

Slide 1-Title Page

Welcome to the training course for researchers required to work with biohazardous materials at Biosafety Level 2. As a requirement of the University of Tennessee Health Science Center Institutional Biosafety Committee and the NIH Guidelines all researchers working with recombinant DNA, transgenic organisms, or material that may be infectious to humans must complete this training before working on an IBC approved protocol or handling biohazardous materials at BSL2.

As a prerequisite to completing this course all UTHSC researchers are required to complete the course “Laboratory Safety and Chemical Hygiene.” That course introduces the concept of biohazards and describes the work practices and containment requirements necessary for work at Biosafety Level 1. Each successive biosafety level builds incrementally on the previous level, applying progressively more protective precautions. If you have not completed Laboratory Safety and Chemical Hygiene and acquainted yourself with the requirements for work at Biosafety Level 1 you should postpone the completion of this program until you have completed the basic lab safety course.

Slide 2 Purpose and Objectives

Biohazardous materials known to be associated with moderate risk to human health often fall into Risk Group 2 and are frequently handled using biosafety level 2 work practices and containment precautions.

The purpose of this program is to promote the safe handling of biohazardous materials at biosafety level 2. The completion of this training is a requirement for work on protocols approved by the Institutional Biosafety Committee and is necessary to comply with the requirements of the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). This training must be refreshed every three years and is intended to coincide with the renewal of IBC protocols.

Upon completing this training program, you should be able to:

Obtain hazard information and the exposure response procedure for the BSL2 agents that you handle.

Identify containment requirements necessary for work at BSL2.

Perform work practices required for work at BSL2.

Describe the role of the Institutional Biosafety Committee (IBC).

UTHSC BSL2 Training
Standard Precautions and Containment

This presentation is part one of the two-part training requirement. It focuses on standard precautions and containment practices for work at biosafety level 2.

Slide 3- Hazard Specific Checklist

The second part of the training requirement for work with BSL2 agents consists of a hazard-specific checklist. This must be completed with your supervisor or senior laboratory personnel familiar with the biohazardous agents or, in the case of recombinant or synthetic nucleic acids, the genetic sequence, the you will be handling.

Due to the great variety of biohazardous agents handled at BSL2 and the array of genetic materials that may present a risk to researchers that handle them it is outside of the scope of the Office of Research Safety's ability to provide information for every possible agent or genetic sequence in a single computer-based presentation. Instead it is the responsibility of the Principal Investigator or a knowledgeable lab supervisor to provide the necessary information.

The checklist is intended to guide your supervisor to provide you with the information necessary to handle these materials. Ensure the communication of hazards, the necessary precautions and response actions. Provide researchers with the opportunity to ask questions and Document that training has been provided.

Slide 4- BMBL

At this point the international biohazard symbol should be readily recognizable. This symbol is often found on red bag waste, sharps containers, or labeling equipment that may have come in contact with human blood or other potentially infectious material. Be sure to take note of this symbol whenever you see it and ensure that all of the necessary equipment in your lab is properly labeled to identify this hazard.

The Biosafety in Microbiological and Biomedical Laboratories guidelines, or BMBL, is the national guideline for work with biohazardous agents. This document is prepared by the Center for Disease Control and is accessible on the internet. All organizations that receive NIH funding must comply with the BMBL. This document is accessible online and is an excellent resource for researchers that handle biohazardous materials of all risk groups and at all biosafety levels. The discussion of BSL2 work practices and containment precautions covered in this presentation is based on information contained in the BMBL.

Slide 5-Risk Group Comparison

A pathogen is a microorganism, nucleic acid, or protein that is capable of causing disease in humans or animals. Using a pathogen risk assessment process, pathogens are assigned to a risk group (RG) category based on the risk to the individual/animal and the risk to public health or animal populations. These categories range from risk group 1 to 4. You may recall the discussion of Risk Groups included in the Lab Safety and Chemical Hygiene training. Many of the agents handled in BSL2 laboratories, including bloodborne pathogens, fungi, many bacterial and viral vectors are risk group 2 organisms. Depending on how you are handling or manipulating biohazardous materials the BIOSAFETY LEVEL that you will work at depends not only on the risk group of the biological agent you are handling, but how you plan to manipulate that agent. The manipulation of biohazardous material may affect the potential for hazard or risk of exposure and may warrant additional precautions.

