Biosafety Training
Training Overview

- DEFINE Biosafety, Biohazard, Biosafety Levels
- Protection (PPE, biosafety cabinets)
- CELL CULTURE
  - Hazards (Blood Borne Pathogens)
- Aseptic technique
- Sharps
- Waste
- Autoclave
- Liquid nitrogen
- Centrifuge
- Microscopy
What is Biosafety?

- Measures used when handling biohazardous materials to avoid harm to oneself or the environment.

  - Engineered controls
    - Containment (biosafety cabinet)

  - Personal Protective Equipment

- Practices and procedures
  - Aseptic technique
  - Labeling, MSDS, facility approval
What is a Biohazard?

- A potential hazard to humans, animals or the environment caused by a biological organism or by material produced by such an organism

- Examples: virus, bacteria, fungi, parasites and their toxins/allergens

- Blood and body fluids as well as tissues from humans and animals **

- Transformed cell lines and certain types of nucleic acids
Biosafety Levels

- 4 Biosafety levels
  - BSL1
  - BSL2
  - BSL3
  - BSL4

- Biosafety level defines lab requirements, protective clothing and work practices
Biosafety Level 1

- Not known to consequently cause disease in healthy human adults

- Requires:
  - Basic laboratory (no eating or drinking)
  - No special design features
  - Biosafety cabinets are not required (work can be done benchtop)
  - Safe handling of sharps
  - Decontamination of work surfaces
  - PPE: Lab coat, gloves, eye protection when necessary
Biosafety Level 2

- Associated with human disease

- Clinical, diagnostic, research and teaching laboratories with level 2 agents

- Requires:
  - Class 1 or class II biological safety cabinet if any potential for aerosol or splash exists
  - An emergency plan for handling spills must be developed
  - Controlled access
  - Written biosafety procedures
  - All personnel are advised of the hazards
  - PPE: lab coats, gloves, eye protection when appropriate
Biosafety Level 3

- Work with exotic or indigenous agents that may cause serious harm or potentially lethal disease as a result of inhalation or exposure

- Requires:
  - Specialized room design (2 doors in a series to access the lab)
  - Specialized training
Biosafety Level 4

- Work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease

- Requires:
  - Class III biosafety cabinets or positive pressure suits
  - Clothing is usually autoclaved
  - Shower/change rooms
  - Airlocks
Protection - Behavior

- No food or drink EVER in the lab!!
- Proper attire
- Proper behavior
Protection - Personal Protective Equipment

- Gloves!!!
  - Nitrile for cell culture
  - Cryogenic for liquid nitrogen handling
  - Heat gloves for autoclave

- Lab coat

- Closed toe shoes

- Aseptic technique

- Face shield and cryogenic apron for liquid nitrogen handling
Protection - Biosafety Cabinet

- Physical containment for biological agents, especially when aerosols are generated
- HEPA filters remove particles with 99.7% accuracy
- Three classes of cabinets
  - I – Does not protect the work from contamination, air entering cabinet is not filtered
  - II – Airflow intake & exhaust is HEPA filtered. Either hard ducted or exhausted into room
  - III – totally enclosed, ventilated cabinets, work through portals with attached gloves
- Do not restrict the airflow of the cabinet
Protection – Practice & Procedures

- All cell lines, reagents and protocols must be approved by BNRF manager
- All items that remain in the lab must be clearly labeled
- MSDS must be supplied

- No food or drink ever!
- Proper apparel
- Proper behavior
Cell Culture

- Tissue culture cell lines have the potential to contain pathogenic organisms
  - Human, non-human primate, mycoplasma-containing cell lines are LEVEL 2
Cell Culture

- Use biosafety cabinet (BSC) for all work with BSL-2 procedures

- Class II, Type A2 BSCs provide personnel, environmental, and sample protection

- Always clean before and after use
Bloodborne Pathogens (BBP)

- A pathogenic microorganism that is present in human blood, fluids, tissues and cells and can cause disease in humans

Examples:
- Hepatitis B
- Hepatitis C
- HIV

Precautions
- Education
- Vaccination if available or must sign declination form
- Aseptic technique & hand washing
- Wearing protective barriers
- Use safe work practices
Bloodborne Pathogens (BBP)

- All personnel handling mammalian cell cultures are required to have a Hepatitis B vaccination
  - Mon Health Dept (304) 598-5119

