

Science at the heart of medicine

Laboratory Safety Training

Albert Einstein College of Medicine
Department of Environmental Health & Safety
<http://www.einsteinmed.edu/ehs>

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Training Outline

- Laboratory Safety Concepts
- Chemical Hygiene Plan
- General Concepts of Chemical Toxicology
- Personal Protective Equipment (PPE)
- Chemical Storage/Disposal
- Waste Disposal
- Emergency Procedures

Laboratory Safety

- Occupational Safety and Health Administration (OSHA) promulgated the:
 - Hazard Communication Standard (HCS) [29 CFR1910.1200]
 - Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR 1910.1450)
- These OSHA regulations, part of the Hazard Communication Standard (HCS), were issued to:
 - “Protect people from injuries and illnesses associated with using hazardous chemicals in the workplace”.
 - Requires employers to write and have available to all employees a Chemical Hygiene Plan.

Laboratory Safety – Chemical Hygiene Plan

- Describes Einstein's laboratory safety program and is a tool to coordinate laboratory safety procedures; personal protective equipment (PPE); engineering controls; use, storage, and handling of hazardous chemicals; waste management and disposal procedures; and what to do in case of spills or an emergency.
- Goal is to:
 1. Protect employees from health hazards associated with hazardous chemicals in the laboratory.
 2. Reduce or keep potential employee exposures to hazardous chemicals at concentrations below the action levels or Permissible Exposure Limits (PEL).

NOTICE

**HAZARD
COMMUNICATION
POLICY
LOCATED HERE**



<https://intranet.einsteinmed.edu/download/?token=F3U4mtNk56VTr4YwmOleltOEedZjiAS3%2fX4zi1xF6i4>



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Laboratory Safety

- Laboratory employees can be exposed to hazardous chemicals while conducting their job/research duties.
- Exposure to toxic substances in the workplace could have chronic or acute health effects on individuals.
- Safeguards to protect workers while using a specific chemical included the use of engineering controls, wearing personal protection equipment (PPE), and to follow good personal hygiene (wash hands) and housekeeping practices in the work area.

Laboratory Safety (Continued)

- All chemicals must be properly stored in laboratory shelves or appropriate chemical storage cabinets.
- Laboratory employees must:
 - Properly use emergency equipment (fire extinguishers, eye wash stations, emergency showers),
 - Be familiar with the appropriate emergency procedures,
 - Know what to do in case of a fire,
 - Know evacuation routes,
 - Know spill cleanup and proper waste disposal procedures.

Laboratory Safety – Plan the work

Before conducting any experiment

- Assess the hazards related to the work including:
 - Worst possible things that could go wrong,
 - How to deal with them, and
 - What are the prudent practices, protective measures, and equipment necessary to minimize the risk of exposure to the hazards.
- Order the correct amount of chemicals; order what you need to prevent storage and disposal problems.
- Know the hazard characteristics of the material you work with.
 - ✓ Corrosive, flammable, reactive, or toxic.

Laboratory Safety – Know the Hazards

- Have available and read the Safety Data Sheets (SDS) for hazards information on all chemicals you plan to use.
- Wear and have available the appropriate PPE when using chemicals:
 - Laboratory coat, aprons, gloves, eye/face protection, foot protection.
- Post a sign on the door to notify others of the hazards present in your laboratory:
 - Biohazardous materials
 - Radioactive materials
- Inspect electrical equipment and cords for frayed wiring or damage before use.
- Discard or repair damaged equipment before use.

Laboratory Safety – Know the Hazards

- Never use mouth suction to fill a pipette or siphon. Use a pipette bulb or other suitable device.
- Handle needles, syringes and other sharps carefully. Do not recap needles.
- Use self-sheathing needles or needless systems whenever possible.
- Dispose of all sharps in an appropriate sharps container.

Laboratory Safety – Know the Hazards

- Do not dispose of chemicals down the drain. Most chemicals must be disposed of as hazardous waste.
- Compressed gas cylinders must be secured to prevent them from being knocked over.
- Cylinders must be capped when the regulator is removed or not in use.
- Inspect the laboratory weekly for hazardous conditions.

Einstein - SDS Stations Locations

Forchheimer:

Ground & 4th Floor

Kennedy:

3rd Floor

Price:

Basement

1st, 4th & 5th Floors

Van Etten

Basement



Employee Responsibilities

- Attend training classes.
- Learn the chemical hazard by reading the SDS.
- Know the signs and symptoms of exposure.
- Know the proper personal protective equipment (PPE) to use.
- Know emergency procedures.

Hazards at Einstein



- Chemical

- Approximately 600 different chemicals on campus
- Flammable
 - ✓ Examples: Xylene, Ethanol
- Corrosive
 - ✓ Examples: Nitric Acid, Sodium Hydroxide
- Reactive
 - ✓ Examples: Sodium Metal, Sodium Azide, **Picric Acid (dry)**

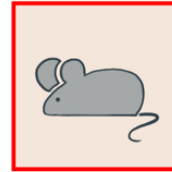


- Biological

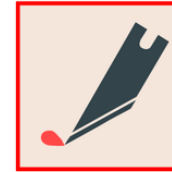
- Examples: Allergens, Infectious Diseases, Toxins

Hazards at Einstein

- Physical
 - Temperature Extremes
 - Electrical
 - Fire
 - Explosive
 - Cryogenic Liquids
 - Compressed Gasses



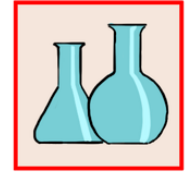
ANIMAL
HAZARD



SHARP INSTRUMENT
HAZARD



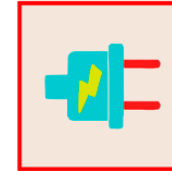
HEAT HAZARD



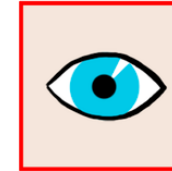
GLASSWARE
HAZARD



CHEMICAL
HAZARD



ELECTRICAL
HAZARD



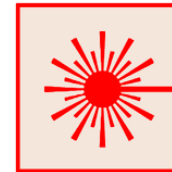
EYE & FACE
HAZARD



FIRE
HAZARD



BIOHAZARD



LASER RADIATION
HAZARD



RADIOACTIVE
HAZARD



EXPLOSIVE
HAZARD

Hazards at Einstein

- Picric Acid
 - In a dry powdered state, it can become explosive when jolted (shock sensitive)