Slide 6- Biosafety Containment Levels

Biosafety is a combination of containment principles, technologies and operational practices that, together, help prevent exposure to, or release of, infectious material or toxins that can cause harm to humans or animals. BSL2 is appropriate for work with biohazardous materials that are associated with moderate human disease. BSL2 is also appropriate to provide containment for materials that must be prevented from entering the environment.

Materials that must be handled at BSL2 include:

- any human cells, tissues, blood or other potentially infectious material including all human body fluids except sweat
- Lentivirus
- CRISPR Cas9 modified with certain genetic sequences
- Genetic materials modified for selective antibiotic resistance
- Or other genetically manipulated biohazardous materials

This training focuses on containment practices and work procedures appropriate for work at Biosafety Level 2.

Slide 7- BSL2 Facility Requirements

UTHSC BSL2 Training
Standard Precautions and Containment

BSL2 facilities include a wide variety of laboratories and animal work areas, including diagnostic and health-care laboratories (public health labs, clinical or hospital-based) and many biological research laboratories in universities.

BSL2 is appropriate for biohazardous materials of moderate human risk whose primary routes of exposure include **ingestion, inoculation, and contact with mucous membranes**. These organisms are not generally transmitted by the airborne route, but you **MUST** take care to avoid splashes and the generation of aerosols.

Biosafety requirements build incrementally as risk increases. Requirements for BSL2 facilities include all of the requirements necessary for work with biohazardous agents at BSL1, this includes chemically resistant, non-porous work surfaces, no cloth furniture and no consumption or storage of food or drink for human consumption.

Not all laboratory facilities on campus are suitable for work at BSL2. Due to the increased risk associated with biohazardous agents that must be handled at BSL2 facility requirements for BSL2 labs have been established to emphasize **CONTAINMENT**.

Slide 8- BSL2 Facility Requirements Continued...

Consider for a moment your basic laboratory.

The objective of laboratory design is to facilitate containment. To do this you must consider what are the pathways through which materials, including biohazardous agents, can escape the lab?

First, Materials can move with you – they can be intentionally transported, transported as contamination on your clothing or PPE, or – in the unfortunate event that you have been inoculated with or exposed to an agent - they can move inside of you. Next, it is possible that biohazardous agents can exit the laboratory through plumbing. Biohazardous liquids can flow down drains or even be drawn into the building's vacuum line. Finally, if aerosols are created through activities like pouring, pipetting, injecting or centrifuging biohazardous materials may also be able to move with air currents within the building. Each of these possible pathways must be addressed to ensure proper containment of biohazardous materials.

Slide 9- Restricted Access

Lab staff must be able to restrict access to BSL2 facilities.

It is important that doors to BSL2 work areas remain closed at all times. Keeping doors closed may prevent unauthorized individuals from entering.

The building's directional airflow should be directed into BSL2 labs. Keeping doors closed will help maintain a pressure differential and direct the flow of air into the lab. In the event of a spill or accidental production of hazardous aerosols this will help contain the release. Allowing the

door to remain open compromises this directional airflow and may put individuals at risk of exposure to biohazardous aerosols.

Be aware of the risk of contamination within the room. Due to the handling of materials associated with human disease it is particularly important to ensure that doorknobs are not handled while wearing gloves. Either transport hazardous materials to and from the BSL2 lab using a clean secondary container or use the one glove method by using one gloved hand to carry materials and keeping on glove-free hand to operate the door knob.

Slide 10- Warning Sign

A warning sign must be posted at the entrance to BSL2 work areas. This sign is intended to alert entrants to the potential hazards that may be encountered within the room.

