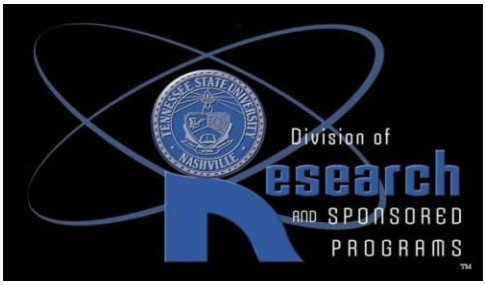
BIOSAFETY TRAINING
COURSE

TENNESSEE STATE UNIVERSITY
BIOSAFETY OFFICE



Biosafety Office
Tennessee State University

Welcome to the Bloodborne Pathogens/Biosafety annual online training module for TSU laboratory personnel. This module is comprised of 8 sections and contains a total of 21 questions in a multiple choice format. Each section contains one or more pages covering the following topics: Bloodborne Pathogens, Modes of Disease Transmission, Legislation Updates, Prevention, Regulated Medical Waste Handling, Emergencies, Select Biological Agents, and Shipping Biological Materials and Dry Ice.

Successful completion of this training module is accomplished by answering all questions in the module correctly and completing the training documentation form at the end. There is no quiz. A confirmation e-mail will be sent to you upon completion of this module.

An alternative to this online module, classroom-style training, is also available for persons who prefer this format. A schedule of the classroom training sessions can be found by following the campus specific links at the Biosafety Website.

Questions about the module should be directed to Dr. M. Karim at mkarim@tnstate.edu 615-963-5344.

Biosafety Office
Tennessee State University

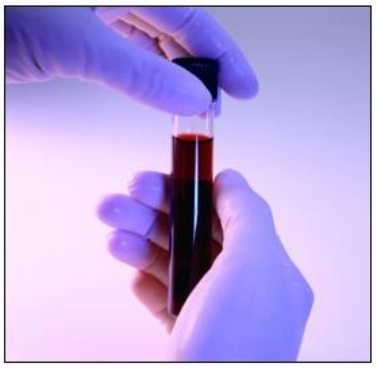
Section 1 of 8: Bloodborne Pathogens

(*You must answer each question correctly before proceeding to the next page*)

INTRODUCTION:

Bloodborne pathogens are microorganisms that are present in human blood and can cause disease in humans. Such pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Other pathogenic microorganisms in blood and body fluids that can cause disease are:

1. Staphylococcus and Streptococcus
2. Salmonella and Shigella
3. Neumonia
4. Syphilis
5. TB
6. Malaria
7. Measles
8. Chicken Pox
9. Herpes



Because of the potential to contain pathogens, OSHA considers uncharacterized human cell lines to fall under the BBP standard. Commercial suppliers such as ATCC do not characterize their cell lines for all bloodborne pathogens. Therefore, even commercially available cell lines are regulated under the BBP standard.

OCCUPATIONAL EXPOSURE

The OSHA/TOSHA Bloodborne Pathogens Standard applies to all TSU employees who have occupational exposure to blood or other potentially infectious materials.

Occupational exposure is defined by the standard as "**reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or Other Potentially Infectious Materials (OPIM) that may result from the performance of the employee's duties.**"

The three main bloodborne pathogens of concern are human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus.

A blood/body fluid exposure incident occurs when blood or OPIM enters the body via one of the following routes:

1. Being stuck with a used needle or contaminated sharp object. This is known as a percutaneous injury.
2. Contact with mucous membranes, such as the eyes.
3. Contact with non-intact skin (especially when the exposed skin is chapped, abraded or afflicted with dermatitis).
4. Contact with intact skin for a prolonged period of time, or contact involving an extensive area.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV Incidence and Prevalence

The National Institute of Allergy and Infectious Diseases (NIAID) estimates that the prevalence of HIV in the USA is 900,000 people, a quarter of whom are unaware of their HIV-positive status. The annual incidence of HIV in the USA is 40,000 cases.

HIV Symptoms

When first infected with HIV, up to 70% of people have a flu-like illness within a month after exposure to the virus. This illness may include fever, headache, tiredness and enlarged lymph nodes. These symptoms usually disappear within a week to a month and are often mistaken for those of another viral infection. During this period, people are very infectious, and HIV is present in large quantities in body fluids.

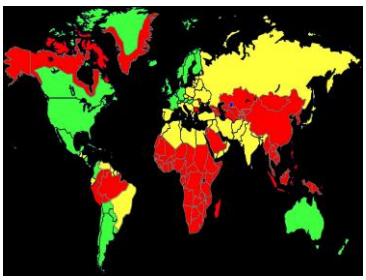
More severe symptoms may not appear for 10 years or more after HIV first enters the body in adults. Some people may begin to have symptoms within a few months, while others may be symptom-free for more than 10 years.

As the immune system is weakened by the HIV virus, a variety of complications may develop. The first signs of infection for most people are lymph nodes that may be enlarged for more than three months. Other symptoms, often experienced months to years before the onset of AIDS, may include lack of energy, weight loss, frequent fevers and sweats, and short-term memory loss.

To date, no vaccine is available to prevent AIDS, and no antiviral drugs are available to cure AIDS, although post-exposure prophylaxis is available. Some drugs have been found to inhibit the action of the virus, and others are able to fight certain opportunistic infections. Currently, prevention is the only approach to control the spread of the disease.

HEPATITIS

Hepatitis B virus (from www.cdc.gov)



Hepatitis B distribution: green = low, yellow = medium, red = high (from www.cdc.gov)

In 2001, an estimated 78,000 persons in the USA became infected with Hepatitis B. People of all ages get hepatitis B, and each year approximately 5,000 die from associated illnesses. The US

Department of Health and Human Services has also classified Hepatitis B virus as a known human carcinogen.

Hepatitis Symptoms and Incidence

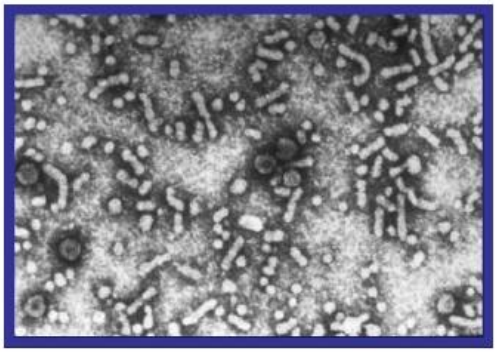
short-term (acute) illness	**long-term (chronic) illness**
loss of appetite	liver damage (cirrhosis)
diarrhea and vomiting	liver cancer
tiredness	Death
jaundice (yellow skin or eyes)	
pain in muscles, joints, and stomach	

About 1.25 million people in the USA have chronic HBV infection. Each year it is estimated that:

1. 73,000 people, mostly young adults, get infected with HBV.
2. More than 11,000 people are hospitalized because of hepatitis B.
3. 4,000 to 5,000 people die from chronic hepatitis B infections.