Hazards at Einstein

- Cryogenic Liquids
 - Liquid Nitrogen



- Compressed Gas Cylinders
 - Oxygen
 - Carbon Dioxide

Hazards at Einstein

■ Radiation

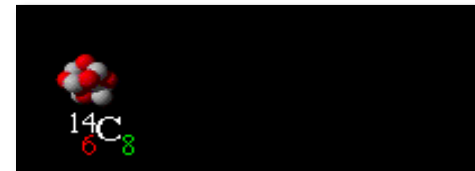
➤ Non-ionizing

- Near Ultraviolet
 - ❖ Damage to skin and eyes.
- Lasers
- Microwave



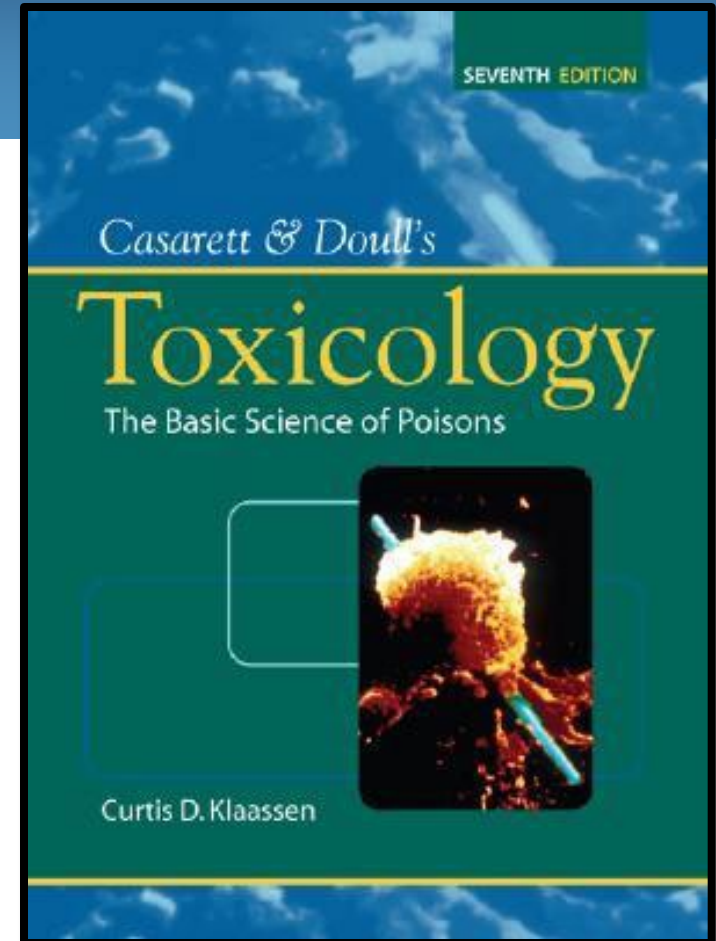
➤ Ionizing

- Alpha, Beta, Gamma and X-rays
 - ❖ Generates free radicals and ion pairs in living tissue, producing damaging intermediates.
 - ❖ Direct hits to DNA



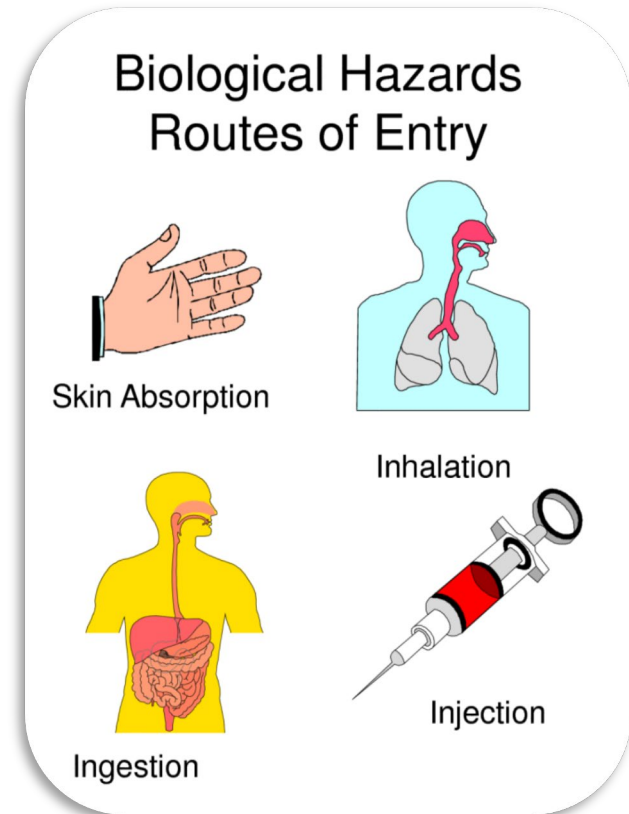
Chemical Toxicology

- Toxicology is the study of the adverse effects of chemicals on living organisms.
- Know the signs and symptoms associated with exposure to the chemicals in your workplace.
- Be observant for these signs and symptoms.
- Know what to do if you are exposed.



Chemical Toxicology

- Routes of Entry
 - Inhalation (Breathing)
 - Absorption (Direct Contact)
 - Ingestion (Eating)
 - Injection



Chemical Toxicology

- **Inhalation**

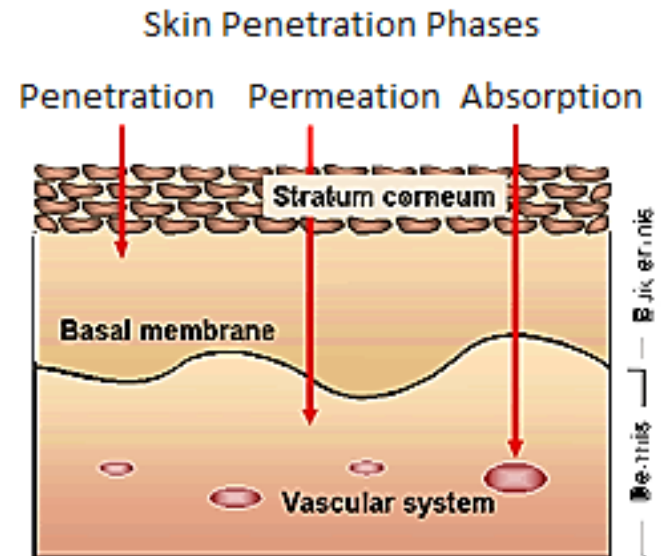
- The most common route of exposure.
- Can be in the form of a gas, vapor or dust.
- Can be deposited in the airways or absorbed through the lungs and into the blood stream.
 - ✓ Blood can then circulate the toxin to the rest of the body.



Chemical Toxicology

- Absorption

- Skin acts as a barrier between the environment and the organs of the human body.
- Skin can be attacked directly.
 - ✓ Examples: Acids and Bases
- Skin can be penetrated.
 - ✓ Examples: Phenol, Nitrobenzene



Chemical Toxicology

- Ingestion

- Rarely takes place by deliberate swallowing of toxic substances.
- Food and drinks can become contaminated by dust, mist and fumes.
- No eating or drinking in laboratories or storing food in laboratory refrigerators or cold rooms.