- Students – WVU Student Health (304) 293-2311
  - $71 dose

- Postdocs, Faculty & Staff – Occupational Medicine at the

  WVU Medical Surveillance Program

  Employee Information

  NAME: [Name]  SSN: XXX-XX-   TODAY'S DATE: [Date]

  ADDRESS: [Address]  DOB: [Date of Birth]
Recombinant DNA & Viral Vectors

- Genetic engineering is *in vitro* incorporation of non-native genetic material into a cell

- Adenovirus, Herpes virus, Retrovirus, Transgenes
Carbon nanotubes

- Carbon nanotubes (CNTs) have undetermined health risk and should be handled accordingly
Aseptic technique

- Keep areas clean and free of unnecessary equipment

- Wash your hands
  - upon entering and leaving the lab
  - before and after cell culture work
  - before touching an unprotected part of your body
  - as needed

- Tie your hair back

- Pull back sleeves

- Pay attention to what you touch!!
Aseptic technique – Decontamination

- Spray down with 70% ethanol
  - Surfaces
  - Hood
  - Media bottles

- Use diluted bleach as necessary

- Use the biosafety cabinets and fume hoods

- Disinfect all contaminated surfaces after procedures
Aseptic technique – Disposable glove use

Keep in mind what is “clean” and what is not.

- “Clean” areas are places where microorganisms and samples are not allowed
- Clean areas such as keyboards, doorknobs, drawers and instruments must not be touched with contaminated hands. If there is any doubt, replace gloves.
- Contaminated gloves should be disposed of properly
- Only wear gloves where needed
Biohazard Waste

- Discarded biological material
  - Cells and used media → liquid waste
  - Solids → biohazard trash  NO BLEACH!

- All contaminated cell culture gloves, petri dishes, pipets and pipette tips should be discarded appropriately

- Dispose of noninfectious waste in regular trash
Biohazard Waste

- Only “treated” waste is no longer considered hazardous and can be disposed of in the regular waste

  - SOLID WASTE → Autoclave waste cycle in clear plastic bag with indicator tape
  - NO BLEACH!

  - LIQUID WASTE* → Treat with 10% bleach for 30min

- Waste containers must be closed when not in use

* as long as no heavy metals, viruses, solvents, alcohols or chemotherapeutics used
Sharps

- Includes needles, syringes, razor blades, cover slips, slides, scalpels, glass and plastic pipets, broken plastic, glassware or other devices capable of cutting or piercing skin.

Plastic Sero-pipets are considered a sharp and should be placed in the cardboard box container underneath the biosafety cabinet.

Glass or pasteur pipettes are considered glass sharps and should be placed in plastic sharps container.
Autoclave

- CAN autoclave:
  - Contaminated cultures, stocks and media
  - Contaminated solid items: eppendorf tips, glassware, incubator racks

- Use only approved autoclave bags

- Do not overfill bags

- Do not autoclave bleach, DMSO, volatiles, solvents or corrosives
Autoclave

- **Safety concerns:**
  - Burns
  - Be careful opening autoclave after cycle

- **Before use:**
  - Get proper training
  - Wear PPE!!
  - Do not autoclave bleach
Liquid Nitrogen & -80 Freezer

- Used for cryogenic storage of mammalian cells in both ESB & CRL

- Safety concerns:
  - cryogenic burns
  - Wear proper PPE!!!
  - No open shoes in the lab ever
Centrifuge safety

- Hazards: Leakage and spills creates aerosols of contents (*inhalation concern*)

- Before use:
  - Get proper training
  - Set temperature, spin time and speed correctly
  - Ensure that caps and tubes are sealed
  - Balance tubes correctly
Microscopy

Safety concerns:
- Sample handling (biohazards, chemical reagents)
- Sharps (broken glass, needles, razor blades)
- UV light (fluorescence scope)
Using your samples and reagents in the lab...

- All items brought into the BNRF have to be approved to manage & determine risk assessment.
- All samples & reagents stored in the lab must have a "Biomaterial & Sample Tracking Form” submitted.

http://sharedresearchfacilities.wvu.edu/facilities/srfFacilityBioNano.html
Be Smart, Be Safe

- Carefully plan out all procedures and experiments

- Notify other users of unsafe practices before they hurt themselves or others.

- Contact a facility manager if there is a dangerous situation

- Don’t be afraid to ask for help
In case of emergency……

- In case of emergency….
  - Call 911 (9-911 from campus phone)
  - Call emergency contact numbers listed on door placards, logbooks or Chemical Hygiene Plan

- Reporting
  - In the event of any accidents, incidents or spills please let SRF managers and staff know immediately!!

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