# Biological Safety: Principles & Applications for Lab Personnel

Presented by: Biological Safety Office http://biosafety.utk.edu



Introduction

# OVERVIEW OF BIOSAFETY PROGRAM

## **The Institutional Biosafety Committee**

Institutional Biosafety Committees (IBC) are required by the National Institutes of Health Office of Biological Activities (NIH; OBA) for institutions that receive NIH funding and conduct research using recombinant or synthetic nucleic acids.

The UT IBC is composed of 13 faculty members and 3 community representatives, who are selected to offer a broad range of experience in research, safety and public health.

### Functions of the IBC

- Performing both initial and annual reviews of research proposals for Risk Assessments and compliance with state, local and federal guidelines
- Setting Biosafety levels for research studies
- Notifying the Principal Investigator of IBC review and approval
- Report any significant problems, violations, research related illnesses or accidents to UT and the NIH/OBA
- Develop emergency plans covering accidental spills and personal exposures

### **Projects requiring IBC review**

- Those involving recombinant or synthetic nucleic acids
- Those utilizing agents infectious to humans/animals/plants
- Projects using human derived materials
- Studies which use biological toxins
- Use of novel nanoparticle conjugates
- Venomous animals/toxic plants

## The Biosafety Office

The mission of the Biosafety Office is to help manage and facilitate research in the life sciences at the University of Tennessee while ensuring the safety of laboratory personnel and regulatory compliance

- Training for researchers and staff
- **Risk assessment** of research materials & use
- Assistance with IBC registration of research projects
- **Inspection** of laboratory spaces and assistance with the remediation of problems
- Shipping biological hazards
- Maintenance of institutional records
- Compliance oversight for the University regarding federal, state, and local regulations



Brian Ranger, MS, CBSP Biosafety Officer



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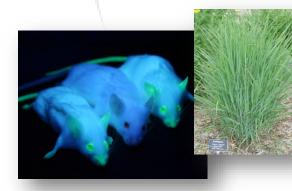
# **Regulations & Standards**



# **Biological Safety Training**

Based on federal and institutional guidelines, faculty, staff and students are required to complete both initial and annual refresher trainings if their research involves the following:

- Agents infectious to humans
- Animal/plant pathogens requiring enhanced containment
- Acute biological toxins
- Human derived materials
- Soil and water samples that likely harbor infectious agents
- Recombinant or synthetic nucleic acids



Section I

# RISK ASSESSMENT AND MITIGATION

## **Introduction to Laboratory Safety**

When entering the laboratory environment you are likely to contact hazards that you would not encounter on a daily basis. These can include:



# **Biohazards**









Any biological agent or condition that poses a threat to human, animal, or plant health, or to the environment. Examples include:

- Human, animal and plant pathogens
- Toxins of biological origin
- Materials potentially containing infectious agents or biohazards
   Blood, tissues, body fluids etc.
   Waste, carcasses etc.
- Recombinant DNA (depends)
- Nanoparticles w/biological effector conjugates (depends)





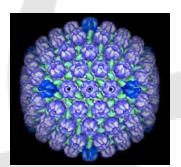


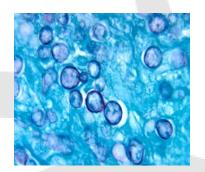


## Types of Biohazards:

### What they are....









How they'll look....



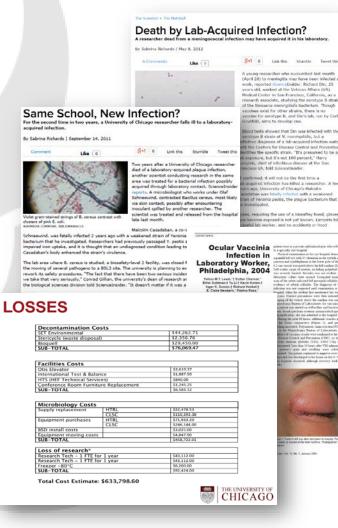
## Lab Acquired Infections: A Real Risk

Unintentional exposure of personnel to infectious agents is a major concern when working with Risk Group-2 or higher agents

The most comprehensive historical survey of **lab acquired infections** (**LAIs**) was conducted by Pike et. Al. in 1976

- Reviewed 4079 LAIs and documented exposures to159 separate organisms
- Only ~15% LAIs come from known exposures
- Agents in use at UTK-area campuses:
  - Staphylococcus aureus 29 cases, 1 death (1973)
  - Streptococcus pyogenes 78 cases, 4 deaths (1976)
  - Toxoplasma gondii 28 cases, 1 death (1976)
  - Hepatitis B virus 234 cases, 1 death (1976)
  - Adenovirus (1-4, 7)10 cases (1988)
  - Candida albicans 2 cases

On top of the human cost associated with these illnesses, there can also be substantial financial consequences associated with LAIs. For example, the loss of work, medical care recovery, and facilities remediation at UC was estimated to be > \$625,000 following an accidental release and exposure to *Bacillus cereus* 



## **Understand the Hazards you are Working With**

The overall goal of this training is to inform personnel of risks associated with work conducted with potentially infectious and recombinant agents and encourage the development of a more safety conscious environment. However, the ultimate responsibility for safe conduct in the lab falls to the personnel themselves.

Prior to working in the lab, it is important to become informed about the environment and agents that you will be working with. As such, the Office of Biosafety recommends that prior to beginning any new project, personnel:

### <u>Review pathogen safety data sheet and other information</u>

- History of LAI's & routes of transmission
- Signs/symptoms of infection
- Methods of inactivating the agent
- Specific features of your biohazard materials.
- In the event of incomplete information we recommend the following resources:
  - Public Health Agency of Canada
  - Centers for Disease Control
- Review lab-specific standard operating procedures (SOPs) prior to use.
- **Review** these training slides.
- Locate and Familiarize yourself with the Lab Biosafety Manual



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#### SECTION II - HAZARD IDENTIFICATION

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### **The Risk Assessment**

A **risk assessment** uses information provided by the investigator to set a level of containment and precaution that is intended to reduce the chance of accidental exposure or release of recombinant or infectious agents. The level of containment is referred to as the **Biosafety Level.** 

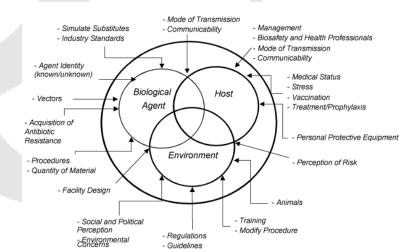
### **Basic Considerations:**

### Agent

- Host range
- Pathogenicity
- Availability of prophylaxis
- Route of transmission
- Viability in the environment
- Origin of the source
- Additional for recombinant DNA:
  - Nature of insert
  - Environmental impact

### Procedure

- Concentration of organisms
- Scale
- Use of animals
- Potential for the generation of aerosols
- Experience level of personnel



### Classification of Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research Involving Recombinant DNA Molecules	World Health Organization Laboratory Biosafety Manual
Risk Group 1	Agents not associated with disease in healthy humans.	(No or low individual and community risk) A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human Disease that is rarely serious and for which preventative or therapeutic interventions are <i>often</i> available.	(Moderate Individual Risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to lab workers, the community, livestock or the environment. Lab exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventative or therapeutic interventions may be available	(High individual risk, but low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventative measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventative or therapeutic measures are <i>not</i> usually available.	(High individual and community risk) A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventative measures are not usually available.

Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition.

## Comparative Routes of Transmission With RG-2 and RG-3 Organisms

### Risk Group-2

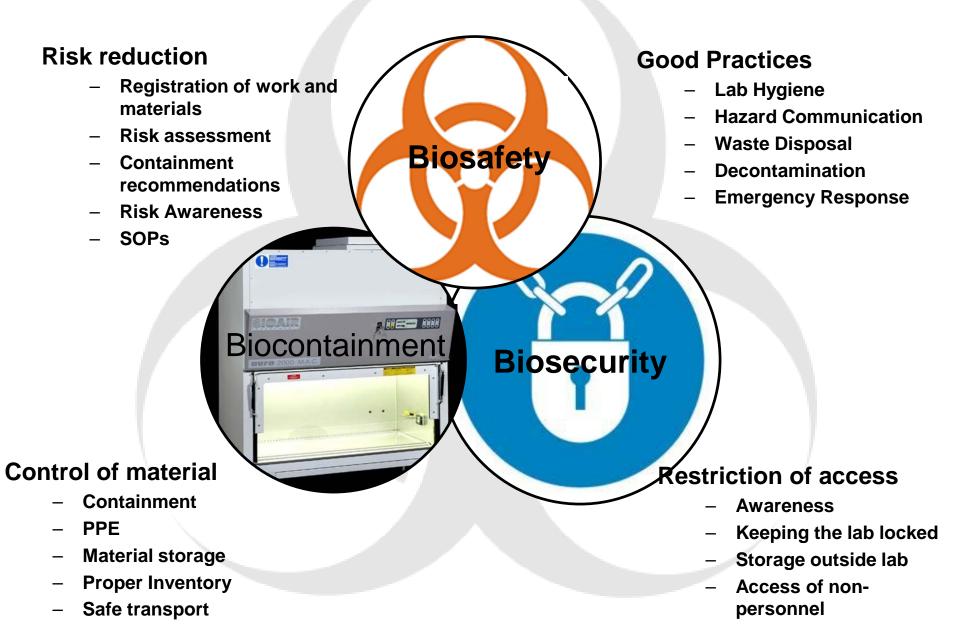
- Direct contact to:
  - Broken skin
  - Eye
  - Mucosal membranes
- Sharps injury (i.e., needle stick)
- **Bites** from infected animals or arthropod vectors
- **Ingestion** of liquid suspension of infectious agent (e.g., hand to mouth exposure)
- Aerosol contamination

### Risk Group-3

- Direct contact to:
  - Broken skin
  - Eye
  - Mucosal membranes
- Sharps injury (i.e., needle stick)
- Bites from infected animals or arthropod vectors
- Ingestion of liquid suspension of infectious agent (e.g., hand to mouth exposure)
- Aerosol contamination
- Potential for Respiratory Transmission

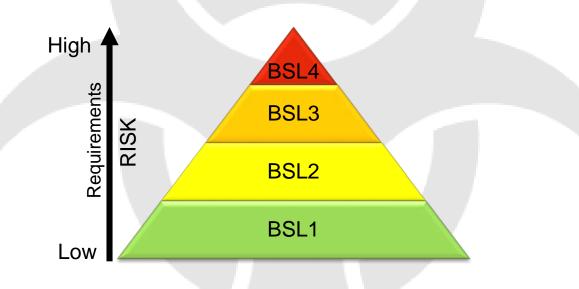
Good lab practices focus on infection prevention by incorporating barriers (e.g., **personal protective equipment**, **biosafety cabinets**) or procedures that reduce the likelihood of accidental exposure.

### **Risk Reduction for Biohazardous Agents**



## **Biosafety levels**

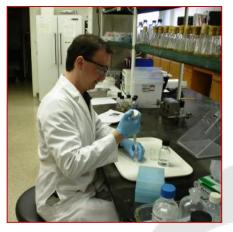
The **Biosafety Level (BSL)** of a procedure or lab can be thought of as a short hand for the recommended safety requirements and is a combination of lab practices and techniques, safety equipment and lab facilities which are used to Minimize potential exposure to a biohazard for lab personnel and the environment.



As the potential **Risk** to human health increases, so do the required levels of containment.

### **Biosafety Levels Permitted at UT**

### BSL-1



Used for work with biological agents and materials that pose **minimal risk** to people or the environment.

#### Features:

- Work on open bench
- Lab coat & gloves
  recommended
- Decontamination
  procedures in place

BSL-2



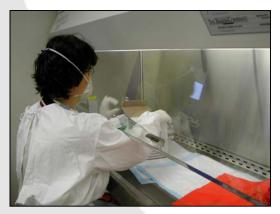
Used for work with biological agents or materials that pose **moderate risk** to people or the environment.

#### Features:

- Aerosol-generating procedures performed in a **biosafety cabinet (BSC)**
- Lab coat & gloves required
- Biosafety manual with labspecific procedures/training and restricted access

Note: The BSL refers to containment practices. The agents themselves are categorized into **Risk Groups (RG 1-4).** With increasing RG corresponding to increasing hazards.

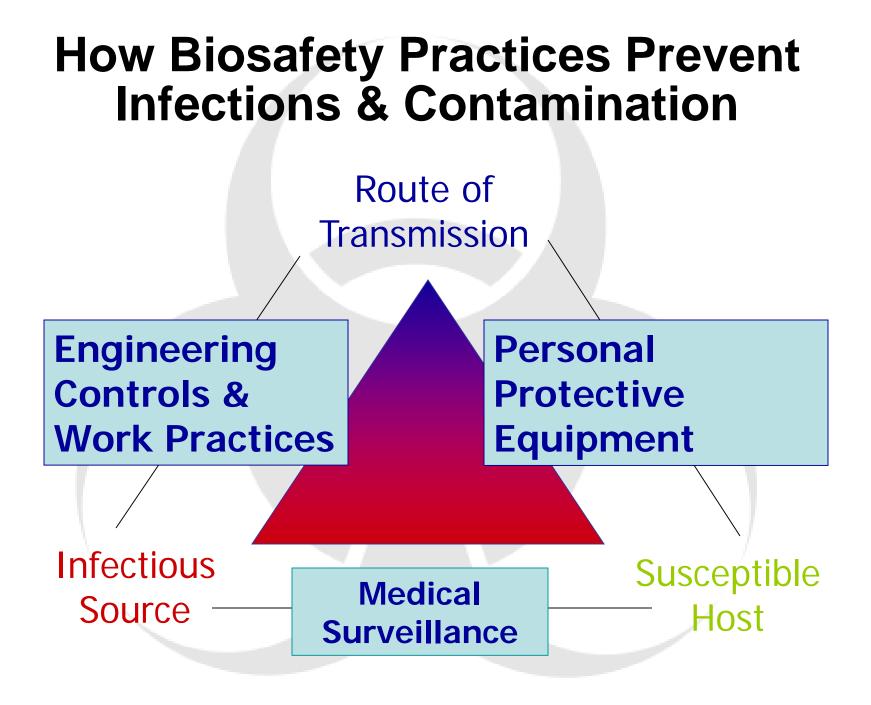
**BSL-3** 



Used with indigenous or exotic biological agents with potential for airborne transmission <u>or</u> for procedures involving aerosolization, concentration or large quantities of moderate risk materials.