Chemical Toxicology

- **Injection**

- Occurs through accidental needle sticks, puncture wounds or through broken skin/open wounds.
- May produce rapid response when injected because the chemical/toxin is introduced directly into the blood stream.



Health Hazards Effects

Acute Health Effects (Short-term):

* Adverse health effect that develops immediately or within minutes, hours or even days after an exposure. Includes dizziness, skin irritation, and throat irritation.

Examples:

- Burns
- Headache
- Vomiting
- Nausea

Chronic Health Effects (Long-term):

* Adverse health effect resulting from long-term exposure to a substance. Symptoms do not stop when the exposure stops.

Examples:

- Asthma
- Cancer
- Asbestosis, Mesothelioma



Personal Protective Equipment

- Laboratory Coat and Aprons
- Gloves
- Safety Glasses/Face Shields
- Hearing Protection
- Respirators



Personal Protective Equipment

Lab Coats and Aprons



Personal Protective Equipment

■ Glove Selection

- Gloves are made of many different types of materials, yet no one material type affords protection against all chemicals.
 - **Latex gloves provide little to no chemical protection.**



Butyl rubber



Neoprene



Nitrile



Latex



Personal Protective Equipment Ansell Glove Guide

Standards for Color Coding (“Degradation Rating”)

- Green** - means that the glove is very well suited for application with that chemical:
 - An “Excellent” or “Good” degradation rating,
 - A permeation rate of 30 minutes or longer, and
 - A permeation rate is “excellent”, “Very Good”, or “Good”, OR
 - Permeation rate is no specified,
 - Permeation breakthrough time is 240 minutes or longer,
 - The degradation rating is “Excellent”, Very Good”, or “Good”.
- Red** – The Degradation Rating is “Poor” or “Not Recommended”, regardless the permeation rating. Avoid using the glove with the associated chemical.
- Yellow** – The glove is suitable for that application under careful control of its use. This code is issued for any glove-chemical combination not meeting either set of conditions required for “Green”, and not having a “Red” degradation rating of either “Poor” or “Not Recommended”.
- Chemicals highlighted in “Blue” are experimental carcinogens
- And chemicals highlighted in “Gray” are listed as suspected carcinogens, experimental carcinogens at extremely high dosages, and other materials which pose a lesser risk of cancer.

Permeation/Degradation

The first square in each column for each glove type is color coded to provide an overall rating for both Degradation and Permeation. The letter in each colored square is for Degradation alone.

GREEN: The glove is very well suited for application with that chemical.

YELLOW: The glove is suitable for that application under careful control of its use.

RED: Avoid use of the glove with this chemical.

SPECIAL NOTE: The chemicals in this guide highlighted in BLUE are experimental carcinogens, according to the ninth edition of Sax' Dangerous Properties of Industrial Materials. Chemicals highlighted in GRAY are listed as suspected carcinogens, experimental carcinogens at extremely high dosages, and other materials which pose a lesser risk of cancer.

CHEMICAL	LAMINATE FILM			NITRILE			UNSUPPORTED NEOPRENE		
	BARRIER*	SOL-VEX®	29-SERIES	BARRIER*	SOL-VEX®	29-SERIES	BARRIER*	SOL-VEX®	29-SERIES
	Degradation Rating	Permeation Breakthrough Time	Permeation Rate	Degradation Rating	Permeation Breakthrough Time	Permeation Rate	Degradation Rating	Permeation Breakthrough Time	Permeation Rate
1. Acetaldehyde	E	300	E	F	---	---	E	10	---
2. Acetic Acid, Glacial, 99.7%	E	150	---	G	150	---	E	300	---
3. Acetone	E	>480	E	NR	---	---	E	10	---
4. Acetonitrile	E	>480	E	F	30	F	E	20	---
Acrylic Acid	---	---	---	G	120	---	E	---	---
Acrylonitrile	E	>480	E	---	---	---	---	---	---
Alcohol	E	>480	E	F	140	F	---	---	---
Ammonia, 40%	E	19	E	E	>480	E	---	---	---
Ammonia (75-90%)	E	>480	E	E	>300	---	---	---	---
Ammonia (25-30%)	E	30	---	F	---	---	---	---	---
Ammonia (10-15%)	E	470	---	---	---	---	---	---	---

Personal Protective Equipment

- Safety Goggles/Face Shields



Personal Protective Equipment

Respirators

- N95 particulate respirator
- Filters 95% of particles 0.3 microns or greater
 - Not to be used for gases, vapors, or oxygen deficient areas



Personal Protective Equipment

- Hearing protection
 - Earplugs
 - Single use
 - Earmuffs



Engineering Controls

- Chemical Fume Hood - Primary engineering control for containing and removing chemical gases, vapors, mist and fumes.
 - Fume Hoods must be able to remove hazardous vapors and odors from the breathing zone
 - Inspected annually
 - Face velocity of 80 - 120 feet per minute.
 - Sash height 12-18 inches
 - Close the sash when finished



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Engineering Controls



- Do not overload the hood with extraneous equipment or chemicals.
- Fume hood is not to be used as storage
- Do not evaporate chemicals
- A cluttered hood can compromise the airflow patterns and negate the hoods safety features

Chemical Storage/Disposal

- Order only what is needed
 - Each lab is required to store their own materials
- Make sure chemicals are labeled properly
 - As well as all secondary containers
- Avoid floor and top shelf storage
 - Nothing above eye level
- Store flammables in appropriate cabinets
 - 15 gallons (56 liter) limit per lab
 - 25 gallons (94 liter) limit per lab with sprinkler system (Golding, MRRC & Price Building)

Chemical Storage/Disposal

- Separate acids and bases
 - Organics vs. Inorganics



- Examples:
 - Hydrochloric Acid (HCl) and Sodium Hydroxide (NaOH)
 - Sulfuric Acid (H₂SO₄) and Formic Acid (HCOOH)
- Perchloric acid must be stored in glass containers separated from organic materials.
- Segregate oxidizers from organic liquids.
 - Examples: Hydrogen peroxide, Permanganates, Nitrate compounds

Chemical Storage/Disposal

- Nitric acid must be isolated from other acids and bases.



Chemical Storage/Disposal

- Dispose of used / expired chemicals via EH&S.
 - Examples: Ethyl Ether, 2-Propanol
- May form explosive peroxides after one year. Exposure to light and/or air significantly increases the rate of peroxide formation.
 - Complete the required peroxide tests and forms



Chemical Storage/Disposal

- Acid Storage

- Store in plastic secondary containers, not directly on metal shelves



- Flammable Storage

- Do not store cardboard or Styrofoam



Chemical Storage/Disposal

- Chemical disposal is free
- Use the proper waste container
- Rinse empty bottles and deface label
- Label all chemical waste

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HAZARDOUS WASTE

START DATE: _____ END DATE: _____

SUPERVISOR: _____ EXT: _____

CHEMICAL COMPONENTS

AMOUNT

_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

FLAMMABLE TOXIC REACTIVE CORROSIVE

Please handle with care.