The sign must include the following:

- The biohazard symbols
- The identity of the hazardous agent(s) being handled.
- The necessary entry precautions that must be taken – such as the use of PPE
- The availability of and possible requirement for vaccinations prior to entry
- And Contact information for that work area

The UTHSC BSL2 lab door sign is accessible through the Safety Information page of the Research Safety website. The sign can be printed or downloaded.

Slide 11- Building Vacuum Line Filters

Building vacuum lines in BSL2 facilities must be equipped with filters to protect building facilities from contamination.

This is necessary to ensure that infectious materials are not drawn into building systems where they could possibly infect workers performing service or maintenance activities on building systems.

Filters must have a 1 micron or smaller pore size

Filters are available from various vendors or for purchase at General Stores in the GEB.

Slide 12- No Plants or Animals

When animals are housed in a space where the room itself provides the primary containment, the requirements for these types of zones are referred to as ABSL2.

UTHSC BSL2 Training
Standard Precautions and Containment

Animals not associated with the research being performed are not permitted in BSL2 labs. Similarly, plants that are not associated with research being conducted are not permitted in BSL2 labs.

Slide 13- Equipment

Equipment used with BSL2 agents must be labeled with the biohazard symbol to identify the potential risk associated with exposure to contaminated surfaces or materials within the equipment. Refrigerators, freezers, biosafety cabinets, centrifuges and any other equipment used to **handle** BSL2 agents must be labeled. Biohazard symbol stickers are the most common way to do this.

Centrifuges used to handle materials at BSL2 must receive special attention. Aerosols can be released during centrifugation even when using sealed tubes. Micro-fractures in tubes can turn into cracks during centrifugation resulting in a loss of containment. Exposure to personnel and contamination of equipment can occur from the resulting spray, splatter or aerosols. This is of greatest concern when centrifugation of substances containing microbes, toxins or human material which may be infected with blood borne pathogens. To address this risk only use centrifuges equipped with safety cups or sealed rotors with BSL2 materials. If such equipment is unavailable and may be possible to place the centrifuge inside of a biosafety cabinet to limit the risk associated with aerosol production. If BSL2 material spills or something breaks during centrifugation turn the unit off. Keep the centrifuge closed for AT LEAST 30 minutes to allow any aerosols to settle. The proceed by treating the material with 10% bleach or another suitable disinfectant. Allow at least 30 minutes of contact time before proceeding to clean the spill. While cleaning required PPE includes at least gloves, a lab coat and eye protection.

Slide 14- Biosafety Cabinet for Aerosols

If the work being performed presents a risk of aerosol generation the facility must have the ability to contain these potentially inhalable biohazardous agents. One means of containing potentially infectious aerosols includes performing work inside of a biological safety cabinet.

Examples of activities that may produce aerosols includes:

- Pouring of liquid cultures or supernatant fluids
- Filling syringes and injecting animals
- Mixing a fluid culture with a pipette
- Vortexing or using high speed mixing devices
- Dropping a tube or flask of liquid culture and
- Breaking tubes during centrifugation

UTHSC BSL2 Training
Standard Precautions and Containment

These procedures should be performed in a biosafety cabinet when they involve liquids known to present a risk of human infection.

When using a biosafety cabinet be sure to disinfect work surfaces before and after use.

There are several types of biosafety cabinets. The Class A2 cabinet shown in this picture provides protection to the worker, the environment and products being handled on the work surface from contamination with particulates or aerosols. This cabinet relies on high efficiency air filters to remove contaminants from the air stream. Biosafety cabinets must be certified annually to ensure that it properly contains contaminants while providing a contaminant free work surface. Additional information about the proper use of biosafety cabinets is provided later in this course.

Slide 15- BSL2 Standard Practices

BSL2 standard practices build upon BSL1 practices to provide additional precautions for the handling materials known to be associated with human illness. This section will provide a brief review of standard work practices for BSL1 and a discussion of additional practices to be employed when working at BSL2.