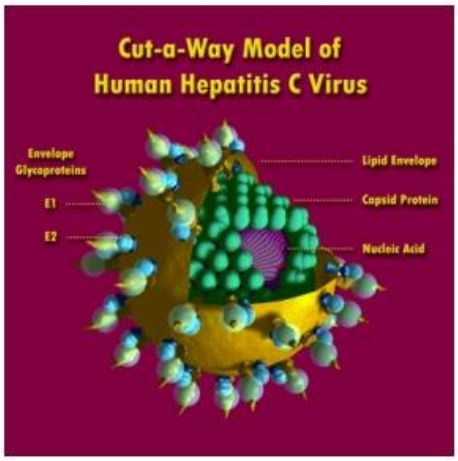
Since 1982, a vaccine for Hepatitis B has been available to prevent the HBV infection. Medical, scientific and public health communities strongly endorse using hepatitis B vaccine as a safe and effective way to prevent disease and death. Scientific data show that hepatitis B vaccines are very safe for infants, children and adults.

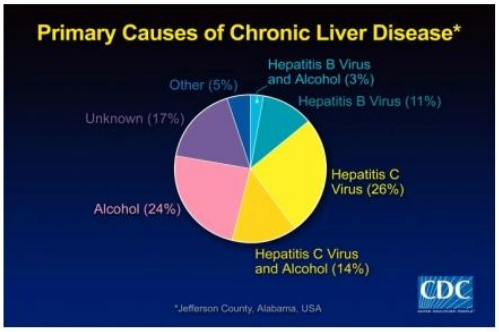
All tsu personnel who have a reasonable chance of exposure to blood or body fluids are required to receive the hepatitis B vaccine or to demonstrate immunity. The vaccine is available at no cost to potentially exposed employees. The first injection can be given any time, the second injection one month later and the third injection within 6 months of the initial injection.



HEPATITIS C VIRUS

(from www.hepcprimer.com)





(from www.cdc.gov)

Hepatitis C virus (HCV) was previously referred to as "Non-A, Non-B Hepatitis Virus." Even though the incidence of HCV has declined by about 85% since 1989, it remains the most common chronic bloodborne infection in the United States. Eighty-five percent of cases become chronic compared to fifteen percent of Hepatitis B cases. HCV is a major cause of chronic liver disease worldwide. Its cumulative prevalence in the US is estimated to be 3.7 million. About 30,000 new cases of HCV were identified in 2003. The US Department of Health and Human Services has also classified Hepatitis C virus as a known human carcinogen.

Currently, occupational HCV is predominantly transmitted by percutaneous exposure to infected blood. Needlestick injuries involving HCV-positive body fluids in the health-care setting result in a 1.8% risk of HCV transmission.

The Two Phases of HCV

Acute Phase - Occurs just after a person becomes infected and can last from a few weeks to several months. Of those who become infected, only about 20% become symptomatic with nonspecific symptoms such as malaise, anorexia, vomiting, fever, rash and polyarthritis. These symptoms last 3 -10 days. This is followed by the onset of jaundice and/or dark urine. Fulminant viral hepatitis is defined as the development of severe acute liver failure with hepatic encephalopathy within 8 weeks of the onset of symptoms with jaundice.

Chronic Phase - At least 85% of newly infected adults become chronically infected. Chronic liver disease with persistently elevated liver enzymes develops in approximately 70% of those with chronic infection. Chronic hepatitis C is a risk factor for cirrhosis and primary hepatocellular carcinoma.

REFERENCES

OSHA/TOSHA Bloodborne Pathogen Standard (1992):
http://www.osha-slc.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

NIAID HIV/AIDS Fact Sheet (2005): http://www.niaid.nih.gov/factsheets/hivinf.htm

Hepatitis B Fact Sheet (2006): http://www.cdc.gov/ncidod/diseases/hepatitis/b/fact.htm

Hepatitis C Fact Sheet (2006): http://www.cdc.gov/ncidod/diseases/hepatitis/c/fact.htm

National Toxicology Program 11th Report on Carcinogens (2005):
http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932

Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis (2005):
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5409a1.htm

Guidelines for the Management of Occupational Exposures to HBV, HCV and HIV and Recommendations for Postexposure Prophylaxis (2001):
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5011a1.htm

Viral Hepatitis C:
http://www.cdc.gov/ncidod/diseases/hepatitis/c/plan/HCV_infection.htm

QUIZ QUESTIONS

1. Which of the following is currently the most common chronic bloodborne infection in the United States?

- a. Hepatitis B virus
- b. Hepatitis C virus
- c. Human immunodeficiency virus (HIV)

2. True or False: The Hepatitis B vaccine is a series of three injections and is available at a reduced cost to TSU employees potentially exposed to blood or OPIM.

- True
- False

3. True or False: Human cell lines are treated as Bloodborne Pathogens by OSHA/TOSHA.

- True
- False

Biosafety Office
Tennessee State University

Section 2 of 8: Modes of Disease Transmission

(*You must answer each question correctly before proceeding to the next page*)

INTRODUCTION

- A percutaneous exposure can occur as a result of contact with a sharp object or by needlestick or when infectious materials come into contact with non-intact skin. This could be due to rashes, cuts, punctures, abrasions, acne, dermatitis, cold sores or bites.
- A permucosalexposure is contact with mucous membranes (eyes, nose or mouth). This can occur from splashes, aerosol generating procedures or droplet exposure.
- Aerosol transmission occurs through contact with inhaled fine-particle aerosols. "Exhaled bioaerosols" may also carry airborne pathogens and thereby magnify the spread of certain infectious diseases, such as influenza, tuberculosis and severe acute respiratory syndrome (SARS).

AEROSOLS

Aerosols are defined as dispersions of particles in a gaseous medium (e.g., air). Aerosols can be generated when pipetting, centrifuging, vortexing, sonicating, pouring liquids or blending tissue. When using BSL-2 or BSL-3 organisms, all of these operations should be conducted in a biosafety cabinet. **The picture here** is a high-speed photograph of aerosol generation from a pipette. An aerosol of approximately 15,000 droplets, most under ten micrometers in diameter, is produced when the last drop of fluid in the tip of a pipette is blown out with moderate force.

Measures to decrease aerosol hazards from pipetting biohazardous material:

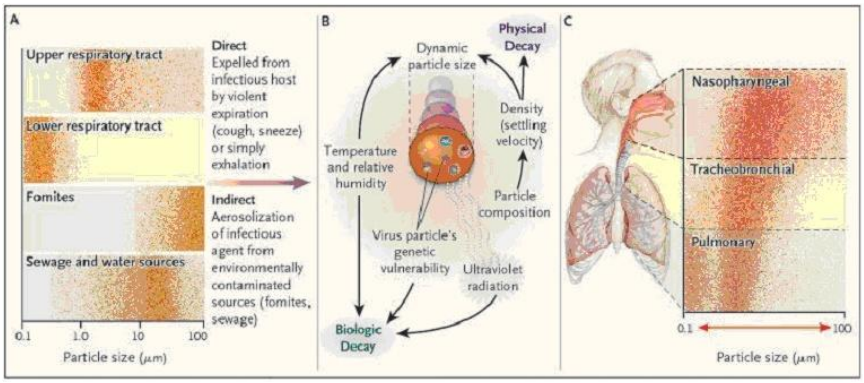
- Use aerosol-resistant tips.
- Pipetting biohazardous materials should be done in a biological safety cabinet.
- Drain a pipette with tip against the inner wall of the receiving vessel.
- Never forcibly expel any hazardous material from a pipette.

- Place reusable pipettes horizontally in a pan filled with enough liquid disinfectant to completely cover them.
- Never mouth pipette. Always use some type of pipetting aid.