If you have any questions call:
(718) 430-4150



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Hazardous Materials Spill Cleanup



MINOR CHEMICAL SPILLS CLEANUP PROCEDURES:

- Do not clean up the spill yourself, unless you know what spilled and how to properly clean it up.
- Immediately, notify others in the area that a spill has occurred.
- Turn off ignition and heat sources.
- Prevent others from coming in contact with the spilled chemical(s).
- Wear proper PPE (i.e., goggles, gloves)
- Use the appropriate material to confine or contain the spill to avoid spreading
- Absorb inorganic acids and bases and neutralize.
- Sweep up the absorbed spill from the outside toward the middle. Scoop up and deposit in a leak-proof container. Label and dispose of the container through the hazardous waste management program
- Collect the residue, place in a container, and dispose as hazardous waste.



NEVER PLACE HAZARDOUS MATERIALS OR SPILL CLEANUP MATERIALS IN THE BIOHAZARD BIN OR REGULAR TRASH.

- Clean spill area with water.
- Report spill to EH&S at X4150



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Hazardous Materials - Spill Cleanup

MAJOR CHEMICAL SPILLS CLEANUP PROCEDURES:

- If the chemical spill presents an immediate danger; turn off ignition and heat sources, evacuate all personnel, exit the room/area, and close the door behind you.
- Attend to anyone who may have been contaminated or injured and remove them from exposure.
- Instruct contaminated person to remove any contaminated clothing and wash the affected area for at least 15 minutes.
- Use the safety shower if necessary – know the location of the nearest safety shower and eye wash station in your work area.
- Notify EH&S at X4150 and Security at X4111

NEVER PLACE HAZARDOUS MATERIALS OR SPILL CLEANUP MATERIALS IN THE BIOHAZARD BIN OR REGULAR TRASH.



Waste Disposal

- Evaluate what you need to conduct the experiment and consider the types of waste that will be generated in the procedure.
- Consider alternative chemicals; consider less hazardous chemicals that may be safer and generate a less costly hazardous waste stream.
- Order only the amount of material needed for the experiment. Excessive stocks of hazardous chemicals, radioactive materials, and infectious agents presents serious storage, safety, and disposal problems.
- Consider using non-hazardous material alternatives to conduct your research.



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Waste Disposal Guidelines and Waste Disposal Training

- Waste Disposal Policies and Procedures is available from our document library

<https://intranet.einsteinmed.edu/departments/environmental-health-safety/policies/>

- EH&S provides a comprehensive Waste Disposal Training to all our staff, students, and faculty.
- Contact EH&S and Call X4150 for additional information or to sign up for trainings at

<https://www.einsteinmed.edu/administration/environmental-health-safety/training/>

The image shows the cover of a document titled "Waste Disposal Policies and Procedures" from Albert Einstein College of Medicine. It includes a "Table of Contents" with the following items and page numbers:

Section	Page
I. Purpose	3
II. Scope	3
III. Policy	3
III.A. Ordering	3
III.B. Ordinary Trash	3
III.C. Sharps	3
III.C.1. Sharps, Non-Infectious, Non-Contaminated	4
III.C.2. Sharps, Infectious Agents or Materials Including rDNA or Trace Chemicals*	4
III.D. Labware	5
III.D.1. Labware, Non-Infectious, Non-Contaminated	5
III.D.2. Plastic Labware, Infectious, Chemically Contaminated	5
III.E. Infectious Materials	6
III.F. Bottles and Containers	6
III.F.1. Bottles, Non-Infectious, Not Chemically Contaminated	7
III.F.2. Broken Glass/Bottles, Non-Infectious, Not Chemically Contaminated	7
III.F.3. Bottles, Chemically Contaminated	7
III.G. Chemicals	7
III.G.1. Chemicals, General	7
III.G.2. Chemical Consolidation, Non-Chlorinated Solvents*	8
III.G.3. Trace Chemicals*	8
III.G.4. Photographic and Chemical Fixer Disposal	8
III.H. Animal Waste	9
III.H.1. Animals and Bedding, Infectious Agents or Materials Including rDNA	9
III.H.2. Animal Waste and Bedding, Non-Infectious	9
III.I. Disposal of Mead Waste	9
III.I.1. Radioactive Waste	10
III.I.2. Radioactive Waste, Dry Solid (P-32, S-35, I-125, Cr-51, and P-33)	11
III.I.3. Radioactive Waste, Dry Solid (H-3 and C-14)	11
III.I.4. Radioactive Waste, Lab Dewy-in-Storage	11
III.I.5. Radioactive Waste, Liquid Scintillation Vials (LSV)*	12
III.I.6. Radioactive Waste, Sink Disposal of Liquids	12
III.I.7. Radioactive Waste, Disposal of Large Quantities of Liquid Waste	14
III.I.8. Universal Waste	14
III.I.9. Universal Waste, Animals/Animal Bedding	15
III.K. Computers and Electronics	15
III.L. Paint	15
III.L.1. Paint-Related Materials	15
III.M. PCB Waste	16
III.N. Refrigeration Units	16

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1



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Biosafety Training

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Biosafety

- Biosafety is defined as “The discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials” that have the potential to cause disease in humans.
- The practice of safe handling of pathogenic microorganisms and their toxins in the biological laboratory is accomplished through the application of containment principles and the risk assessment.

Regulations

- OSHA Bloodborne Pathogen Rule requirements:
 - Exposure Control Plan
 - ✓ List of job titles exposed/not exposed
 - Annual training
 - Offer the Hepatitis B Vaccine
 - Personal protective equipment
 - Utilize engineering controls
 - Provide exposure follow-up/counseling



Other Regulatory Guidance

- NIH Recombinant Guidelines
 - Apply to all institutions receiving NIH funding
 - Covers rDNA work, but also includes risk group listing
- CDC's BMBL -Biosafety in Microbiological and Biomedical Laboratories
 - Guidelines that are not optional
- Select Agent law (covers high consequence agents and toxins)
- Dangerous goods shipping regulations
- Animal Welfare Act
 - IACUC review of animal protocols