Slide 16- Standard Practices

Standard practices for work at BSL1 were covered in the Laboratory Safety and Chemical Hygiene course. If you do not recall these practices it would be appropriate to review that module rather than relying on the cursory description provided here.

Briefly though, Standard BSL1 practices that must also be adhered to when working at BSL2 include the following:

- Using the proper technique for doffing gloves. Gloves are intended to be single use disposable. Once a pair of gloves come out of the box, they should either be on your hands or in the garbage. Do not leave contaminated gloves lying around your work area and do not reuse gloves after you have worn them – even if you think that they are probably clean.
- Wash your hands after removing gloves or handling materials in BSL2 work areas. Proper hand hygiene is the single most effective way to prevent the spread of disease.
- No eating drinking smoking, applying contact lenses or makeup in lab areas.
- Employ work practices that minimize the potential for aerosol creation.
- Decontaminate work surfaces at least once a day when materials are handled in that location. This means changing absorbent paper at least daily if it is used.

UTHSC BSL2 Training
Standard Precautions and Containment

- Decontaminate cultures, stocks and other infectious liquids prior to disposal. Decontamination consists of treating with a 10% solution of bleach for at least 30 minutes prior to disposing of liquids down the drain and flushing with an ample amount of water. Liquids must not be disposed of in red bags.
- Ensure that you have received the necessary immunizations for the materials that you will be handling. If this includes human cells, tissues, blood or other potentially infectious materials this includes immunization for hepatitis B.

Slide 17-BSL2 Standard Practices

A significant requirement for researchers performing work at BSL2 is the degree of training and supervision that they must be provided. This is detailed at length in the BMBL guidelines. All researchers working in BSL2 work areas must complete training the required training. You must also be trained by your principal investigator or other experienced lab personnel how to safely perform the work that you are conducting.

All employees conducting research at BSL2 or working with recombinant DNA must be listed on the institutional biosafety committee, or IBC, protocol for the work that they are performing. As new researchers join the lab the IBC protocol must be amended to include them. Researchers performing BSL2 work and listed on IBC protocols must review the protocols on which they are listed. All IBC protocols include a detailed risk assessment describing the health and safety risks associated with the work. The IBC protocol also describes the safety precautions that must be applied. It does this by simply referencing BSL1 or BSL2 for lab work and ABSL1 or ABSL2 for animal work. It does not often elaborate on specific safety requirements. This means each worker must be familiar with the full set of work practices and containment measures that are required at each biosafety level.

Finally, work must be supervised by competent scientists. **Direct** supervision is not required at all times. But researchers must have received adequate training prior to performing independent work under the general supervision of a more experienced investigator. The BSL2 hazard specific checklist that must be completed as part of this training ensures that researchers are provided with the minimum amount of health and safety information necessary. Supervisors should also provide detailed information about how to perform the scientific procedures necessary for their work. Ideally the health and safety information will be imbedded within this important operational information.

Slide 18- BSL2 Standard Practices Continued...

When working at BSL2 work surfaces must be decontaminated after completion of work. If absorbent pads have been placed in the work area they must be picked up and discarded upon

UTHSC BSL2 Training
Standard Precautions and Containment

the completion of work. As is the case with gloves, once an absorbent pad is used it is considered contaminated and must be discarded as potentially infectious waste. It is not up to the discretion of the researcher whether or not the pad was contaminated in the course of this work.

Like surfaces equipment must be decontaminated after use. Similarly, equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

Potentially infectious material must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility. Remember to remove gloves before exiting the work area and either using a clean transport container or the one glove method for transporting materials. Door knobs, elevator buttons, etc. must not be touched or handled with gloved hands!

When generating potentially infectious liquids these must be chemically treated prior to drain disposal. Liquids can be consolidated into a larger volume container or treated in their current container provided it is constructed of a material of suitable chemical resistance. Use an appropriate chemical decontaminant as instructed by your supervisor – usually a 10% bleach solution or Spok Klenz – and be sure to allow sufficient contact time prior to disposal. The typical contact time for inactivation with 10% bleach is at least 30 minutes.