#### Features:

- Lab designed to contain airborne hazard
  - double-door entry,
  - airflow in lab is negative to surrounding areas,
  - no recirculation of air
- All open manipulations of materials in BSC
- Respiratory protection
  usually required
- Facility design & operational procedures documented; annual functional verification

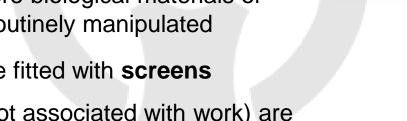


Section II THE BIOSAFETY LEVEL-2 LABORATORY

### **General notes on BSL-2 lab facilities**

To reduce the likelihood of environmental contamination and unintended release of agents, the NIH has provided the following guidelines:

- Bench tops must be solid, impervious to water, and resistant to heat and chemicals
- Spaces between benches, cabinets, and equipment should be easily accessible for cleaning
- Floor carpeting or throw rugs are not permitted
- Fabric furniture where biological materials or other hazards are routinely manipulated
- Windows should be fitted with screens
- Pets and plants (not associated with work) are prohibited.

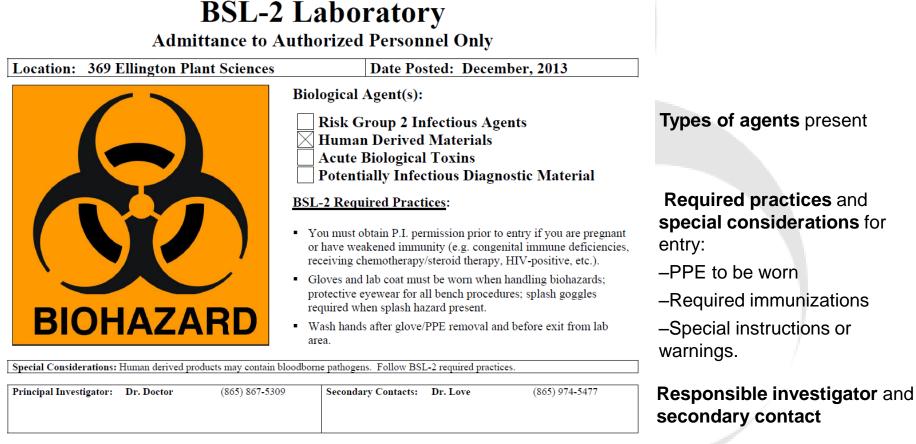


• Integrated Pest Management Program in place

Please help reduce the spread of infectious agents by insects or feral rodents. Report any signs of these pests to lab supervisors and the UT Facilities Services

## **Hazard Communication: Door Signage**

All laboratories designated as BSL-2 must have a door sign Indicating the following:



Institution EmergencyUT PoliceUTK SafetyBiosafetyRadiation SafetyContact Information:974-3114974-5084974-1938974-5580Emergency contact information

### **Hazard Communication: Biohazard Labels**

Equipment used for procedures with RG-2 (or higher) agents must be posted with a biohazard label. For example, hoods, freezers, incubators, and centrifuges, etc.

In addition, **transport containers**, **biohazardous sharps** and **biohazard waste containers** must display a **biohazard** label on the **outermost part**.



# WORK PRACTICE CONTROLS & PREVENTION OF ACCIDENTAL EXPOSURES

Section III

## **Good Lab Hygiene = Exposure Prevention**

Transmission of RG-2 organisms occurs through **mechanical transmission**, aerosols, and puncture wounds with contaminated sharps.

By becoming more aware of actions that lend themselves to unintentional exposures, a majority of **lab acquired infections** (LAIs) can be avoided.

In short, this means keep your hands away from your face and avoid activities that require those actions. For example:

- **<u>Do not</u>** store or consume food or drink in lab.
- **<u>Do not</u>** apply facial cosmetics, adjust contact lenses, etc.
- Keep personal items such as cell phones or tablets <u>out</u> of the work area.



## **Personal Protective Equipment (PPE)**

Beyond good lab practices, **Personal protective equipment** (**PPE**) is used as a primary barrier between personnel and an infectious agent, preventing **mechanical transmission** of agents.

PPE includes the following:

- Gloves (required for all manipulations of RG-2 organisms)
  - Fluid resistant
  - Disposable, i.e., SINGLE USE
  - Inspect gloves for defects while donning
  - Pick the right size for your hands
  - Do not touch "clean items" (phones, doorknobs, etc.)

Lab coat

- Long sleeved, preferably with gathered cuff
- Do not wear lab coats in common areas
- Launder frequently and when contaminated

### Eye protection

- Safety Glasses are recommended as a minimum level of protection for all activities
- Splash goggles should be worn when working with large quantities of materials



## Hand Hygiene

Transmission of RG-2 organisms occurs through **mechanical transmission**, aerosols, and puncture wounds with contaminated sharps.

In our day to day life we are exposed to a number of organisms that could cause illness. One of the simplest measures we take to protect ourselves is hand washing. Likewise, in the BSL-2 lab, Hand washing is <u>extremely</u> important in preventing LAIs and minimizing the spread of infectious materials. As such:

### Hands must be washed:

- After glove removal
- Before leaving the work area
- Anytime the gloves may have become

contaminated with infection-risk material.

## How is it properly done?



Running water, soap Lather 20-30 seconds!

## Hand Hygiene (cont.)

Transmission of RG-2 organisms occurs through **mechanical transmission**, aerosols, and puncture wounds with contaminated sharps.

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Alternatively, in case of <u>limited access</u> to soap and water, waterless hand sanitizers may be used as a <u>temporary</u> means of reducing contamination until a source of running water and soap can be reached.



Note: Because of the variety of products and organisms, the Office of Biosafety cannot adequately validate waterless hand sanitizers for efficacy in all cases. Therefore, these products are **<u>NOT</u>** a replacement for hand washing with soap and water and should not be used as such.

### The Eyewash



At minimum eyewashes should be flushed and tested for function **monthly** or this is what the water coming out may look like.



These flush/checks should be documented on attached eyewash tags or on log sheets kept in proximity to the eyewash fixture.



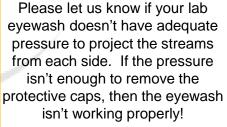
- A functional eyewash should project streams of clear, tepid water high enough to easily reach the eyes of the user.
- Handles should be clear of obstruction and located so that they are easily found



Eyewashes should be kept free of obstructions and easily accessible in case of emergency.



Remember: These devices are in place for your protection. Monthly flushes keep sediment and debris from accumulating in the lines which could potentially cause harm to your eyes in case of emergency use!!



## **Guard against Fomite Transmission**

Transmission of RG-2 organisms occurs through **mechanical transmission**, aerosols, and puncture wounds with contaminated sharps.