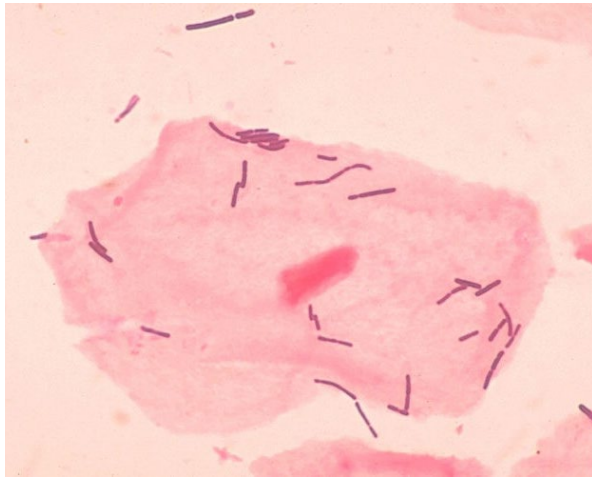


Risk Group/Biosafety Level

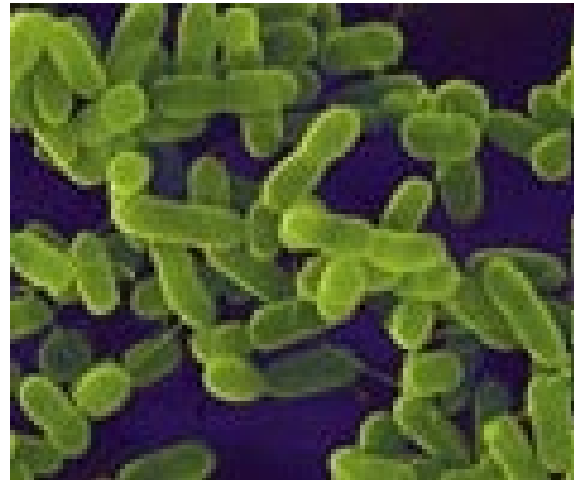
- Risk Group corresponds to the level of hazard associated with the agent
 - NIH Guidelines
 - American Biological Safety Association webpage
- Biosafety Level corresponds to the facility design, PPE and practices required to handle the agent.
- As described in CDC/NIH, "Biosafety in Microbiological and Biomedical Laboratories (BMBL) there are four biosafety levels (BSL) 1-4.

Biosafety Level 1

- **Agents handled in BSL1**
 - Not known to cause disease in healthy adult humans
 - e.g., non-pathogenic *E. coli*, Lactobacillus



Lactobacillus



E. coli

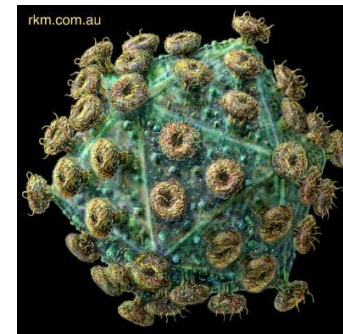
Biosafety Level 2

- **Agents handled in BSL2;**

- Are associated with human diseases
- e.g. *Salmonella typhimurium*, *Cryptococcus neoformans*, HIV and HCV clinical samples
- Human products (blood, tissue, human cell lines)



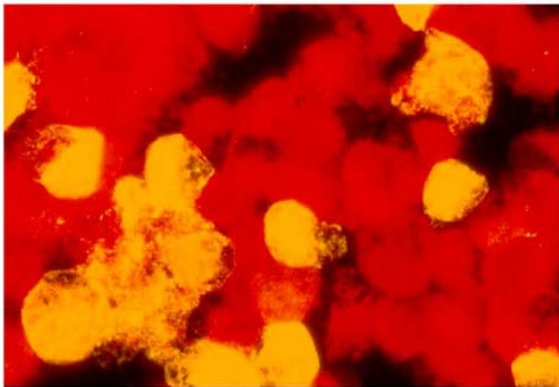
Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells



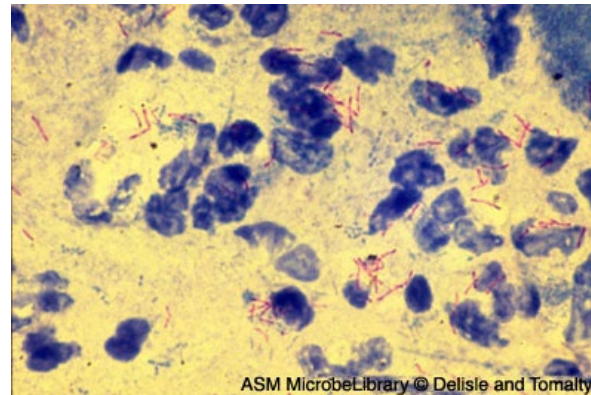
Model of HIV virion

Biosafety Level 3

- **Agents handled in BSL3;**
 - > Indigenous/exotic agents associated with human disease
 - > Potential for aerosol transmission in the lab
 - > e.g. *M. tuberculosis*, Lyme disease, West Nile virus



West Nile Virus



Acid Fast Stain of *M. tb*

Biosafety Level 4

- **Agents handled in BSL4**
 - > Most dangerous/exotic agents of life threatening nature
 - > e.g., Ebola virus, Marburg virus, Lassa virus

Lassa Virus

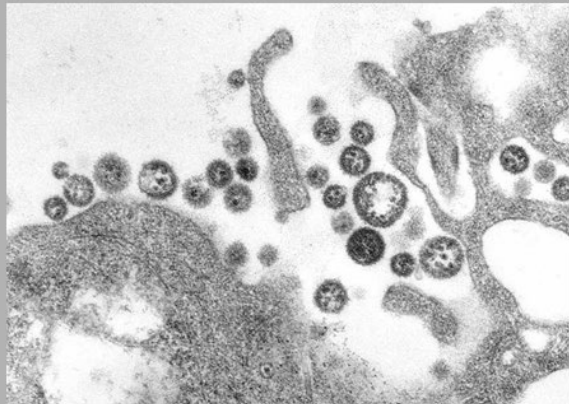
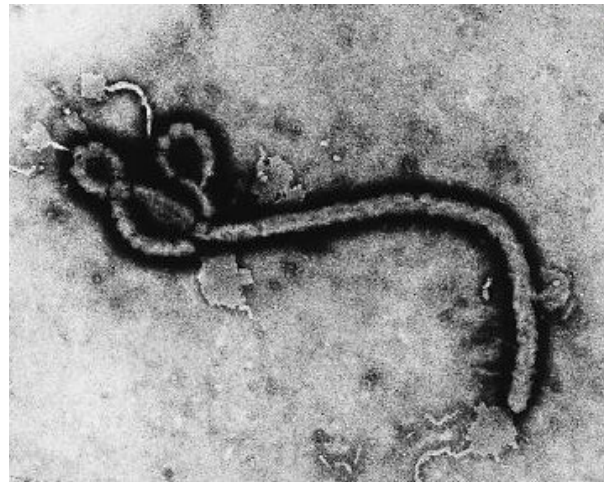