DROPLET NUCLEI

Droplet nuclei are particles that are expelled when a person with an infectious disease coughs or sneezes. *M. tuberculosis* can take the form of droplet nuclei that are approximately 1-5 µm. Because of their small size, these droplet nuclei can remain suspended in the air for substantial periods. This characteristic increases transmission of *M. tuberculosis* when other persons breathe in the droplet nuclei.

The diagram below describes the factors that influence aerosol transmission of a biological agent. The graphic and description were published in the *New England Journal of Medicine*, April 2004, in an article entitled "Airborne Transmission of Communicable Infection -- The Elusive Pathway."



Many factors, including particle size, particle composition and environmental conditions, can influence the length of time a particle remains in the air. This length of time is known as physical decay.

The ability of a particle to maintain its infectivity, or biological decay, depends on environmental factors.

The area of the respiratory tract in which inhaled particles are deposited is a function of the particles' aerodynamic size. Depending on their size, particles may be deposited in both the upper and the lower airways.

BIOSAFETY LEVELS

The CDC-NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) created the assignment "***biosafety levels***" for laboratories. Biosafety levels 1-4 describe the

combination of microbiological practices, laboratory facilities and safety equipment appropriate to types of infectious organisms.

Biosafety Level 1 (BSL-1): A basic level of containment that relies on Standard Microbiological Practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Biosafety Level 2 (BSL-2): Clinical, diagnostic, teaching, research and production laboratories that work with a broad spectrum of moderate-risk agents that may be present in the community and that are associated with human disease of moderate severity are classified as requiring biosafety level 2 containment. In many cases, vaccines or drug treatments are available for these agents.

BSL-2 is also appropriate when work is done with any human-derived blood, body fluids, tissues or primary human cell lines where the presence of an infectious agent may be unknown. Specific requirements for BSL-2 are the following:

1. Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.
2. Access to the laboratory is limited when work is being conducted.
3. Extreme precautions are taken with contaminated sharp items.
4. Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.
5. All surfaces, cultures, stocks and other regulated wastes are decontaminated, as appropriate, before disposal.
6. Work with some agents may require vaccinations, if available.

In most cases, laboratory transmission of biosafety level 2 (BSL-2) organisms occurs by self-inoculation, exposure to non-intact skin or exposure via mucous membranes from aerosol-generating procedures such as centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, pipetting/pouring, loop inoculation, cage changing or dumping, or cell sorting.

Biosafety Level 3 (BSL-3): Clinical, diagnostic, teaching, research and production facilities that work with indigenous or exotic agents that may have a potential for respiratory transmission and may cause serious and potentially lethal infection are classified as requiring biosafety level 3. Primary hazards to personnel working with these agents relate to autoinoculation, percutaneous injuries and exposure to infectious aerosols.

BSL-3 is appropriate when work is done with *Mycobacterium tuberculosis, Francisella tularensis,* and *Bacillus anthracis*. Specific requirements for BSL-3 are:

1. All of the requirements of BSL-2, plus the following;
2. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices. If a biosafety cabinet is not available, personnel are required to wear appropriate personal protective equipment.

3. Entry to the lab must be through an interlock.
4. Waste must be decontaminated before being removed from the lab.
5. A ducted exhaust air ventilation system is provided.

Examples of Various Organisms and their Biosafety Levels

The table below shows many commonly encountered laboratory pathogens and their major modes of occupational transmission and corresponding biosafety levels.

Organism	***Primary Modes of Transmission***	***Biosafety Level***
Adenovirus types 1, 2, 3, 4, 5 and 7	*Percutaneous/permucosal Aerosol*	*2*
CMV, Human (beta) herpesvirus 5	*Percutaneous/permucosal*	*2*
Hepatitis B virus	*Percutaneous/permucosal*	*2/3**
Herpes simplex virus (HSV)	*Percutaneous/permucosal*	*2*
Human Immunodeficiency Virus	*Percutaneous/permucosal*	*2/3**
Influenza Virus	*Aerosol, Percutaneous/permucosal*	*2/3**
Murine retroviral vectors	*Percutaneous/permucosal*	*1/2**
Mycobacterium tuberculosis	*Aerosol, Percutaneous/permucosal*	*2/3**
Severe acute respiratory syndrome (SARS)	*Aerosol, Percutaneous/permucosal*	*3*
Staphylococcus aureus	*Percutaneous/permucosal*	*2*
Vaccinia virus	*Percutaneous/permucosal*	*2*

(BSL 2/3 indicates that work with an agent can be conducted in a Biosafety Level 2 facility using Biosafety Level 3 practices.*

REFERENCES

CDC's Biosafety in Microbiological and Biomedical Laboratories:
http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

NJEM, Volume 350:1710-1712, April 22, 2004, Number 17, Airborne Transmission of Communicable Infection - The Elusive Pathway, *CJ. Roy, DK. Milton*

QUIZ QUESTIONS

1. Which of the following statements is FALSE:

    a. Pipetting of BSL-2 materials should be done in a biosafety cabinet.
    b. In a BSL-2 lab, the biohazard symbol must be posted on the door and on potentially contaminated equipment.
    c. A biological safety cabinet must be present in the lab when using BSL-2 materials.

d. All items potentially contaminated with BSL-2 materials must be autoclaved or chemically disinfected before they are disposed of.

e. Safety cups or sealed rotors must be used when centrifuging BSL-2 materials; otherwise, the centrifuge must be used inside a biosafety cabinet.

2. Material Safety Data Sheets for Infectious Substances are available from Canada's Health Protection Branch - Laboratory Centre for Disease Control. They can be accessed via the EOHSS website. Please review the MSDS for Vaccinia to determine which of the following statements is FALSE.

a. The primary laboratory hazards are ingestion, parenteral inoculation, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

b. For spills it is best to use a 1% sodium hypochlorite solution, allowing a 30-minute contact time for decontamination, before cleanup.

c. Vaccination is not recommended.

d. The containment requirements for activities with cultures or potentially infectious clinical materials are Biosafety Level 2 practices, containment equipment and facilities.

Biosafety Office
Tennessee State University

Section 3 of 8: Legislation Updates
(*You must answer each question correctly before proceeding to the next page*)

TUBERCULOSIS AND LABORATORIES

In 2005, the CDC published new "Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings." The scope of settings in which the guidelines apply has been expanded to ***include laboratories***. The guidelines describe who should be included in a tuberculosis (TB) surveillance program, as well as the appropriate biosafety precautions for various laboratory procedures.

- Risks for transmission of M. tuberculosis in laboratories include aerosol formation during any specimen manipulation and percutaneous inoculation from needlesticks.
- All specimens suspected of containing *M. tuberculosis* should be handled in a biological safety cabinet (BSC).
- Laboratories that manipulate clinical specimens with the potential to contain *M. tuberculosis* are considered "medium risk" facilities. CDC recommends annual screening with the Tuberculin Skin Test for employees in these labs.
- For all laboratory procedures, disposable gloves should be worn. Face protection (e.g., goggles, full-facepiece respirator, face shield or other splatter guard) should also be used when manipulating specimens inside or outside a BSC. Use respiratory protection when performing procedures that can result in aerosolization outside a BSC.
- In general, BSL-2 practices, procedures, containment equipment and facilities are required for non-aerosol producing manipulations of clinical specimens. BSL-3 practices, procedures and containment equipment are necessary for certain aerosol-producing manipulations.