Besides direct contact with contaminated fluids, infectious agents may also be transmitted by **fomites**, or inanimate objects

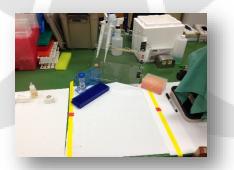
### Examples of fomites:

- Work surfaces
- Doorknobs
- Tools such as pipets
- Telephones
- Lab equipment
- Storage containers



### Ways to protect yourself:

- Set up a "contamination zone"
- Routine surface disinfection
- Wear gloves when handling tools or equipment (such as micropipettes)
- Disinfect storage containers before and after sample retrieval



### Ways to protect others:

- Hazard communication
- Routine surface decontamination
- Don't touch common surfaces (telephones, doorknobs, etc.) with gloved hands
- Remove PPE before leaving the lab space



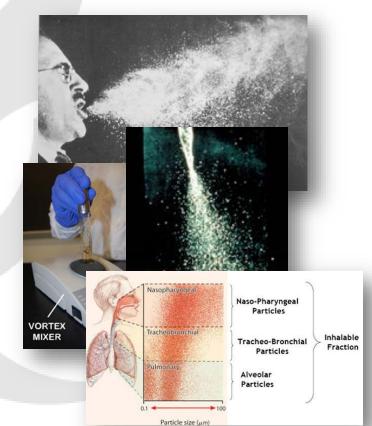
### **Reduction & Control of Bioaerosols**

Transmission of RG-2 organisms occurs through mechanical transmission, **aerosols**, and puncture wounds with contaminated sharps.

Aerosol droplets are formed with virtually any activity that disrupts the surface tension of a liquid.

As liquid particles bearing infectious agents may remain in the air for extended periods, it is important to consider methods of aerosol control. These can include:

- Confine work with viable biologics to a designated work space
- Use a **Biosafety Cabinet** (**BSC**) for procedures with a high likelihood of aerosol generation
- Minimize volumes of materials when feasible
- Use care when opening recently vortexed or centrifuged liquids, preferably do so under a BSC



Deposition regions of the respiratory tract for the various particle sizes. From Roy CJ, Milton DK. N Engl J Med. 2004;350:1710-1712.

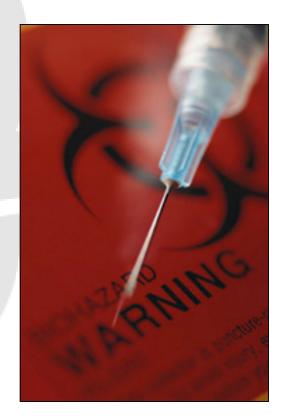
### **Biohazardous Sharps Management**

Transmission of RG-2 organisms occurs through mechanical transmission, aerosols, and **puncture wounds** with contaminated **sharps**.

In the lab, a **sharp**, refer to any object that is contaminated with a biologically hazardous agent and is sharp enough to puncture the skin.

While **needles** and **scalpels** could be considered the most apparent objects in this category, other items may also meet this definition. Some examples include:

- Broken glassware
- Serological pipets (especially if broken or damaged)
- Pasteur pipets
- Metal edging
- Unpolished glass



In the lab, handling and disposal of contaminated sharps requires special attention

## Eliminate, Reduce, Contain

Transmission of RG-2 organisms occurs through mechanical transmission, aerosols, and **puncture wounds** with contaminated **sharps**.

Considerations with handling sharps:

- Are there safer sharps options available?
  - If available, consider disposable sharps devices
  - Plastic ware instead of glassware
  - Devices such as needles, scalpels, etc. that have protections in place (i.e., safer sharps)
- Keep sharps tasks organized to limit exposure
  - This may include working with sharps in a given block of time to help personnel be mindful of tasks requiring sharps
- Ensure that both sharps supply and disposal methods are easily accessible, preferably within arms reach
- If using disposable items, promptly remove them to the waste bin after use
- If using reusable sharps, contain the sharp end "down and away"



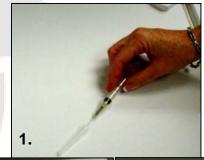
## Get the Point-DON'T RECAP

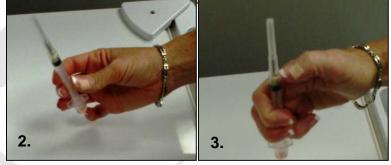
Transmission of RG-2 organisms occurs through mechanical transmission, aerosols, and **puncture wounds** with contaminated **sharps**.

Deep puncture wounds with **contaminated needles** present a high risk for accidental infection. Therefore, special considerations are given to their use in the research lab.

- Special precautions with needles:
- Avoid recapping needles. Dispose of in sharps container along with empty cap
- Always handle uncapped needles with one hand
- Avoid passing uncapped needle from one hand to the other
- Minimize motions that will put fingers or hands in front of needle
- If research activities create the need to re-cap needles, your lab should submit a needle re-cap exclusion form to the Office of Biosafety







In rare instances where re-capping is permissible, utilize the one-handed scoop method. First, place the cap on a flat surface. Second, using one hand, scoop the cap up with the needle. Third, using the same hand "click" the cap in place by pressing with your thumb at the base of the needle cap.

### **Decontamination & Disinfection**

Just as practices such as **hand washing** and the use of **PPE** are important for the protection of the individual, proper approaches to **disinfection** and **decontamination** are important for the protection of other staff and for the prevention of environmental release of organisms.

Removal of infectious agents by surface decontamination is a critical component of biological safety in the lab.

While the **immediate work space** is an obvious area requiring regular decontamination procedures, there remain various other critical items which might not be as apparent. These can include:

- Outer surfaces of storage and sample containers Interior
- Vortexes
- **Centrifuges** (both the housing and rotor)
- Outer surfaces of incubators

Additionally, surfaces should be disinfected:

- When retrieving sample containers or after removing items such as cell stocks from water baths
- Upon completion of procedures involving infectious agents
- When completing work in a biosafety cabinet
- Any time a spill occurs
- If common surfaces are touched with gloved hands

## Interior surfaces of the biosafety cabinet

 Any surface that you may have touched with gloved hands

## **Decontamination & Disinfection**

Just as practices such as **hand washing** and the use of **PPE** are important for the protection of the individual, proper approaches to **disinfection** and **decontamination** are important for the protection of other staff and for the prevention of environmental release of organisms.

When selecting a **disinfectant**, it is important to understand the compound that you are using. For example:

- Is the product appropriate for the organisms that are being targeted?
- Is it safe for the given surface?
- Are there personal hazards to consider that would warrant the use of PPE?
- Are there special instructions for the preparation of the compound?

Currently there are a wide variety of disinfectant options available. These products generally fall into the following categories:

- Chlorine compounds (Clorox)
- Iodophors (Providine-iodine solution)
- Ethyl and isopropyl alchohol
- Phenols (Clorox bleach-free, Vesphene)
- Quaternary Ammonium (Cavicide; Lysol)
- Gluteraldehyde (Cidex)
- Formaldehyde



Remember these disinfectants may have significant chemical hazards. Read labels and understand the compounds before you use them.

http://www.cdc.gov/hicpac/pdf/guidelines/Disinfection\_Nov\_2008.pdf



# **Decontamination & Disinfection**

Just as practices such as **hand washing** and the use of **PPE** are important for the protection of the individual, proper approaches to **disinfection** and **decontamination** are important for the protection of other staff and for the prevention of environmental release of organisms.

Of all products found in the lab, by far the two most common are **ethanol/water (70% vol/vol)** and **bleach (1:10 or 1:100)**.