Image source: C.S. Goldsmith and M. Bowen (CDC)



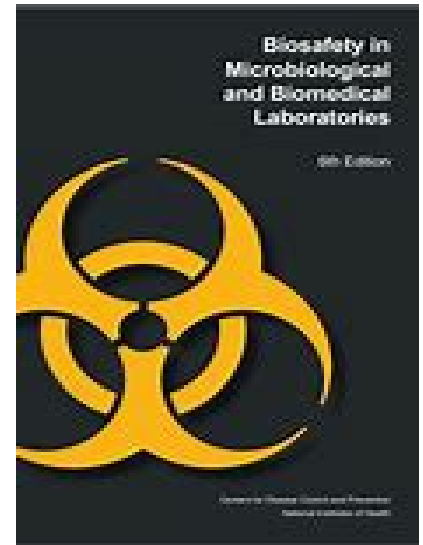
Ebola virus



BSL4 Lab Suit

Facility Design (BMBL)

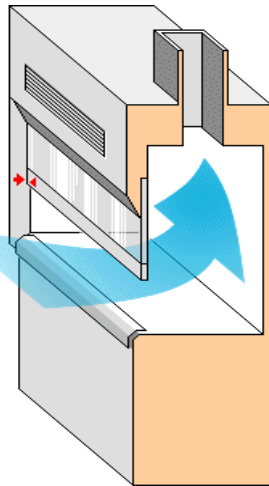
- BSL-1
 - Basic lab with sink for handwashing
- BSL-2
 - Sink for handwashing, biosafety cabinet, door signage, non-recirculated air, etc.
- BSL-3
 - Hand free sink, biosafety cabinet, door signage, ventilation negative pressure for containment, monolithic, sealed, 2X door entry, security, alarms, integral autoclave, etc.



How Do You Protect Yourself?

- Engineering controls
 - Biological Safety Cabinets, sharps containers, safe sharps, centrifuge safety cups, etc.
- Work practices
 - Handwashing, aerosol avoidance, decontaminating work surfaces, etc.
- Personal protective equipment (PPE)
 - Lab coat, gloves, safety glasses, respirator

Know Your “Hoods”!



Chemical Fume Hood

- Closes completely
- Not meant for sitting
- Negative pressure
- May have chemical storage underneath



Biological Safety Cabinet (BSC)

- Fixed sash opening (8 in.) (alarmed)
- Designed for seated work
- Negative pressure
- Check manufacturer label for type of cabinet



Laminar flow Hood

- HEPA filter visible in rear or top of unit
- Usually no sash
- Positive pressure – air blowing into face or breathing zone

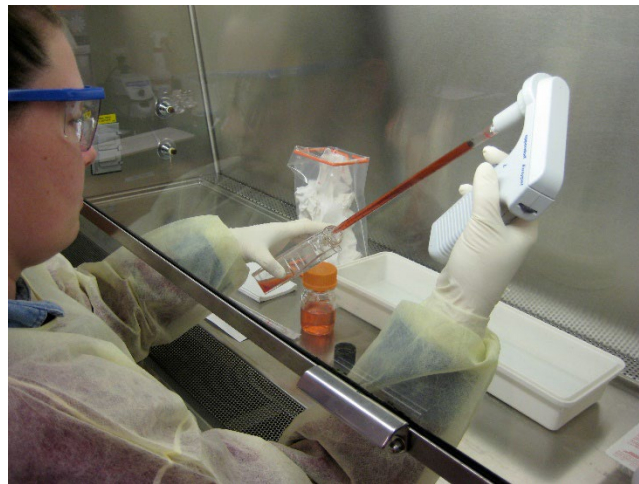


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Biological Safety Cabinet (BSC)

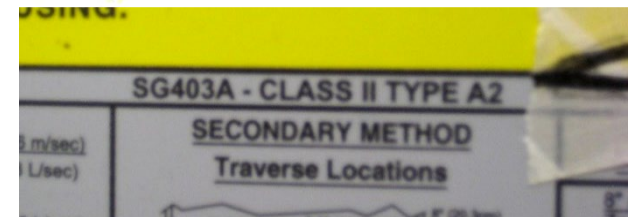
Primary Containment

- Role:
 - To protect the user from the samples
 - To protect the environment from the samples
 - To protect the samples from external elements



Biosafety Cabinets

- There are 3 classes of BSC
 - Class I: not often used
 - Class IIA and B: most often found in laboratories
 - Class III: primarily BSL3 or 4 labs
- Certification required:
 - When new and then annually
 - When hoods are moved
 - After repairs, filter changes
 - Vendor: TSS (Technical Safety Services)
 - ✓ Telephone: 866-536-5656



Clean Bench (Laminar Flow Cabinet)

- Not to be confused with Class I BSC
- Inflow air is HEPA filtered
- Exhaust air is not filtered
- Used for Microbiology clean preparation (making agar plates) and molecular work (PCR)
- Air flow can be vertical or horizontal



Class I BSC

- Is a negative-pressure ventilated cabin
 - User and the environment is protected
- Open in the front with a sash
- Inward flow air is not filtered
 - Sample not protected
- Cabinet air HEPA filtered and 100% exhausted to the lab or outside
- Used with low risk agents
- Used to contain mixers, blenders, etc.



Class II BSC

- Most commonly found BSC in laboratories
- HEPA-filtered vertical laminar air flow
 - User and sample protected
- HEPA-filtered exhausted air
 - User and environment protected
- There are 2 types and 2 subtypes (A1, A2 and B1, B2)



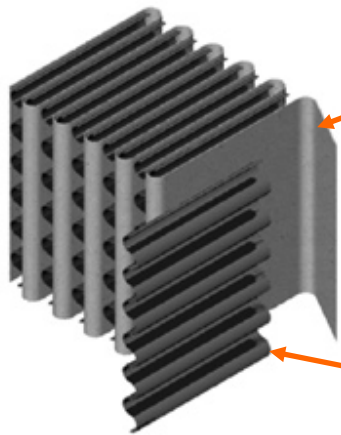
Class III BSC

- Are totally enclosed
- Ventilated cabinet of gas tight construction
- Works under negative pressure
- Supply air is HEPA filtered
- Exhaust air is filtered by 2 HEPA filters or 1 HEPA filter 100% exhausted to the outside
- Offers the highest protection
- Work is done through integrated gloves
- All equipment is integrated in the BSC