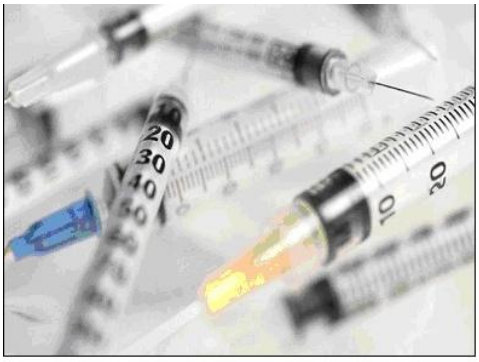
BLOODBORNE PATHOGEN STANDARD

Review of the Requirements of the OSHA/PEOSH Bloodborne Pathogens Standard
The OSHA/TOSHA Bloodborne Pathogen (BBP) standard, 29 CFR 1910.1030, applies to all employees who are exposed or potentially exposed to blood or other potentially infectious material (OPIM). This includes laboratory personnel who work with blood or OPIM. The standard requires that TSU Biosafety Office educate all exposed employees on the following topics:

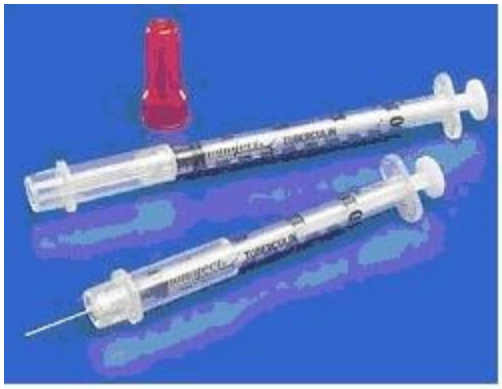
- Bloodborne Pathogens Exposure Control Plan
- Routes of exposure, examples of BBP, symptoms of disease
- Tasks that may involve exposure to BBP

- Measures to reduce, minimize or eliminate exposure
- Hepatitis B Vaccination, Post-Exposure Follow-up
- Spill Cleanup, emergency response
- Labeling
- Training and Recordkeeping

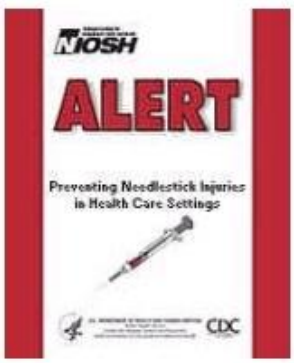
If, after completing this training, you have any questions regarding any of the above topics, contact your campus Biosafety office.

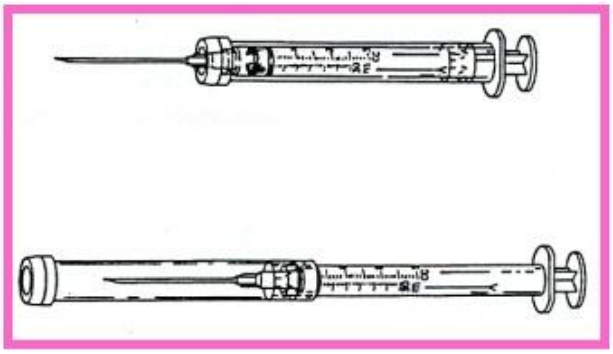


The OSHA Bloodborne Pathogens standard includes requirements for the use of engineering controls such as sharps containers and safer medical devices. This requirement was strengthened by a federal law, the Needlestick Safety and Prevention Act of 2000. This law requires healthcare facilities to evaluate and utilize safe medical devices that eliminate or minimize the risk of exposure to blood and other body fluids. The law also requires that the facility involve employees in identifying and choosing the devices. These devices can also be used to prevent needlesticks and other sharps injuries in laboratories. For example, they should be used when injecting a lab animal with potentially hazardous materials to prevent the lab worker from being accidentally inoculated.



1 cc Monoject Tuberculin Syringe





Bending, recapping or removing contaminated needles is prohibited, except where required by a specific medical procedure or no alternative is feasible. Shearing or breaking contaminated needles is completely prohibited.

Click here to see a List of Safety-Engineered Sharps Devices

EXPOSURE CONTROL PLAN

The Bloodborne Pathogens Exposure Control Plan (ECP) is a written guide that describes how the University implements the OSHA/TOSHA BBP standard. The components of an ECP are: exposure determination, control methods, housekeeping and laundry practices, regulated medical waste disposal, labeling, training, HBV vaccine, post exposure follow-up procedure and record keeping.

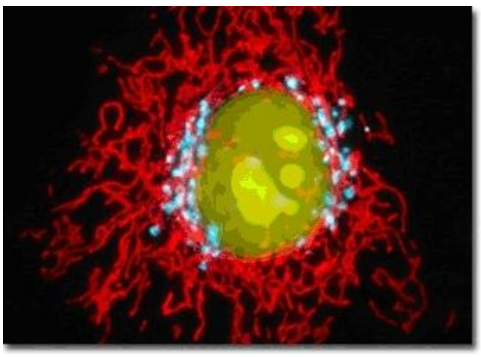
A BBP Exposure Control Plan for laboratories for each of the University's main campuses is posted on the Biosafety website. This binder should be present in each laboratory.

OTHER POTENTIALLY INFECTIOUS MATERIALS (OPIM)

OSHA has compiled a list of "Other Potentially Infectious Materials (OPIM)," which along with human blood, are always to be handled as infectious. OPIM includes semen, vaginal secretions, amniotic fluid, cerebrospinal fluid and any body fluid contaminated with blood. Unfixed tissue and most human cell lines are considered OPIM.

The OPIM list does not include certain materials such as saliva which, although they may contain bloodborne pathogens, have not been implicated in occupational transmission.

HUMAN CELL LINES



(HeLa cell from www.microscopyu.com)

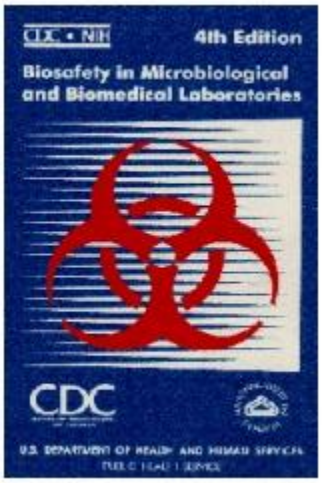
The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human tissues or fluids. Non-human primate cells and tissues also pose risks to laboratory workers and are covered by the Bloodborne Pathogens Standard.

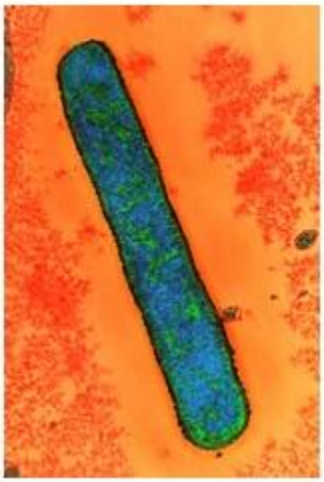
Cells transformed with viral agents, such as SV-40, EBV and HBV, as well as cells carrying viral genomic material, should also be treated as infectious materials and worked with at BSL-2. Self-inoculation of tumorigenic human cells is also a potential hazard.

Appendix H of the CDC's Biosafety in Microbiological and Biomedical Laboratories states that

personnel working with human cell lines must be covered under the institution's Bloodborne Pathogens program. An OSHA clarification notes that human cell lines must be treated as being covered under the Bloodborne Pathogens standard unless they have been characterized to be free of all bloodborne pathogens. OSHA provides a rigorous definition of "characterization", and most commercial vendors are reluctant to certify that their cell lines are free of bloodborne pathogens. See ATCC's statement regarding their testing procedures.