Any potentially contaminated solid materials must be discarded in red or orange bags as infectious waste. At this time, it is not typical at UTHSC to autoclave waste prior to disposal **unless you are working inside of the RBL**. All red bag waste generated on-site is handled with the same precautions as if it was regulated medical waste. Your Principal Investigator or supervisor may instruct you otherwise with respect to the practices of autoclaving waste. However, before doing so they should speak with the biosafety officer to confirm appropriate practices and autoclave validation.

Do not use ethanol to disinfect work surfaces. This is specifically prohibited by the UTHSC IBC and expressed on the all IBC protocols. While ethanol does have the ability to inactivate certain microorganisms it is not effective in the presence of a high concentration of organic matter such as may be found on the surfaces of benchtops and BSCs. It may also evaporate before an adequate contact time has elapsed. A 10% bleach solution or SporKlenz are the recommended disinfectants. Any questions or concerns about identifying a suitable surface disinfectant should be directed to the Institutional Biosafety Officer.

Slide 19- BSL2 Standard Practices Continued...

As with any work at BSL1 safely working at BSL2 requires a conscientious observation of sharps handling practices. Needle sticks were the most common research related injury at UTHSC last year.

Do not use bent, sheared, or broken needles.

Do not recap needles.

Used needles discarded in puncture resistant, FDA approved sharps container.

No direct handling of broken glass.

UTHSC BSL2 Training
Standard Precautions and Containment

Secure sharps including razor blades, scalpels and similar materials when not in use.
Full/closed sharp containers MUST be disposed as regulated medical waste (Stericycle box).

Slide 20- BSL2 Standard Practices Continued...

Any handling of material at BSL2 that may generate an aerosol must be performed in a biosafety cabinet or other physical containment such as a fume hood as directed by your supervisor. Many common laboratory procedures have the potential to generate aerosols or micro-aerosols even when no spray is visible. Such procedures include:

- Pipetting,
- Centrifuging,
- Sonicating
- Mixing
- Shaking
- Inoculating animals intranasally
- Loading or using syringes
- Opening containers of infectious materials

The potential for generating aerosols during these procedures also necessitates the disinfection of all surfaces upon the completion of work.

Slide 21-Using a Biosafety Cabinet

Proper containment of hazardous materials within a BSC is dependent on proper use of the equipment by the researcher. The biosafety cabinet relies on air filters and directional airflow to contain and remove aerosols or particulate contaminants from the work surface. First the air is drawn into the cabinet and beneath the work surface so unfiltered air does not reach the area where materials are being handled. It then passes through a high efficiency particulate air filter before ventilating the work surface in a laminar airflow pattern. This should provide a contaminant free work surface where cells can be cultured, and other materials handled without the fear of contamination. A second HEPA filter removes contaminants from the exhaust air stream. The cabinet relies on filters that only remove particulates. Hazardous gases or vapors are not removed from the air. Unless you are working in a ducted BSC these devices are not appropriate for the handling of chemicals that produce vapors.

Slide 22- Using a Biosafety Cabinet, Continued...

UTHSC BSL2 Training
Standard Precautions and Containment

Biosafety cabinets must be certified annually to ensure that filters are working properly, and the airflow pattern is appropriate. Only in a certified biosafety cabinet do you have a measure of assurance that biohazardous materials are properly contained and that the materials you are handling are not likely to be exposed to contamination within the biosafety cabinet. When signing IBC protocols Principal Investigators attest to the fact that they will conduct operations within a CERTIFIED biosafety cabinet. If the BSC in your work area was last certified more than one year ago, please notify your supervisor and email BSC@uthsc.edu to schedule a re-certification.