HEPA Filters

High-Efficiency Particulate Air filter

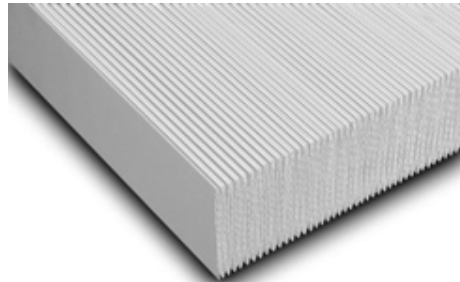


Borosilicate filter medium

Aluminum separator



Wooden frame



Continuous sheet of flat filter medium

Figure 3. Air Filtration Theory Particle Collection Mechanisms

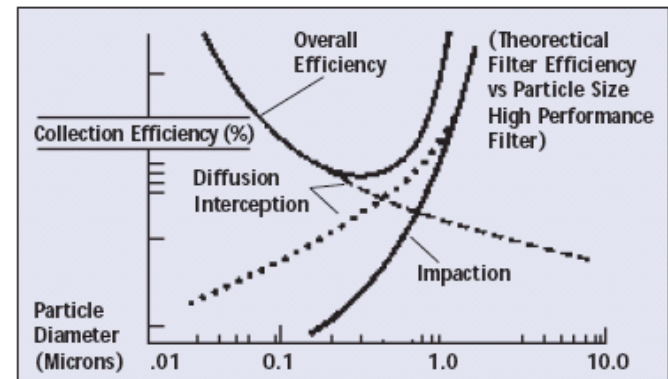


Figure 4. Relative Effect of Particle Collection Mechanism

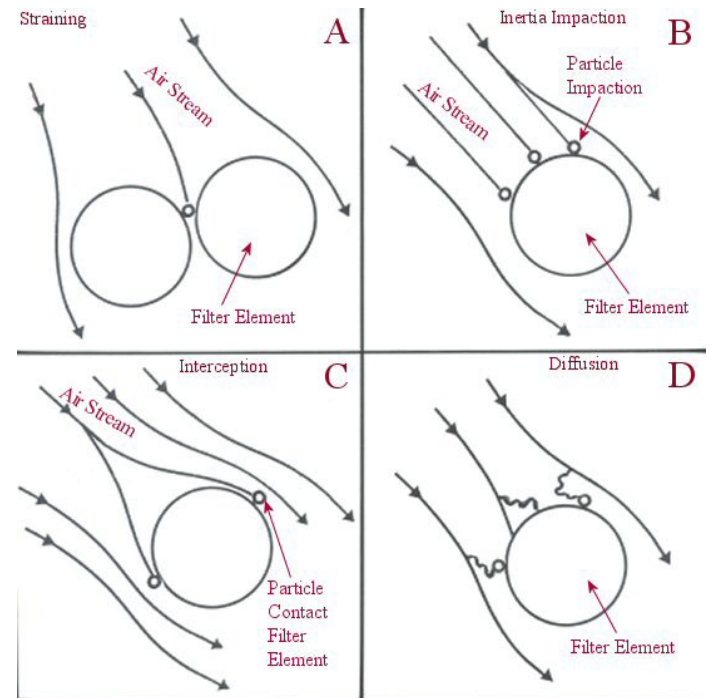
Labconco



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Function of HEPA

- Particles removed by:
 - > **Inertia impaction**
 - > **Interception**
 - > **Diffusion**
 - > **Straining**
- Particles attach by electrostatic (Van der Waals) forces
 - > **difficult to dislodge**
 - (unlikely that a properly functioning HEPA is the cause of contamination)



Particle Size Ranges

Typical aerosolized bacteria

Droplet nuclei

Fungi

Viruses

Bacteria

.01

.10

0.3

1.0

10

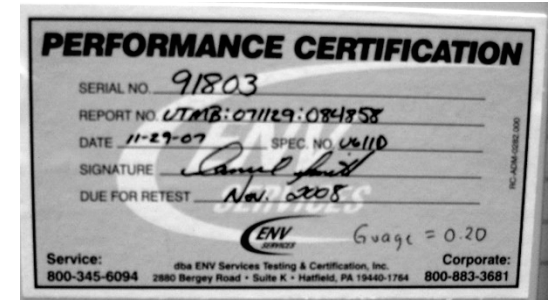
50

X

microns

Proper Use of a BSC

- Before starting
 - If BSC off, turn on 20 min before using for infectious agents
 - Can set up work during this time
 - Check the monitors of the BSC
- Set up work area
 - Decontaminate all surfaces of the BSC
 - Walls, work surface, glass
 - Place the essential material needed for a specific experiment in the BSC after surface decontamination
 - Organize work place
 - Wait 5 min before starting work



Completion of Work



- Surface decon and remove experimental cultures
- Discard infectious waste into the waste container
- Cover, and secure waste containers
- Surface decon all equipment and remove from BSC
- Surface decon and remove waste containers
- Surface decontaminate all surfaces of the BSC
 - Consider use of a dry “Swiffer” to reach all surfaces

BSC Reminders

- Use BSC for manipulating infectious agents or human materials
- Keep work areas free of unnecessary clutter including equipment and supplies.
 - Ignoring this may result in a loss of proper airflow and an increased risk of contamination
- Organize your work time to avoid rushing
- Keep amount of work to be done realistic
- If you feel yourself getting tired, take a break



DO

- **ALWAYS** surface decontaminate all surfaces and material coming out of BSC
- **ALWAYS** change or decontaminate gloves before taking hands out of the biosafety cabinet
- Decontaminate any surface, glove, lab coat that may become contaminated by biological material
- Always use mechanical pipetting aids
- For centrifugation: place the rotor inside the BSC, then wiped down before removal from BSC



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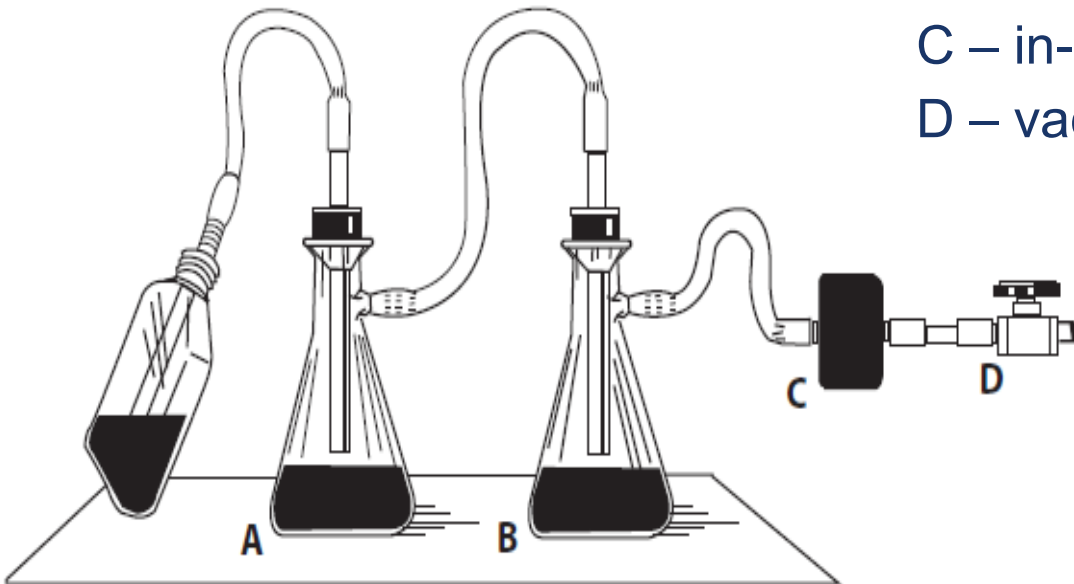
Don't