REGISTERING YOUR EXPERIMENTS





Registration of Pathogens, Select Agents and Human Cells/Tissues

A Pathogen Registry Form must be completed for every laboratory that uses the materials listed below. The form is used to evaluate the safety of the protocol and to ensure that each laboratory is equipped to safely handle the material.

- Human blood or Other Potentially Infectious Materials (OPIM)
- Non-human primate blood or body fluids
- Human tissue samples
- Human cell lines
- Pathogenic organisms
- Xenotransplants

Recombinant DNA

NIH Requirements

The NIH Recombinant DNA Guidelines govern the safety of recombinant DNA research in facilities that receive funding from the US National Institutes of Health (NIH). However, the term NIH "guidelines" is a misnomer. The Guidelines are not optional, they are the terms and conditions of the NIH funding for recombinant DNA research. If a NIH-funded institution is found to be out of compliance with NIH's guidelines, the NIH has the ability to rescind all NIH funding.

NIH grant requirements stipulate that the Institutional Biosafety Committee (IBC) must review recombinant protocols to ensure that appropriate safety measures are in place. "As a condition for NIH funding ... institutions shall ensure that research conducted by the institution ... shall comply with both the NIH Recombinant DNA and Biosafety Guidelines." (NIH Guidelines, Section 1-D).

The NIH rDNA guidelines describe categories of experiments involving recombinant DNA. Some experiments require NIH and IBC approvals prior to initiation, some require notification of the IBC, and some experiments are exempt from the guidelines.

- Experiments requiring **NIH and IBC approval prior to initiation**.
  - Human Gene Therapy (IRB approval also required)
  - Transfer of drug resistance to organisms
- Experiments that require IBC **approval prior to initiation**.
  - Experiments using Risk Group 2, 3, or 4 agents as host-vector systems.
  - Experiments in which DNA from Risk group 2, 3, or 4 microorganisms is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
  - Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
  - Experiments involving transgenic animals and experiments involving viable r-DNA-modified microorganisms involving whole animals or whole plants.
- Experiments that require **notification** of the IBC at the time of initiation.
  - Propagation and maintenance in tissue culture of r-DNA containing <2/3 of the genome of any eukaryotic virus in the **demonstrable** absence of helper virus.
  - The generation of transgenic rodents requiring BSL-1 containment.

REFERENCES

OSHA BBP Regulation:
http://www.osha-slc.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

Needlestick Safety and Prevention Act:
http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=106_cong_public_laws&docid=f:publ430.106

TSU Laboratory Safety Plan: www.tnstate.edu/research

OSHA Guidance Regulating Human Cell Lines as BBP:
http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21519

ATCC Statement Regarding Testing for BBPs:
http://www.atcc.org/TechnicalInfo/faqCellBiology.cfm#Q53

CDC Guidance on Research Involving Human Cell Lines:

http://www.cdc.gov/od/ohs/biosfty/bmbl4/b4ah.htm

QUIZ QUESTIONS

1. TSU's Bloodborne Pathogens Exposure Control Plan and Recombinant DNA requirements can be accessed through which of the following methods?
    - Visiting the Biosafety website.
    - Calling the Biosafety office.
    - In the TSU Laboratory Safety Plan binder which should be in each laboratory.
    - All of the above.

2. Human cell lines ordered from ATCC:
    - Are considered other potentially infectious material (OPIM) by CDC and OSHA/TOSHA.
    - May only be used in a BSL-2 laboratory that has registered with the campus IBC by completing a pathogens registration form.
    - May be used with no restrictions.
    - Are only to be handled by personnel who have proven immunity to Hepatitis B virus or who signed a Hepatitis B Vaccination declination form.
    - e. a, b and d

3. A researcher at TSU is working with a retroviral vector purchased from Invitrogen to transfect human cell lines. Choose the best answer that describes the necessary steps before conducting this work.
    - This work must be carried out at BSL-2, but registration is not required with the IBC.
    - This experiment is exempt from the NIH rDNA guidelines and registration with your campus IBC is not required.
    - An rDNA and Pathogen Registry Form must be submitted to your IBC.

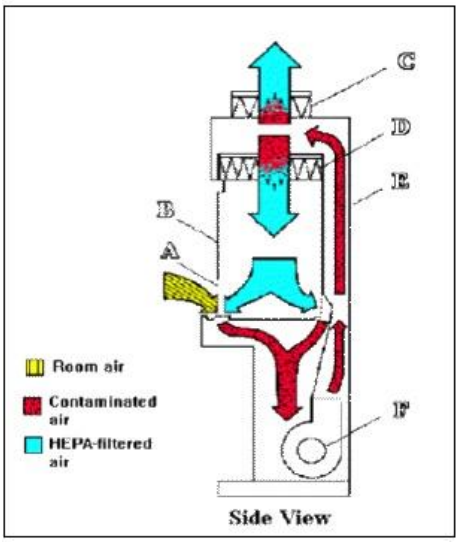
Biosafety Office
Tennessee State University

Section 4 of 8: Prevention
(*You must answer each question correctly before proceeding to the next page*)

INTRODUCTION

Biological safety cabinets, good work practices, personal protective equipment, hazard warning signs, and proper regulated medical waste disposal can all help to prevent bloodborne pathogen exposures.

ENGINEERING CONTROLS.



Airflow Diagram for a Class II Type A Biological Safety Cabinet (BSC). When working with human or primate cell lines, assume that infectious agents are present and follow BSL-2 procedures. The use of a Class II BSC (also referred to as a tissue culture hood) has become an integral component of the modern biology laboratory. The proper use of a BSC will provide protection for you, your cultures and the laboratory environment against possible infection or contamination.

The proper mindset, protective equipment and proficiency in experimental technique are essential to your safety and the viability of your cell cultures.

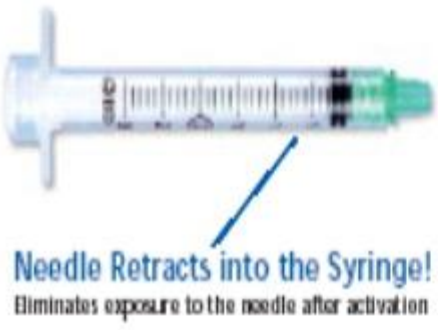
- **Prevent turbulence in the biosafety cabinet.** Use slow and deliberate motions when you move your hands into and out of a biosafety cabinet.
- **Do not place supplies on the airflow grills.** This will severely disrupt airflow.
- **Do not use a Bunsen burner.** Heat convection currents will significantly disrupt airflow and increase the risk of contamination and exposure. Also, most biological

safety cabinets have a motor in the cabinet, which could act as an ignition source if the gas is accidentally turned on. (Related incidents)

- **Pipetting technique.** Discharge pipettes against the flask or tube wall to avoid splashes. Take great care to avoid aerosols and splashes when mixing fluids with a pipette. Use disposable pipettes when your tissue culture procedure involves an infectious agent. Place used pipettes into a bleach solution before discarding them as regulated medical waste.
- **Handling sharps.** Avoid using needles, capillary tubes, scalpels, and other sharp instruments. When you must use them, handle with caution to prevent punctures and cuts. Use needles with safety features when available. For example, there are 1 cc safety syringes available, which could be used with laboratory animals. Discard used needles and disposable sharps into a puncture-resistant container with a lid.
- **Remove soiled gloves.** If your gloves become soiled or contaminated while you are doing your experiment, stop, and remove them carefully. Wash your hands and put on a new pair of gloves. If you double-glove, remove the soiled glove, replace it with a new one and continue your work.