After checking to ensure that a BSC has been certified you may turn the unit on. You must then wait for at least 5 minutes with the biosafety cabinet running before beginning to load in the materials that you plan to use. This will give the unit time to purge and filter any unfiltered room air that may have been present when the BSC was turned off. While waiting you can decontaminate the sides, sash and work surface of the BSC while wiping the unit down with a suitable chemical decontaminant like SporKlenz or 10% bleach. Be aware that bleach may tarnish the stainless-steel finish of the cabinet. After wiping with a bleach solution, it is recommended that the cabinet surfaces also be wiped with a solution of sodium thiosulfate to neutralize the oxidizing power of the residual bleach.

Slide 23- Using a Biosafety Cabinet, Continued...

To ensure that the biosafety cabinet is functioning properly do not block the front grill. Do not place papers, clipboards or other materials over the grate. This may create a channel through which contaminants may enter the BSC. Handle all materials at least 4 inches (10 cm) from the front grill. Blocking the back grill may disrupt the airflow pattern within the cabinet. Be careful not to store materials or equipment along the back grill.

You must enter and exit the biosafety cabinet straight on using smooth hand and arm movements to avoid fanning contaminants either into or out of the cabinet.

Always wear personal protective equipment when working in a biosafety cabinet. This includes at least gloves and a lab coat. Even if you are handling non-hazardous materials inside of the biosafety cabinet a lab coat should still be worn. To do otherwise it to potentially contaminate the inside of the cabinet with cells and other material that may be shed from your arms and clothing.

Eye protection is also required if handling hazardous materials inside of the biosafety cabinet. The sash is not a guarantee that your eyes will be protected from splashes or splatters.

More detailed training on how to properly use a BSC is available through the Office of Research Safety Affairs. That BSC training must be completed by any researcher working in a LACU facility. Check the Office of Research Safety Affairs training website to find out where this training is available.

Slide 24- BSL2 Personal Protective Equipment

This section covers requirements for personal protective equipment to be worn by individuals working at BSL2.

The CDC Guidelines for Biosafety in Microbiological and Biomedical Laboratories specifies the personal protective equipment that individuals handling BSL2 materials are required to wear. Although the type of personal protective equipment may vary somewhat depending on specific procedures the use of PPE is a requirement and must be enforced by your supervisor.

Slide 25- Personal Protective Equipment, Continued...

Lab coats or an equivalent such as disposable gown must be worn at all times when working with materials at BSL2. Your employer is required to provide you with this important PPE. The purpose of wearing a lab coat is to provide a barrier between the researcher and the biohazardous materials that they are handling.

To provide the proper protection Lab coats must be buttoned up. Spills, splashes or aerosolized material may settle onto lab coats – or onto clothing if a lab coat is not worn.

Remove your lab coat before leaving the work area. After use lab coats should be assumed to be contaminated and should be handled accordingly. Lab coats worn when handling BSL2 material must be autoclaved prior to being washed. You must not take lab coats home with you for laundering!

If using a disposable lab coat dispose of it properly as potentially infectious waste.

Lab coat sleeves should be tucked into the top of your gloves to cover any exposed skin. This is especially true when working inside of a biosafety cabinet.

It should be noted that it is appropriate to wear a lab coat whenever you are working at a biosafety cabinet. Even when you are not actively using the biosafety cabinet for the handling of BSL2 agents the use of a clean lab coat will help prevent you from shedding potential contaminants off of your street clothes into the clean, controlled environment within the BSC.

Slide 26- Personal Protective Equipment, Continued...

Gloves must be worn while handling materials at BSL2. Gloves should be changed if they become damaged or visibly contaminated. You may choose to wear a double layer of gloves so that the outer layer can easily be changed while retaining a protective inner glove that is clean. Gloves that have come in contact with potentially infectious materials must be treated as soiled and discarded in red biohazard bags as infectious waste. Remove gloves using the proper

UTHSC BSL2 Training
Standard Precautions and Containment

doffing technique that was covered in the initial Lab Safety and Chemical Hygiene class. Using this technique will ensure that you do not come in contact with the potentially contaminated outer surface of the gloves.