#### Ethanol 70% (vol/vol) in water:

- Bactericidal, tuberculocidal, fungicidal and virucidal agent
- Activity is a result of protein denaturation
- Short contact time required (neutralization time for most organisms reported to be < 1 minute)</li>
- Cidal activity decreases rapidly when concentration is <50%</li>
- Not sporicidal
- May damage some surfaces such as plastics
- Flammable, keep away from ignition sources

#### Bleach (1:10 or 1:100) in water:

- Bactericidal, tuberculocidal, fungicidal, virucidal and sporicidal
- Strong oxidizer, although the actual mode of action is not well characterized
- Contact time of 10-15 minutes sufficient to destroy most bacteria and spores
- Light sensitive: exposure to light sources will break down sodium hypochlorite
- Low stability: prepare every 1-2 weeks
- Bleach is corrosive use caution when handling and when using on stainless steel.

Household bleach contains 52,500-61,500 ppm available chlorine (~5.25-6.15% hypochlorite). The effective cidal concentration ranges from 100-5,000 ppm depending on the organism.

http://www.cdc.gov/hicpac/pdf/guidelines/Disinfection\_Nov\_2008.pdf

### **Spill Prevention and Response**

Spills are a part of life in the laboratory and will occur. This means that when working with infectious or recombinant agents, **precautions** should be taken to **reduce the number of spills** and that a **spill response plan** should be in

place.

#### Spill prevention strategies:

- Use shatter resistant containers with tight fitting lids or stoppers
- If handling multiple tubes, employ a tube rack or other device to prevent drops
- Use a leak-proof secondary container such as a cooler or tray to contain leaks or spills (critical for movement outside the lab)
- Always use a cart when transporting large volumes of materials or when moving from floor to floor



# For spills involving potentially infectious materials:

- Notify others and leave the area to allow aerosols to settle. If sharps hazard is present, use appropriate precautions.
- Put on the appropriate PPE (typically, gloves and lab coats)
- Cover the spill with paper towels
- **Decontaminate** by spraying the area and towels with **disinfectant** (follow instructions for contact time and precautions
- Remove towels to Biohazardous waste bin
- Repeat disinfectant and clean-up steps once.

Note: The response outlined here refers to spills that are <500mL. For larger spills, please contact the Office of Biosafety.

# **Spill Prevention and Response (cont.)**

Transmission of RG-2 organisms occurs through mechanical transmission, aerosols, and **puncture wounds** with contaminated **sharps**.

Breaks involving contaminated glassware and other types of materials occur in the lab. As a part of lablife, it is important to know the appropriate response.

If the container was used to house, culture or manipulate infectious materials:

- Leave the general area to allow aerosols to settle, let others know the break/spill occurred
- Spray disinfectant on the broken materials
- Using a broom/dustpan or other mechanical device, remove the broken glass to a biohazardous sharps waste container
- Drop towels on any liquid and allow time for absorption to occur
- Using mechanical means, remove towels to biohazardous waste bin



Remember: Do not attempt to remove broken glass by hand, always employ a mechanical device (broom, tongs, broken cardboard)

# PRIMARY CONTAINMENT OF MATERIALS: THE BIOSAFETY CABINET

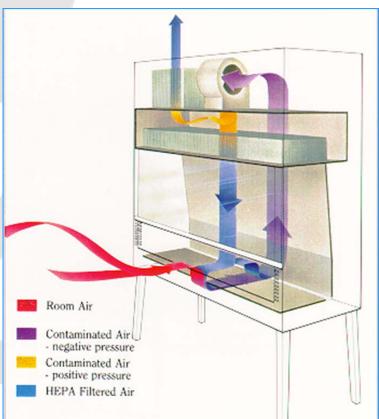
Section IV

Biological Safety Cabinets (BSCs) are designed to protect personnel from accidental exposure to contaminated **aerosol** droplets through the use of HEPA filters.

**BSCs** are sorted into three classes (**Class I, Class II, and Class III**) based upon their levels of protection for both personnel and product. The most common cabinets in use at our institute are the **Class II** A1 and **Class II** A2.

#### Class II BSC

- Provides both **personnel** and **product** protection from contamination
- Class II A1 cabinets use positive pressure to pass air through the system while Class II A2 cabinets use negative pressure
- Room air is drawn into ventilation system housed within the cabinet walls and passed through a High Efficiency Particulate Air (HEPA) filter which is composed of a cellulose/bososilicate glass media
- 70% of the air is circulated through a HEPA stack into the cabinet; while 30% is ejected back into the room through a separate filter stack
- The HEPA filter units are capable of removing 99.997% of particulates of 0.3 microns
- HEPA filters will not remove harmful vapors or gas



# Primary Barriers: Biological Safety Cabinet (BSC) Use and Maintenance

Biological Safety Cabinets (BSCs) are designed to protect personnel from accidental exposure to contaminated **aerosol** droplets through the use of HEPA filters.

Consider the use of a **BSC** when:

- Performing procedures that will likely generate aerosols. This can include activities such as vortexing or pipetting infectious or recombinant agents.
- Manipulating large volumes of potentially infectious material.
- Manipulating concentrated infectious materials.
- Performing necropsy of lab animals that have been exposed to infectious or recombinant agents.



When working with infectious agents inside a BSC, there are several things to be considered.

#### Air intake:

- Air intake at the face of the cabinet is ~100 FPM, so avoid:
  - Disturbances such as high foot traffic
  - Sweeping motions when reaching in or out of the cabinet
- Do not block the front or rear intake grilles
  - Do not place items such paper or absorbent bench cloth over the front grille area
  - Keep items 4" away from front, rear and sides of the cabinet

#### Reduce breaks in the air curtain

- Place items you intend to use inside cabinet
- Place a temporary waste container inside cabinet
- DO NOT PLACE HEAD OR BODY IN CABINET.



The laminar flow of air within the BSC prevents horizontal air mixing, but also produces airflow directed toward the user. Blockages in the front grill area create egress zones that can enable the release of aerosols into the room.

#### Prior to use:

- Turn on blowers and allow cabinet to run for 5-10 minutes to cycle air.
- Check to ensure UV light source is turned off
  - Several UV burns have been reported recently, so make sure the light switch is positioned to the illumination source, <u>not</u> UV, antimicrobial or antibacterial light
- Raise sash to the appropriate level and check grilles for obstructions
- Check pressure gauge for proper function
  - Gauges may be digital or magnehelic
  - These gauges indicate the amount of resistance across the HEPA filter,
  - Higher than normal reading indicates blockages in plumbing or filter DO NOT USE THE CABINET
  - Lower than normal readings indicate a breach in the filter DO NOT USE CABINET
- Check last certification date
  - Cabinets require annual re-certification to ensure proper function

Many BSCs use UV bulbs as a means of decontamination. Be aware that these bulbs may cause sever burns to exposed areas such as the skin and eyes. While newer models have safety interlocks in place to prevent operation of UV sources while the cabinet is in use, this is not always the case. Speak to senior lab personnel before you use the BSC to ensure you understand the equipment.







#### While in use:

- Remember, the airspeed velocity at the front of the cabinet is relatively low
  - Use deliberate motions when placing or removing hands and objects in the BSC
  - Avoid excessively disrupting the air curtain by keeping items and waste inside the cabinet to the extent that is reasonably allowable
  - Don't clutter the cabinet and keep front and rear air intake grilles clear of obstructions

#### NO OPEN FLAMES INSIDE CABINETS

- Turbulence created by flame sources can disrupt the airflow to the point of compromise
- Excessive heat build-up may damage HEPA units or mounting epoxy
- If a spill occurs, leave the cabinet running and follow spill procedures

#### After use:

- Clean-up all items and waste from cabinet
- Cover any items such as pipet tips
- Disinfect all exposed surfaces
  - Check with lab or facility personnel for preferred method of decontamination



Remember that bleach solutions are corrosive and will "pit" and rust stainless steel over time.