The CDC, NIH, and NSF agree that UV lamps are neither recommended nor required in Biological Safety Cabinets (BSC). The use of UV lighting is not an effective means for controlling contamination within a BSC. For more information, see the American Biological Safety Association (ABSA) draft white paper on the Use of UV Lights in Biosafety Cabinets. For more information on biosafety cabinets, please review the Biological Safety Cabinet Factsheet.

"Engineering Controls" also refers to controls (e.g., sharps disposal containers, self-sheathing needles) that isolate or remove the bloodborne pathogens hazards from the workplace. Safety equipment such as sharps containers, sealed rotors or safety cups on centrifuges, autoclaves and biological safety cabinets must be available and used, where applicable.



Examples of Safety Devices:

ADMINISTRATIVE CONTROLS AND WORK PRACTICES

The term "Universal Precautions" is a generally recognized term used to refer to procedures adopted in the 1980s to protect against bloodborne pathogens. Universal Precautions require you to treat all human blood and certain human body fluids as if they were known to be infectious for HIV, HBV and other bloodborne pathogens.

Most healthcare facilities have moved towards using "Standard Precautions." Standard Precautions synthesize the major features of universal precautions and body substance isolation (BSI) guidelines. (BSI guidelines were designed to reduce the risk of transmission of pathogens from moist body substances.) Standard Precautions apply to 1) blood; 2) all body fluids, secretions and excretions except sweat, regardless of whether or not they contain visible blood; 3) non-intact skin; and 4) mucous membranes. Standard Precautions are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection in hospitals.

Hand Washing

Hand washing is a very simple way to prevent disease transmission. Be sure to wash your hands thoroughly using soap and warm water. Always wash your hands after removing gloves, before eating or smoking and after using the restroom.

Since 2002, officials at the Centers for Disease Control and Prevention have recommended that healthcare workers routinely use high quality alcohol-based gels. Studies have shown that use of the alcohol-based rubs on hands that aren't visibly soiled seems helpful in curbing the spread of disease. It is important to note that the alcohol concentration in the gels needs to be between 62 and 95 percent or microbes will not be killed.

Signage

Biohazard labels must be affixed to items which may be contaminated with blood, OPIM or pathogenic organisms, such as:

- Containers used to store, transport or ship infectious materials;
- Incubators, shakers;
- Refrigerators, freezers;
- Biosafety cabinets;
- Waste containers;

- Centrifuges; and
- Animal cages.

Warning signs must also be posted at the entrance of labs using BSL-2 or higher materials. The posting must include the researcher's name and phone number, biosafety level, required protective equipment and emergency contact information.

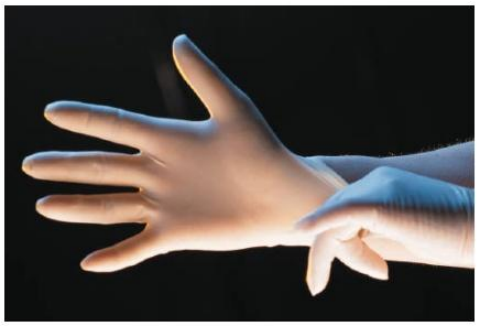
When potentially contaminated laboratory equipment is in need of repair or disposal, it must first be decontaminated. Refrigerators or freezers must have all materials, spills and other visible contamination removed by laboratory personnel. All surfaces must be wiped with a 1:10 bleach solution prior to removal from the lab. Biological safety cabinets must be decontaminated by an outside vendor before being moved, before HEPA filters are changed and before internal repair work is done.

PERSONAL PROTECTIVE EQUIPMENT (PPE)
(from www.CDC.gov)

In addition to other safety equipment such as biosafety cabinets, sharps containers and detailed protocols, **the use of eye protection, lab coat and gloves is the minimum personal protective equipment to be used when working with BSL-2 materials in a UMDNJ laboratory**. Wearing PPE is the last line of defense to prevent your eyes and body from becoming contaminated with the material you are handling.

Gloves

(from www.cdc.gov)



Gloves must be worn whenever handling blood, OPIM or pathogenic organisms. Many types of gloves provide acceptable protection from BBP, including vinyl, latex and nitrile. Some researchers prefer latex gloves because of their cost, availability and tactile sensation. However, latex sensitivity/allergy is increasing among latex glove wearers. For this reason, most UMDNJ laboratories have switched to nitrile as their basic-use glove. Powdered latex gloves should **not** be kept in the laboratory. Latex antigens can become absorbed onto the powder which may become aerosolized and inhaled. This may increase the likelihood of sensitization.

Personnel who notice that they are developing a rash or sensitivity when latex gloves are used should immediately stop wearing latex gloves and should fill out a TSU incident report. An alternative type of glove such as nitrile or vinyl should be used instead of latex.

Gloves should be replaced often, and they should be replaced immediately if they become overtly contaminated, torn or punctured. Hands should be washed after gloves are removed, whether or not the gloves have been compromised.

Care should be taken not to contaminate lab surfaces and objects (e.g., door knobs, phones, pencils) with used gloves. Also, gloves may not be worn outside of the laboratory. Use secondary containment, such as a tupperware container with a tight fitting lid, to carry biological materials so that glove use is not necessary.

Lab Coats

(from www.cdc.gov)

Lab coats must be worn when BSL-2 materials are being used and are recommended whenever any biological materials are used. However, it is inappropriate to wear a lab coat to the lunchroom, cafeteria or seminars.

Soiled lab coats must be laundered through this service. It is the employer's responsibility to provide, launder, repair, replace and dispose of PPE. You should never take your lab coat home to wash it.

Protective Eyewear

Safety glasses with side shields, goggles and face shields are all examples of protective eyewear. Goggles and face shields, rather than safety glasses, must be worn when splashes, splatter, spray or droplets of BBP may be expected to contact the eyes or when working with large volumes of biological material.UV-rated face shields must be worn when looking at gels on the UV box to prevent burns to the face.

REFERENCES

Latex Allergy Prevention Guide: http://www.cdc.gov/niosh/98-113.html

"Hand Sanitizers, Good or Bad? Good, if the Alcohol Content Is Strong Enough": New York Times, March 21, 2006, DEBORAH FRANKLIN

QUIZ QUESTIONS

1. What type of gloves offer superior protection from blood and OPIM with good tactile sensation?
    a) Nitrile gloves.
    b) Powdered latex gloves.
    c) Vinyl gloves.
    d) Unpowdered latex.

2. Match the cause with the each of the three incidents below:
    a) No use of sealed rotors or safety cups
    b) No safety needles
    c) Improper eye protection
    d) Work practices (PPE, decontamination, use of BSC)

    i) A Thomas Jefferson University researcher developed vaccinia infection in her left eye.
        - a.
        - b.
        - c.
        - d.

    ii) Yale researcher developed Sabia Virus.
        - a.
        - b.
        - c.
        - d.

    iii) A post-doc was viewing a gel on a UV-transilluminator and suffered serious burns to face and eyes.
        - a.
        - b.
        - c.
        - d.

