



# Biosafety Training

WVU Shared Research Facilities

2012

# Training Overview

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- ▶ **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?

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- ▶ 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?

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- ▶ 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

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- ▶ **4 Biosafety levels**
  - ▶ BSL1
  - ▶ BSL2
  - ▶ BSL3
  - ▶ BSL4
  
- ▶ Biosafety level defines lab requirements, protective clothing and work practices

# Biosafety Level 1

# BSL1

- ▶ 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

# BSL2

- ▶ Associated with human disease
- ▶ Clinical, diagnostic, research and teaching laboratories with level 2 agents
- ▶ Requires:
  - ▶ Class I 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

# BSL3

- ▶ 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

# BSL4

- ▶ 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

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- ▶ No food or drink **EVER** in the lab!!
- ▶ Proper attire
- ▶ Proper behavior

# Protection - Personal Protective Equipment

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- ▶ **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

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- ▶ 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

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- ▶ 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

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



# Cell Culture

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- ▶ 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)

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- ▶ 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)

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- ▶ 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

TODAY'S DATE: \_\_\_\_\_

NAME: \_\_\_\_\_

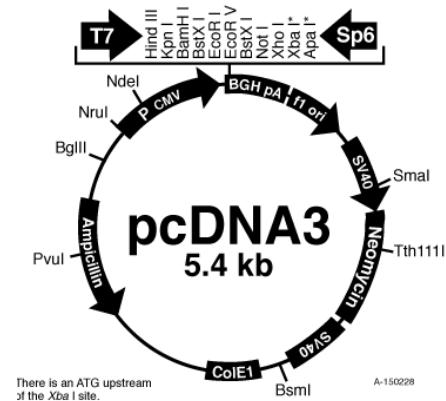
SSN: XXX-XX- \_\_\_\_\_

DOB: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

# Recombinant DNA & Viral Vectors

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

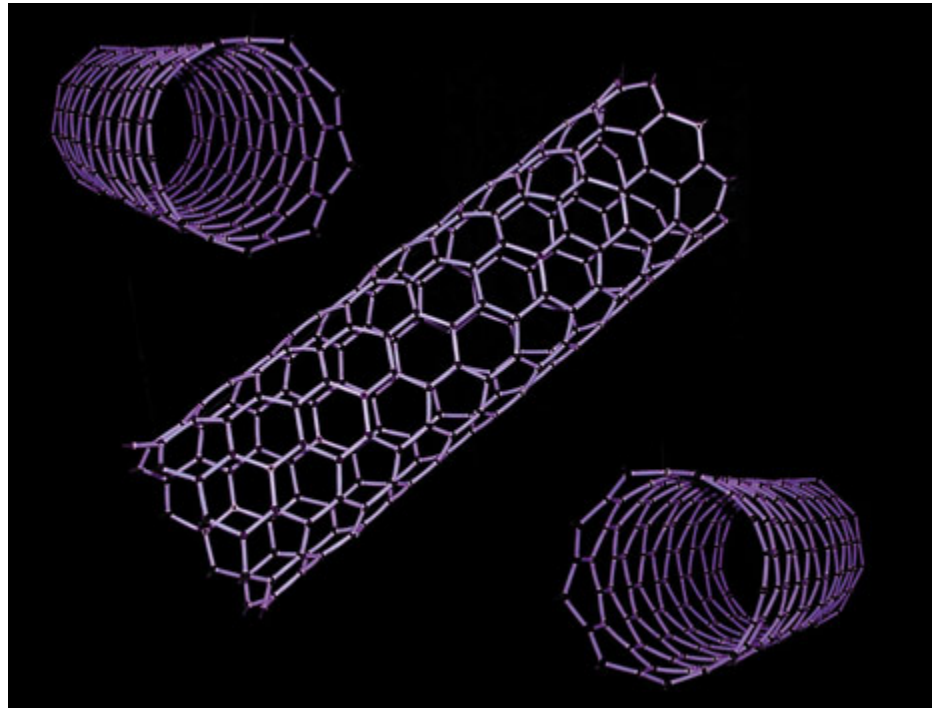


- ▶ Adenovirus, Herpes virus, Retrovirus, Transgenes

# Carbon nanotubes

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



# Aseptic technique

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- ▶ 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

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- ▶ 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

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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

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- ▶ 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

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- ▶ 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

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- ▶ 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

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- ▶ **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

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- ▶ **Safety concerns:**
  - ▶ Burns
  - ▶ Be careful opening autoclave after cycle
- ▶ **Before use:**
  - ▶ Get proper training
  - ▶ Wear PPE!!
  - ▶ Do not autoclave bleach



# Liquid Nitrogen & -80 Freezer

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- ▶ 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

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- ▶ **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

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- ▶ **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...

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- ▶ 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>

<b><u>BIOMATERIAL &amp; SAMPLE TRACKING FORM</u></b>	
This form must be approved before any biological material or sample is brought into or stored within the Bio-Nano Research Facilities. A signed Biomaterial and Sample Tracking Form must be submitted for each type of biomaterial or sample brought into the Bio-Nano Research Facilities. We recommend having this form approved before experimentation and expected storage. For biomaterials and samples that are regularly used, we recommend saving this form.	
<b>PERSONAL INFORMATION</b>	
Name:	
Email:	

# Be Smart, Be Safe

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- ▶ 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.....

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- ▶ 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!!
  
- ▶ Siera Talbott  
(304) 293-0747  
siera.talbott@mail.wvu.edu