If bleach is used, a follow-up rinse with alcohol or mild detergent is recommended.

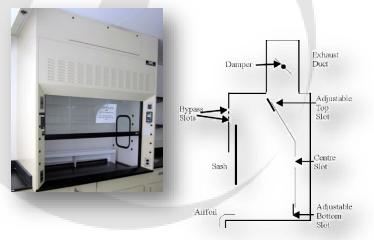
# **Similar Types of Equipment**

#### Laminar flow bench

- Also referred to as a "clean bench"
- Typically, these benches are used for mixing sterile solutions, prepping agarose plates, etc.
- Pulls room air using a blower mounted below a HEPA filter
- Filtered air is passed across bench space to produce a "clean zone"
- These cabinets provide product protection, but no personnel protection
- DO NOT USE IN CONJUNCTION WITH INFECTIOUS AGENTS

#### **Chemical fume hood**

- Chemical fume hoods use directional air flow to remove harmful vapors and gasses from the cabinet and are hard ducted to the building exhaust system.
- Cabinets are typically not equipped with HEPA filters and are not compatible with viable infectious agents
- Incoming air is not filtered, so product sterility is not likely feasible
- Chemical fume hoods are useful for applications such as formalin fixing of cells, as the chemical hazard greatly outweighs the biological. Use discretion.



# WASTE DISPOSAL AND DECONTAMINATION

Section V

# **Disposal of Biohazardous Waste**

In order to reduce the **environmental impact** and avoid the **accidental release** of **infectious or recombinant agents** while working under **BSL-2 containment**, it is required that all labs have a **waste disposal** plan in place.

For all **solid** <u>non-sharps</u> biohazardous waste including **microbiological** and **cell cultures**, **stocks**, **potentially infectious body fluids** and items contaminated with this material please use the following guidelines:

- Waste should be collected in a **labeled** biohazardous waste container
  - Lined with a bag, labeled with a biohazard symbol (BSL-2 only)
  - Have a lid that is closed when not in use and when container is in transport
  - Biohazard symbol should be easily visible on front and/or top of container
- Waste must be neutralized by autoclave prior to disposal
  - Autoclaves must be validated quarterly (see <u>biosafety.utk.edu</u> for details)
  - Leave bags loosely taped and add a small amount of water for steam exposure
  - Place bags in autoclave in secondary container for the appropriate cycle and time (instructions found onsite)
  - As the cycle completes, remove bags, allow to cool and place in white "Autoclaved Waste" bins



Reminder: No bags bearing visible biohazard markings are permitted in UT dumpsters.

### **Pipette Wastes**

- Some items used for manipulating infectious wastes offer unique challenges
- Occasionally these items require a creative approach for accumulation prior to disposal
- Serological pipettes and micropipette tips are good examples, as they may not fit some biohazardous waste bins or may present a sharps hazard if comingles with heavier wastes (e.g. agar plates).





Remember: The goal is to dispose of contaminated items properly and avoid creating a mess. Think about containing solids as well as liquids when accumulating waste.

# **Sharps Waste**

In order to reduce the **environmental impact** and avoid the **accidental release** of **infectious or recombinant agents** while working under **BSL-2 containment**, it is required that all labs have a **waste disposal** plan in place.

# Biologically contaminated sharps have a unique waste stream, so be sure you understand the difference in containers.

# **Biohazardous sharps waste** must be disposed of using the following guidelines:

- Container that are manufactured for the disposal of biohazardous sharps waste
  - Puncture resistant
  - Have a lid that is closed when not in use and when container is in transport
  - Labeled for the disposal of biohazardous sharps waste
- Permanently close container at "full line" when ¾ full or objects no longer freely fall into container
- Submit containers to EH&S during regular waste pick-up times on Knoxville or UTIA campuses
- All others should contact the Office of Biosafety for instructions



#### Remember: <u>NO</u> chemical or radiological hazards in the biohazard containers! If you have mixed waste, contact the appropriate safety officer for assistance!

### **Regulated Medical Waste Contractor**

Certain groups generate a special category of waste (including human derived materials and sharps) that is packaged and sent to a contractor for final disposal.

Regulated medical waste is accumulated in secured locations either outside UTCVM or on the third floor of the Jessie Harris Building.

Disposal of waste in these areas uses the following procedure:

- Locate waste accumulation area
- Securely close waste bags and sharps containers
- Line a vendor supplied container with a Biohazardous waste bag (found onsite)
- Place waste inside lined containers and close container or replace lid
- Lock storage area



# **Biohazardous Liquids and Other Wastes**

#### Liquid biological wastes

- May be collected in a flask or bottle
- Secondary containment should be used if vessel stored on floor or open bench
- Treatment options:
  - Autoclaved at 121C for 20-30 minutes, then allowed to cool.
  - Neutralize with 10% bleach (final vol:vol) for 30 minutes contact time.
- This may be followed by disposal in sink.
- Use care not to create splash; rinse sink thoroughly after discharge.

#### <u>Other</u>

- Some items used for manipulating infectious wastes offer unique challenges
- Occasionally these items require a creative approach for accumulation prior to disposal
- A good example of this is found in serological pipets, as they may not fit some biohazardous waste bins or may present a sharps hazard if stood vertically





Remember: The goal is to dispose of contaminated items properly and avoid creating a mess. Think about containing solids as well as liquids when accumulating waste.

# **Pathological Wastes**

### Animal tissues



- Collect in leak proof, sealed bags.
- Biohazard label required if source contains an infectious agent or recombinant DNA.
- Freeze and store tissues for disposal through your animal care group.
- **Do NOT discard tissues in the trash!** Please contact your animal care facility or the BSO for assistance.

# STORAGE AND TRANSPORTATION OF RESEARCH MATERIALS

Section VI

### Maintaining a secure lab environment

NIH Guidelines and the BMBL require recombinant and potentially infectious agents to be secured when not in use. Beyond this requirement, maintaining a secure lab environment protects both work related materials and private property.

Entry to any BSL-1, -2 or -3 facility is granted at the sole discretion of the laboratory principal investigator. Speak to them or the laboratory supervisor prior to allowing entry to visitors. Be aware of the following guidelines:

- **Contractors, service personnel, and visitors** should be made aware of risks and entry requirements prior to beginning work in the lab
- Children under the age of 16 are prohibited from entering BSL-2 laboratories at the University (excluding STEM or other sanctioned/reviewed circumstances).
- Be aware of your surroundings and do not allow strangers wander through the lab.
  - If you notice someone who seems "out of place", make other personnel aware and offer to direct them to their desired person or location.
  - Note: The above recommendation does not mean for you to put yourself at risk. If you are uncomfortable with the situation or are alone, please do not hesitate to seek help from others in the lab or surrounding labs.
- Lock the laboratory when not in use. Unlocked and empty facilities are easy targets for theft.
  - Please secure the lab if you will be out for more than 5-10 minutes.

**The principal investigator holds the final responsibility** for the activities that occur in their lab. Therefore, it is imperative that they be notified of any visitors prior to allowing entry.