3. Which of the following is FALSE when working in a Class II biological safety cabinet?
    - The person working at the hood is protected because materials used within the cabinet are contained by room air that is drawn into the cabinet.
    - Cultures in the cabinet are kept sterile because the air in the cabinet has passed through a high-efficiency particulate air (HEPA) filter.
    - Using a Bunsen burner within the cabinet will help keep cultures sterile.
    - Air exhausted from the cabinet is filtered by high-efficiency particulate air filters, so contaminants do not enter the lab.

Biosafety Office
Tennessee State University

Section 5 of 8: Regulated Medical Waste (RMW) Handling
(*You must answer each question correctly before proceeding to the next page*)

Picture of Regulated Medical Waste Container

Picture of Sharps Container

All TSU labs are responsible for appropriately disposing of their waste products. Labs must segregate, decontaminate and package waste for disposal.

Regulated Medical Waste (RMW) is defined as any solid waste generated in the diagnosis, treatment or immunization of human beings or animals, in research pertaining thereto or in the production or testing of biologicals (Medical Waste Tracking Act of 1988). Each TSU laboratory is responsible for segregating, appropriate decontamination and packaging of its regulated medical waste.

In laboratory areas, three types of RMW are generated:

- **Liquids**, including cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories; wastes from the production of biologicals; discarded live and attenuated vaccines; and devices used to transfer, inoculate and mix cultures and human blood.
- **Sharps** used in animal or human patient care or treatment, in medical research or in industrial laboratories, including sharp items and items potentially sharp if broken such as, but not limited to, hypodermic needles, all syringes to which a needle can be attached (with or without the attached needle) and their components, scalpel blades, razor blades, blood vials, carpules, needles with attached tubing, culture dishes, and broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.
- **Other Medical Waste**, including animals and bedding used in infectious agent research; tissue, organs or body parts from humans or infected animals; biological agent spill

clean-up materials; Petri dishes; glass and plastic pipettes; pipette tips; and eppendorf tubes.

What is NOT Medical Waste?

- Chemical bottles
- Paper wrappers from pipettes
- Food containers
- Styrofoam and plastic containers
- Memos, newsletters, newspapers

- Regulated medical waste containers are NOT to be used for **regular trash** or **chemical waste** of any kind.

BSL-2 Materials Must be Decontaminated Before Disposal

BSL-2 or higher materials, including pathogens and recombinant DNA, must be decontaminated by autoclaving or treatment with a chemical disinfectant before disposal in the sink or in a regulated medical waste container. Environmental Services workers transport regulated medical waste for disposal; the presence of live organisms in the waste poses unnecessary risks during handling.

All disposable plastic lab-ware (Petri dishes, pipette tips, etc.) must be disinfected before being placed into the RMW container. Glassware, sharps and razor blades must be disposed in a sharps container.

When the Regulated Medical Waste container is 2/3 full, tie the liner bag closed and close the lid. Environmental Services will remove the container and replace it with an empty one.

Agarose and acrylamide gels may be disposed of in the normal trash. (Let them dry out before disposing of them). Mercury thermometers must be disposed of as hazardous waste through EOHSS.

Large quantities of normal trash and recyclable paper are disposed of in Regulated Medical Waste Containers. Do your part. Keep paper and normal trash out of the Regulated Medical Waste bins.

QUIZ QUESTIONS

1. The materials listed below are going to be disposed. Next to each item, indicate the proper trash container for disposal.

A. Normal Trash
B. Regulated Medical Waste Container
    a. unused petrie dishes: A or B
    b. used petrie dishes: A or B

c. gloves used for chemicals (not overtly contaminated): A or B
d. gloves used for biological materials: A or B
e. paper towels used for general lab clean-up: A or B
f. agarose gels: A or B
g. acrylamide gels: A or B

2. True or False: Laboratory supplies that were in contact with human cell lines obtained from ATCC must be decontaminated before being disposed of in the regulated medical waste container.
    a) True
    b) False

3. True or False: Pound for pound, it costs approximately four times as much to dispose of Regulated Medical Waste as normal trash.
    a) True
    b) False

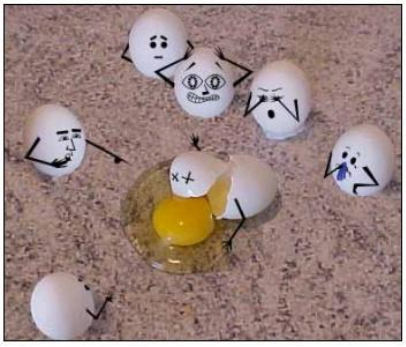
Biosafety Office

Tennessee State University

Section 6 of 8: Emergencies

(*You must answer each question correctly before proceeding to the next page*)

INTRODUCTION



If an emergency happens, it's important that you know what to do. Your primary concern should be your own safety and the safety of those around you. Experiments and equipment can be replaced, but lives cannot.

Each lab should have the most current Emergency Response Guide Flipchart. The flipchart lists the basic steps to take for fires, radiation and chemical and biological spills, as well as for medical emergencies, including exposures to biological materials. The last page of the flipchart lists campus-specific phone numbers for Public Safety, Physical Plant and.

EOHSS.

Most emergencies must be reported to the Public Safety Emergency phone number. Public Safety will contact EOHSS and other emergency responders.

TYPES OF EMERGENCIES.

There are two main emergencies that will be reviewed in this module:

- Biological Exposure Incidents such as a needle stick or splash of BL-2 material to mucous membranes or non-intact skin
- A spill of BL-2 or higher materials.

INJURY/EXPOSURE

(from www.thinkclean.org)

If you are injured or splashed with biological materials in the lab, be sure to follow these steps:

- Immediately wash the area with regular soap and water. If you have been splashed in the eyes, flush them with copious amounts of water for at least ten minutes. Hold the affected eye open while it is being flushed.
- Do not use abrasive cleansers or bleach on the wound. Do not try to bleed the wound after an exposure. This may aggravate the injury site.
- Keep a sample of the blood or OPIM so that it can be tested for bloodborne pathogens, if appropriate. If possible, refrigerate the specimen.
- Notify your supervisor of the incident.
- Obtain medical attention by following the instructions on the Emergency Response Guide Flipchart.

Steps to Take Following an Exposure Incident

Each campus has an "Emergency Response Guide" flip-chart that provides specific instructions for addressing exposures. If you have any questions or concerns, report the incident by calling the Public Safety emergency phone number, and they will contact emergency services for you.

For exposure to blood or OPIM, wash the affected area with soap and water, and seek medical assistance immediately. If the exposure was high risk for HIV then the doctor may prescribe chemoprophylaxis for HIV. Post-exposure prophylaxis for hepatitis B virus is also available but does not need to be started immediately.

Inform the physician as to whether the biological material is likely to contain any bloodborne pathogens. If this information is not known, try to obtain a sample of the biological material so that it can be tested for the presence of bloodborne pathogens. Informed consent is necessary if the source of the biological material is known.

A physician must review the details of the exposure with you as soon as possible to determine if post-exposure prophylaxis is needed. The benefits and risks of post-exposure prophylaxis will be explained. If possible, source specimens will be tested for presence of potential bloodborne pathogens.

POST-EXPOSURE PROPHYLAXIS (PEP)

Post-exposure prophylaxis (PEP) is any treatment that is provided after an exposure to a disease-causing agent. The treatment is designed to prevent the occurrence or recurrence of the disease. The CDC published updated PEP guidelines for HIV, HBV and HCV in 2001.