- Discard materials outside BSC during experiment
- Cross hands while working in the BSC
- Block front and rear intake grills
- Scratch nose/eyes, get hair out of face with gloves on
- Make sudden swift movements of hands in the biosafety cabinet
- Over-fill waste containers
- Do not use Bunsen burners in BSCs
- Work in the BSC if alarm or warning light is on

Vacuum Set-Up

- A – disinfectant containing flask
- B – overflow flask
- C – in-line HEPA filter
- D – vacuum system



Aerosol-Producing Procedures

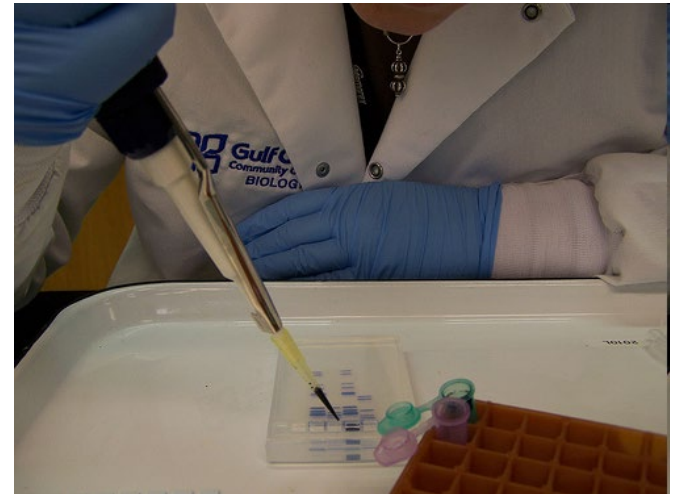
- Centrifuge
- Vortex
- Rocking/shaking cultures
- Lyophilization
- Sonication
- Tissues grinding
- Cell Sorting
- Pipetting
 - Opening tubes



Pipetting



- Open centrifuge tubes carefully
 - Cap openers prevent spills on thumbs
- Pipette in a slow and controlled motion
- Should not hear the “clicking sound” of your pipetter
- Filtered pipette tips do not replace good technique



Centrifuges used for Potentially Infectious materials

- You need a sealed or gasketed rotor if run outside of BSC
 - For blood and infectious agents
- If no seals/gaskets
 - Order “safety cups”
 - Load, unload and run inside the BSC



Safety cups

Proper Use of Centrifuges

- All personnel must be trained on the proper use of centrifuges.
 - Rotors and seals must be checked before and after each procedure.
 - Check tubes for cracks before and after use.
 - Balance tubes
 - Critical for ultracentrifuges
 - Wait 5 min before opening the centrifuge



Disinfection

- Solutions most commonly used
 - 10% bleach (freshly made)
 - Allow sufficient contact time (10-15 minutes) to ensure decontamination.
 - Other EPA registered tuberculocidal disinfectant (Cavicide, Wescodyne, Vesphene, etc.)
 - 70% ethanol (kills environmental contamination -- for items going into BSC)



Disinfection Procedure

- Centrifuges
 - Use damp cloth at the end of each procedure
 - Clean Rotors/bucket
 - Inside-at the end of each experiment
 - Outside-wipe each time it is taken out of the BSC
- Bench top
 - Wipe down
 - Before and after each experiment
 - End of the day
 - After a spill



Spill Procedures In a BSC

- Alert your co-workers
- Change PPE
- Cover with absorbent material
- Flood area with appropriate disinfectant
- Allow 15 min for disinfectant to be effective
- Notify supervisor
- Decontaminate all material
 - Place cleaning waste into waste container in the BSC
 - Disinfect equipment, remove, and clean the BSC
- Place all waste in red biohazard bag
- Change PPE
- Resume work

Spill Procedures in Centrifuge

- If centrifuge is in the BSC, use BSC spill procedure
- If the centrifuge is outside of BSC:
 - Close centrifuge and stop work immediately
 - Notify co-workers and place a note on centrifuge so no one uses it. Leave it closed for 60 min
 - Notify your supervisor
 - Don necessary PPE
 - Absorb spilled material with paper towel, pour disinfectant on spill, and wait 15min
 - Clean all potentially exposed surfaces
 - Remove rotor and decontaminate it in BSC

Spill Procedure in the Laboratory

- Stop work immediately and notify co-workers
- Remove any contaminated clothing
- Wash any exposed skin, flood eyes, etc.
- Allow aerosols to settle for 30 min
- Notify supervisor
- Don PPE
- Contain spill with absorbent material
- Pour disinfectant on spill and wait 15 min
- Clean-up spill
 - Remove broken glass (no bare hands)
 - Soak contaminated area again with proper disinfectant and clean outside spill area
 - Remove waste into biohazard red bag
- Remove PPE
- Return to work



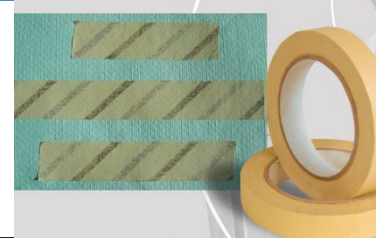
Disinfection Procedure



- Biosafety cabinet
 - Surface decontaminated before and after each experiment
 - Complete decontamination including underneath the tray - monthly
- Incubators should be decontaminated monthly following the manufacturer's guidelines

Autoclave Procedures

- Autoclave is used primarily for solid waste
- 3 ways to verify the autoclave was run
 - Autoclave tape (each run)
 - Biological/chemical indicator
 - Read printout (each run)
 - ✓ Indicates temperature, pressure, and time run
- Autoclave malfunction
 - Do not open door (post sign)
 - Notify administrator and supervisor for repair



Emergency Procedures

- Remain Calm
- Wash any area that may have been exposed for 15 minutes.
- Protect yourself and your colleagues from further harm.
- Notify supervisor
- If needed, notify emergency services (911)
- Notify Security (X4111) and EH&S (X4150)
- Seek medical attention, if necessary

Radiation

- If you handle, store or dispose of radioactive materials, you must take radiation safety training.
- Please contact EH&S at X2243 for training information.

Laboratory Safety Sign in Sheet and Quiz



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