### **Storage of Research Materials**

Recent events at the national and institutional levels have highlighted the importance of the proper storage of research related materials.

Strategies for the **short** and **long term storage** of **research materials** should consider **environmental requirements** to preserve the **viability** of samples, the **security of material**, and provide a means of **clear** and **simple identification of agents by others**.

- Maintain control of research materials and keep them out of reach of the general public by:
  - Storing biohazard materials (including wastes) in the lab.
  - Store stock cultures, tubes, or plates of infectious agents in **lockable devices** whenever possible.
  - Lock all storage devices that are maintained outside of the lab
  - Keep doors closed and locked when the lab is not in use and restrict access to authorized personnel

#### Properly house materials

- Make sure biological hazards are kept in primary tubes and secondary storage boxes that will prevent leaks in the event of containment failure
- Necropsy specimens must be sealed in primary containers and housed in leak-proof secondary containment vessels when possible
- Prevent surprise discoveries of old research materials
  - Recent events at the National Institutes of Health and the Centers for Disease control have demonstrated the need for investigators and students to keep track of materials.
  - Clearly label all prokaryotic and eukaryotic cell stocks, necropsy specimens and other research and teaching materials
  - Neutralize and dispose of old or un-needed research materials at the end of a project or during laboratory check-out procedures

# **Transportation of biological agents**

Transportation of biological agents out of the lab requires lab personnel to take precautions to ensure that lost samples and/or spills in public areas do not occur.

To help achieve this goal, please remember the following:

#### When transporting agents within a building

- Use primary containers with lids or tight fitting stoppers to prevent spills
- When transporting **multiple tubes**, place in a **rack** or other type of holder that will keep them from shifting or tipping during transport.
- Consider the use of a leak-resistant secondary container with a lid to contain any leaks or spills while moving through hallways.
- Use a cart to transport large volumes of materials or when moving from floor to floor
- Avoid contaminating common areas
  - Removal of one glove will allow for the manipulation of door knobs, elevator buttons, etc. while still allowing for the handling of infectious agents



# **Transportation of biological agents**

Transportation of biological agents out of the lab requires lab personnel to take precautions to ensure that lost samples and/or spills in public areas do not occur.

To help achieve this goal, please remember the following:

#### When moving materials outside of buildings

- Follow the guidelines outlined above
- Secondary containment is required.
  - Leak proof
  - Lidded
  - Labeled with biohazard symbol if transporting recombinant or infectious agents
  - Clearly label outer surface of secondary container with contact information and a brief description of contents
- Do not take RG-2 or recombinant agents on public transit
- Keep biohazardous agents secure
  - Place containers in storage areas of vehicles
  - Situate containers to prevent shifting during transit

# Remember: If you have a spill in a public area, contact the BSO for assistance with cleanup.



# **Transportation of biological agents**

Transportation of biological agents out of the lab requires lab personnel to take precautions to ensure that lost samples and/or spills in public areas do not occur.

To help achieve this goal, please remember the following:

#### Movement beyond the University

- Shipment of biological agents by courier (i.e., FedEX or UPS) is permissible, however certain packaging and labeling guidelines apply
- Non-regulated items include:
  - Low risk (RG1) or non-infectious organisms
  - Heat-killed or otherwise-inactivated samples
  - Patient samples from healthy individuals for routine testing
- Regulated items include:
  - Cultures of infectious agents
  - Patient specimens that likely harbor infectious agents
  - Genetically modified organisms (in some cases)
- Training is available from the Biosafety Office for labs that regularly ship materials

Please contact the Biosafety Office for assistance in determining the requirements that will apply

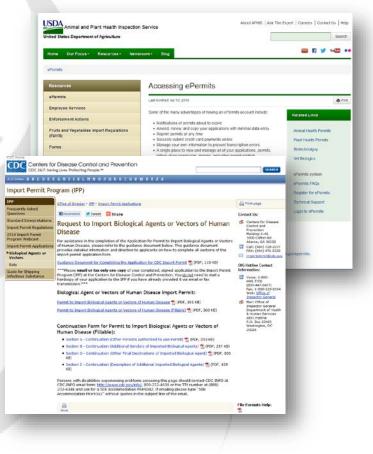






### **Regulatory Reminders: Biological Permits**

- Contact the Biosafety Office if you plan on any of the following permit-restricted activities:
- Import of potentially infectious agents affecting humans, animals, or plants into the U.S.
- Interstate movement of animal pathogens and plant pests, pathogens, & noxious weeds
- Export of potentially infectious agents affecting humans, animals, or plants from the U.S.
- Field release trials for plant pathogens or genetically modified plants and/or plant pathogens
- Import/export of biological materials obtained from wildlife



Biological materials permits may take several weeks to months to obtain. Do not wait until the project is imminent to begin the process of obtaining a permit. Federal agencies will not make exceptions for permits. Do not attempt to transport undeclared biological materials in your carry-on or checked luggage while flying. This may result in substantial fines and/or incarceration.

**BLOODBORNE PATHOGENS AWARENESS** 

Section VII

# The OSHA BBP Standard

The Occupational Safety & Health Administration's (OSHA) Occupational Exposure to Bloodborne Pathogens (BBP) Standard [29 CFR 1910.1030] applies to anyone that will handle or may come into contact with human blood or other potentially infectious materials (OPIM) due to the risk of bloodborne pathogens.

OSHA mandates initial and <u>annual</u> recurrent training for all affected personnel.



# **BBP Exposure Control Plan**

All employers with workers who have a reasonably anticipated risk for BBP exposure must provide an Exposure Control Plan:

- Workplace-specific document that outlines jobs/tasks with BBP risk, methods of exposure control, and employer & employee administrative responsibilities;
- KNOW where your lab's copy of the current Exposure Control Plan is!
  - Can also be found online at <u>http://biosafety.utk.edu/bbp-exposure-control-</u> plan/

# Bloodborne Pathogens (BBPs)

# Definitions:

What is a pathogen?

-Any infectious agent (viruses, bacteria, parasites, etc.) that causes illness and disease. So...

<u>Bloodborne pathogens (BBPs)</u> are disease-causing organisms found in human blood and certain body fluids.

# **Bloodborne Pathogens Examples**

Bloodborne Pathogens include:

- Human Immunodeficiency Virus (HIV)
- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- At least 20 other pathogenic organisms and/or viruses, for example:
  - Viruses: HTLV-1; various arboviruses
  - Parasites: Plasmodium falciparum (malaria)
  - Bacteria: Treponema pallidum (syphilis)

# **Recap of Major BBPs**

Feature	HIV	Hepatitis B	Hepatitis C
Major target organs	Immune system	Liver	Liver
Early symptoms	None to nonspecific "flu- like" symptoms	None to nonspecific "flu- like" symptoms; rarely acutely serious (jaundice, liver failure)	None to nonspecific "flu- like" symptoms; rarely acutely serious (jaundice, liver failure)
Persistence/ chronic disease?	Yes, leads to AIDS	Rarely (~5-15% of cases); long-term cases may lead to liver failure/cancer	Commonly (>85% of cases); leads to liver failure/cancer
Effective vaccine available?	No	YES (see slides below)	No

# BBPs on the Job

Occupations that bear a risk of exposure to BBPs include:

- Healthcare workers
- Emergency responders performing first aid and/or CPR:
  - EMTs, paramedics, life guards
- Custodial personnel
- Researchers/lab personnel



Workers at risk of exposure to BBPs fall under specific OSHA regulations in order to reduce occupational risk!