Do not re-use gloves. Once worn they must be contaminated and discarded directly into the trash or a red biohazard bag – depending on the nature of materials handled while wearing the gloves. Used gloves should not be laid out on benchtops, fume hoods or in other locations. Be sure to remove and discard gloved before leaving the lab.

Slide 27- Personal Protective Equipment, Continued...

The UTHSC Chemical Hygiene Plan requires that eye protection be worn whenever handling hazardous chemicals. The handling of Trizol, formaldehyde, bleach solutions and other disinfectants are all considered hazardous and must be accompanied with the use of eye protection. Prescription eye glasses do not qualify as suitable eye protection unless specifically designed for that purpose and they meet the requirements established by the American National Standards Institute, or ANSI.

When handling biohazardous materials eye protection must be worn when performing activities that can reasonably be anticipated to result in a splash or spray of infectious material. A face shield should be worn whenever handling volumes of infectious liquid that are greater than 4 liters.

After use Eye and face protection must be either decontaminated or disposed of with other contaminated laboratory waste.

Slide 28- Waste Disposal

Biohazardous waste disposal

Infectious waste generated in the course of work at BSL2 is managed identically to contaminate materials handled at BSL1 in all areas except for the RBL. Any potentially contaminated materials must be packaged properly in red or orange biohazard bags or in sharps containers and discarded by Stericycle as regulated medical waste. Training that meets the regulatory requirements is provided in the Lab Safety and Chemical Hygiene module. The following slide provides a brief review of the proper procedure.

Slide 29-Waste Disposal, Continued...

REMEMBER NOT TO DISPOSE OF LIQUIDS IN RED BAGS! Any volume of liquid greater than 25 ml must be chemically treated for at least 30 minutes. The most common chemical disinfectant on campus is a 10% bleach solution. However, your supervisor should provide instructions about what disinfectant has the appropriate activity for the biohazardous materials that you are handling. After chemical deactivation the liquid must be poured down the drain and rinsed with copious amounts of water.

All areas where BSL2 materials are handled should have a waste container available for the disposal of potentially infectious materials including pipettes, disposable plastic ware, gloves and other items. A rigid, non-porous container with a lid must be lined with a red or orange biohazard bag. Any potentially infectious solid waste materials must then be placed in the biohazard bag.

After the red bag is full - or when you have finished working - the waste that you have generated is ready for shipment and disposal. Infectious waste shipping boxes are located in Stericycle pickup locations in buildings throughout campus. Please contact the Institutional Biosafety Officer for assistance finding the location closest to your lab.

Stericycle shipping boxes are all pre-printed with the biohazard symbol and orientation arrows that identify the top of the box. Assemble the box by examining the orientation arrows to determine which side is the top of the box. Tape the bottom of the box shut. In step 2 you can see that each box must be lined with a red bag. Step 3 shows that biohazard bags containing waste or sharps containers that are ready for disposal are then placed inside of the red bag lined Stericycle box. In this manner all infectious waste is contained within two layers of bags. Once the box is full it is taped shut, labeled with a generator sticker (These should be available in your pickup location) and placed neatly in the pickup location to await the Stericycle driver.

Slide 30-Incident Response

It is important that researchers know how to respond to hazardous agent exposures and other emergencies before these incidents occur. Specific symptoms of exposure and response procedures may vary depending on the biohazardous material or infectious agent that you are handling. Your supervisor or a senior researcher in your lab must review this information with you as part of the hazard-specific checklist. The information on the following slides addresses general information and response procedures applicable to all researchers working at BSL2.

Slide 31- Medical Surveillance

Medical surveillance typically consists of blood serum banking to establish a baseline biomarker concentration. Most of the hazardous agents on campus do not require participation in this type of a medical surveillance program. Your Principal Investigator or supervisor should direct

UTHSC BSL2 Training
Standard Precautions and Containment

you in cases where this is appropriate. If warranted a medical surveillance program be administered by the Occupational Health department located in 910 Madison Avenue. The Occupational Health office located on the 9th floor of the 910 Madison Avenue building can provide guidance for health concerns, immunizations or follow-up care in case of exposures. Any individuals handling human cells, blood or other potentially infectious materials should be vaccinated for hepatitis B. If you will be handling other biohazardous materials for which a vaccine may be available this should also be considered in consultation with your PI and the Occupational Health Office.

