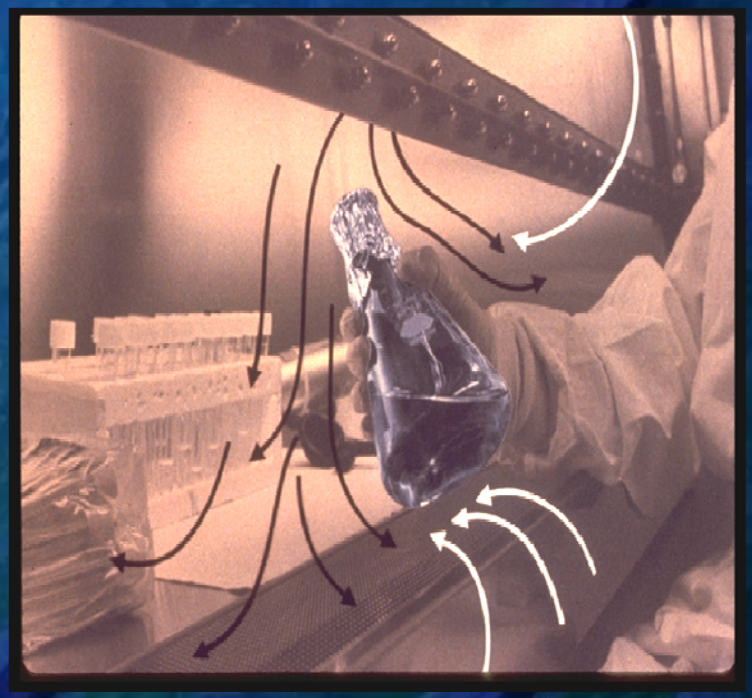
***Not* recommended for decontamination**

- **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**

Working in a BSC

Do

- Move arms in and out slowly, perpendicular to the face opening of the BSC



Working in a BSC

Do

- Clean up spills promptly
- Work in center of work area (or at least 4 inches from the front grille)
- 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

After Work is Completed

- Disinfect materials before removal from BSC
- Seal and remove biohazardous waste
- Disinfect work surface, rear wall, sides, inside front window
- Leave cabinet running if possible

BSC Maintenance

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

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.

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