# **BBP-Infectious Sources**

# BBPs may be present in infectious concentrations in human:

- Blood (highest risk)
- Semen/vaginal secretions
- Fluid from the spine, joints and lungs
- Any body fluid contaminated with blood
- Any body fluid that you can't identify
- ...and OPIMs (other potentially infectious materials)...

# Other Potentially Infectious Materials (OPIM)

### **OPIMs in clinical or lab settings:**

- Human blood products (serum, plasma, albumin, various factors, etc.)
- Unfixed tissue/organ, other than intact skin of human origin
- Cell or tissue cultures that may contain BBPs
  - This includes primary <u>and</u> immortalized or established cell lines regardless of origin/vendor (unless otherwise certified to be free of <u>all</u> BBPs)
- Organ cultures, culture medium or other solutions that may contain BBPs
- Experimental animals infected with BBPs

Other Human Body Fluids The following excreta/secreta are <u>not</u> considered to be a BBP hazard (<u>unless</u> they are visibly contaminated with blood):

- Urine/ Feces
- Vomit



 Sweat/ Tears/ Spit/ Nasal secretions

Even so, use universal precautions and wear appropriate personal protective equipment (PPE) when handling these fluids as they may contain other types of pathogenic microbes!

# Transmission of BBPs Transmission of BBPs can occur through:

- Cuts/punctures with BBPcontaminated sharp objects;
  - needle stick injury



- cut with a scalpel with blood on it
- Contact with blood or OPIM through breaks in skin;
  - blood/OPIM comes in contact with a fresh wound on your arm
  - blood/OPIM comes in contact with chapped skin, acne, eczema, split cuticles, etc.



# Transmission of BBPs (cont.)

- Splashes of blood or OPIM to mucous membranes such as the eyes, nose or mouth;
- BBPs can also be transmitted through sexual intercourse and from mother to unborn child.

## BBP Occupational Exposure: Transmission Risk

Types of exposure incidents from highest to lowest risk are:

- Puncture wound from a contaminated needle
- Cut by contaminated sharp objects
- Contact of blood/OPIM with breaks in the skin
- Contact of blood/OPIM with mucous membranes



**HIGH** 

## How Do I Protect Myself from BBPs?

#### **Practice Universal Precautions!**

- Treat all blood and OPIM as if they were KNOWN to be infectious!
- Communicate the hazard;
- Wear personal protective equipment (PPE) suitable for the task;
- Wash your hands;
- Properly dispose of contaminated materials;
- Clean up and decontaminate all areas and items exposed to blood/OPIMs; and
- Be careful with sharp devices



## Needle Stick Safety & Prevention Act

- This Act is incorporated into the OSHA BBP Standard. It is intended to minimize sharps-related injuries. The Act specifies that:
- Employers must provide safer sharps devices (detailed on next slide) if available;
- Employees who work with sharps and blood must be involved in device evaluation & selection process;
- Initial and annual sharps evaluations must be completed by anyone using sharps on LIVE HUMANS (e.g. phlebotomy, finger sticks). Evaluation forms can be found at <u>http://biosafety.utk.edu</u>.
- A sharps injury log is maintained.

## Safer Sharps Device Features



Safer sharps device features include:

- A barrier between hands and sharp;
  - This barrier is an integral part of the device
- Protection that is in place before and after disposal;
- It is simple to operate;
- It is safe & effective.
- Safer sharps resources can be found at <u>http://biosafety.utk.edu</u>.

## UT Employees with BBP Risk & Hepatitis B Vaccination

OSHA requires employers to offer Hepatitis B vaccine to all at-risk employees as follows:

- Those with risk offered vaccine at no cost.
- Vaccine offered at convenient time and location.
- Offer must be documented (waiver/request).
- If an employee declines offer, they can request vaccination at later date if still at-risk.
- For further information on the hepatitis B vaccine offer, please contact the Biosafety Office or Dr. Amy Knowles, Occupational Health, at 974-5728.

## HBV Vaccination-What's Involved?

- Series of 3 shots over ~6 months
- Titer check about 6 weeks after last shot to confirm antibody response
- A booster is generally not recommended.

## About the Vaccine...

#### **Benefits**

- > 90% of patients who complete series acquire immunity
- Safe

#### **Minor Risks**

- Pain, redness at injection site
- Fatigue, fever, nausea possible
- Possible reaction for individuals with yeast allergies

# EMERGENCY RESPONSE & NOTIFICATION PROCEDURES

Section VIII

#### **Exposure Response**

## If potentially infectious research materials enter your body through a:

- Cut or puncture with a contaminated sharp
- Broken skin
- Splash to the eyes, nose or mouth
- Suspected inhalation (per risk assessment)
- You must:
  - Flush the exposed area (15 minutes)
  - Immediately report to your supervisor
  - Seek medical attention as soon as possible



#### **Medical Follow-up**

- For all other biological material exposures, paid staff are to contact CorVel Corp. at 1-866-245-8588 to obtain a health consultation, claim number, and further instructions, per UT Risk Management procedures. Unpaid students may report to UT Student Health Services (865-974-3135).
- Paid staff must complete the *Report of On-the-Job Injury or Illness* as soon as possible. Unpaid students/volunteers must complete the *UT Report of an Occurrence* form as soon as possible. Forms are to be remitted to the Risk Management Office. For additional information, see <a href="http://riskmanagement.tennessee.edu">http://riskmanagement.tennessee.edu</a> or contact (865) 974-5409.
- For exposure incidents involving human-derived materials (i.e. human blood, tissue, body fluids, cells), report *immediately* to UT Medical Center Emergency Room. Identify yourself to ER staff as a UT employee or student who has had a bloodborne pathogens exposure. Paid staff should report exposure to CorVel Corp. at 1-866-245-8588 to obtain a claim number per UT Risk Management procedures (this step can be concurrent with emergency reporting).

### **BBP Post-Exposure Medical Follow-Up**

#### Why is it so important to seek prompt medial care?

- Allows accurate evaluation of exposure risk by a medical professional;
- Increases the chance of identifying and testing source of blood/OPIM;
- Gives lead time to administer treatments that can reduce chance of infection (if high-risk event):
  - Antiretroviral therapies for HIV-these are most effective if started within 2-4 hours of exposure;
  - Hepatitis B immunoglobulin or vaccine booster.

### **BBP Post-Exposure Medical Follow-Up**

#### What's Involved?

- Risk assessment of exposure
- HBV vaccination or Hepatitis B treatment
- Baseline testing
- Attempt to ID & test source
- Drug therapy for high-risk HIV exposure
- Ongoing counseling

Note: Contact Occupational Health Nurse, Amy Knowles, within 3 days of event to continue follow-up! (865) 974-5728 or <u>aknowles@utk.edu</u>

#### **Reportable Incidents**

To ensure proper agency reporting and other remedial actions, notify your supervisor and the Biosafety Office in the event of:

- Accidental exposures to Risk Group-2 (or higher) and/or recombinant organisms
- Spills in public areas involving Risk Group-2 and/or recombinant organisms
- Biohazardous materials appear to be tampered with or missing.

## **Questions?**

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