Slide 32- Exposure Response

Standard precautions, work practices and PPE are intended to prevent exposure to hazardous materials. Because accidents occur, it is important for all researchers to know the signs and symptoms associated with exposure to the biohazardous materials that you are handling. For example, if you are handling human blood or cells you must know the symptoms of common bloodborne pathogens like hepatitis B, C and HIV. You must know how to respond to exposure risks such as needlesticks, skin contact or splashes to the eyes. This information will be covered by your PI or supervisor when you complete your BSL2 hazard-specific checklist. In general, following an exposure it is appropriate to wash the affected area with soap and water, remove contaminated clothing or PPE, and, if splashed in the eye use the emergency eyewash station to rinse your eyes for at least 15 minutes.

It is also important to be familiar with the incident reporting procedure covered in the next slide. If you are handling an exotic agent, you should also know where to go to obtain follow-up care.

Slide 33- Exposures

If injured or exposure to a hazardous material or infectious agent perform or obtain first aid. If emergency medical care is necessary, call 911 or proceed to the nearest emergency room. All incidents and suspected exposures must be reported immediately to your supervisor. They must also be reported by calling CorVel's 27-7 nurse hotline. This will initiate a claim and provide that responding nurse with an opportunity to direct you to the appropriate healthcare provider for follow-up care. The number for Corvel is 1-866-245-8588.

The Office of Research Safety Affairs has a policy for the reporting of incidents. It is accessible on the Research Safety Affairs policy website and must be reviewed by all research supervisors and PIs. Different committees or individuals must be notified depending on the nature of the incident. For example, any incident or concerns related to animal research must be reported to the chair of the Institutional Animal Care and Use Committee, or IACUC. Incidents involving

UTHSC BSL2 Training
Standard Precautions and Containment

radioactive materials must be reported to the radiation safety officer. And incidents involving the escape of a transgenic animal, illness or exposures involving recombinant DNA, violations of NIH guidelines or any significant problem involving recombinant DNA research must be reported to the Chair of the Institutional Biosafety Committee.

Slide 34- BSL2 Spill Response

In the event that liquids containing biohazardous agents that are handled at BSL2 are spilled outside of a biosafety cabinet you should respond by following this procedure:

Exit the spill area. The act of spilling biohazardous liquids has the potential to generate biohazardous aerosols that may be present a risk of exposure via inhalation. You should notify your colleagues in the room about the spill and the need to exit the room while potentially hazardous aerosols settle.

On your way out of the room be sure to Close the door.

After exiting post, a warning sign on the door notifying potential entrants of the presence of the spill and potentially hazardous aerosols. The sign must instruct fellow researchers to keep out until a time when it is safe to re-enter.

Wait 30 minutes for aerosols to settle.

After 30 minutes have elapsed return to the lab to clean up the spill. While cleaning the spill wear PPE that includes gloves – possibly a double layer of gloves – a lab coat, and eye protection.

Clean spill by:

1. Covering with paper towels or rags
2. Pouring 1-10% solution of household bleach.
3. Waiting 10 minutes for bleach to work.
4. Discard material as infectious waste.
5. Use tongs to handle broken glass and sharp materials.

Slide 35- Questions?

That concludes the information for standard work practices and containment precautions for work at BSL2. To meet your full requirement for BSL2 training you must also complete the unit covering the IBC and NIH Guidelines and review the BSL2 training checklist with your supervisor or PI. Individuals handling human cells, blood or other potentially infectious human materials must also complete the Human Materials and Bloodborne Pathogens unit. You may now proceed to the quiz on BSL2 standard precautions and containment practices.