HIV infection, following an exposure to contaminated blood or OPIM, can be prevented using very effective antiretroviral drug therapies. The CDC recommends that this therapy be started within a few hours of the exposure incident for maximum effectiveness. Antiretroviral drug therapy, sometimes called a "cocktail," usually includes zidovudine and other common antiretroviral medications. Even if there is a delay of days or even weeks, the therapy may still be initiated.

The risk of HBV infection in unvaccinated persons can also be reduced by post-exposure treatment. First, a titer will be obtained from the exposed employee. The titer will determine if the individual already possesses an anti-HBV titer. Only if a person does not demonstrate a titer, will post-exposure prophylaxis be administered. In most cases, treatment with hepatitis B vaccine or hepatitis B immune globulin post-exposure prophylaxis should be started as soon as possible. However, treatment can be started up to one week after the initial exposure to contaminated blood or body fluids.

Currently, there is no recommended PEP for HCV exposures. Prompt follow-up is critical to establish baseline titer. The baseline will be used as a comparison to assess future liver enzyme measurements.

CLEANING A SPILL OF BSL-2 OR HIGHER MATERIALS

If a spill occurs, remain calm. Decide if you can clean the spill yourself. If you need assistance, call Public Safety Emergency phone number.

Cleaning up a biological spill: Before gathering the necessary cleanup supplies, block off the area and warn others in the lab to stay away. If you were working with a respiratory pathogen, you should leave the lab for at least 30 minutes to allow aerosols to settle.

If you decide you can clean up the spill yourself, gather your necessary supplies: absorbent, disinfectant, waste bag, protective gloves and other items. Then proceed to clean the spill:

- Put on gloves and a lab coat and any other necessary equipment.
- Cover spill with absorbent towels.
- Pour 1:10 dilution bleach over spill; avoid splashing.
- Allow 20 min contact.
- Clean spill with towels, outside to inside.
- Use tongs, forceps or a scoop and brush to collect broken glass (no bare hands!).
- Clean any nearby surfaces, equipment or furniture that may have been contaminated with disinfectant-soaked towels.
- Clean area again with disinfectant.
- Place all spill clean-up materials in a biohazard/regulated medical waste bag.
- Place broken glass into a sharps container.

REFERENCES

Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Post-Exposure Prophylaxis:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5409a1.htm

Guidelines for the Management of Occupational Exposures to HBV, HCV and HIV and Recommendations for Postexposure Prophylaxis:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5011a1.htm

QUIZ QUESTIONS

1. If you are unable to clean a biological spill on your own, you can call EOHSS for assistance. What is the best way to reach them?
    a) Call EOHSS directly at their office phone number.
    b) Call Public Safety Dispatch and the dispatcher will call EOHSS.
    c) Send an email to EOHSS requesting assistance.
    d) None of the above.

2. What is meant by the term "exposure incident?"
    a) An exposure to mucous membranes such as the eyes and nose.
    b) An exposure to non-intact skin such as skin that has a rash or is chapped.
    c) A stick from a contaminated needle or sharp.
    d) Any skin exposure to BSL-2 materials, independent of whether the skin is intact or non-intact.
    e) e. a, b and c.

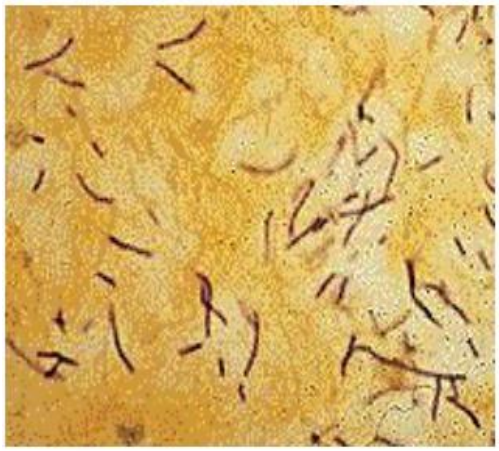
Biosafety Office

Tennessee State University

Section 7 of 8: Select Biological Agents

(*You must answer each question correctly before proceeding to the next page*)

INTRODUCTION



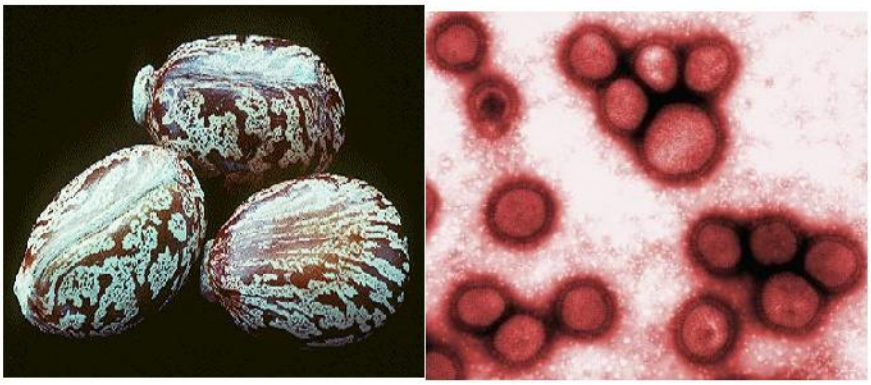
(*Bacillus anthracis* from www.cdc.gov)

The recently reauthorized "*USA PATRIOT Act*" and "*Possession, Use and Transfer of Select Agents and Toxins Final Rule*, also known as the Select Agent Rule," are designed to improve the ability of the United States to prevent, prepare for and respond to bioterrorism and other public health emergencies.

The CDC and USDA oversee monitoring programs established as a result of these regulations. The rules govern the use, handling and transfer of certain select agents that are biologically hazardous bacteria, rickettsiae, fungi and viruses, and certain toxins which have the potential to cause substantial harm to human, animal or plant health.

Laboratories possessing, using or transferring any biological select agents and any select agent toxins over designated threshold quantities *must be registered* with either the CDC or the USDA. Laboratory staff must pass a background check by the Federal Bureau of Investigation (FBI) in compliance with TSU policies. Violations of the select agent regulations can result in fines of up to $500,000 and imprisonment of up to five years.

The first step for any TSU laboratory contemplating obtaining, using or transferring any select agent is to contact its campus biosafety officer:



(Ricin-producing castor beans from City of New York, DHHS)
(1918 pandemic influenza virus, from sciencedaily.com)

SELECT AGENTS

The select agent regulations include both live organisms and biological toxins. **Any quantity of live organisms designated as select agents are subject to the regulations**. Biological toxins listed as select agents are subject to all of the FBI, CDC and USDA criteria listed in the rules if the maximum amounts permitted by the regulation are exceeded. See table below:

Select Agent Toxin Threshold Amounts Per Principal Investigator (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73)

HHS/USDA Toxins	Amount
Abrin	100 mg
Conotoxin	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Tetrodotoxin	100 mg
Botulinum neurotoxins	0.5 mg
Staphylococcal enterotoxins	5.0 mg
Clostridium perfringens epsilon toxin	100 mg
Shigatoxin	100 mg
T-2 toxin	1000 mg

Labs that possess a select agent toxin below the threshold quantity may be exempt from the Federal regulations, but UMDNJ requires that these toxins be registered with EOHSS. In Piscataway and New Brunswick, the Institutional Biosafety Committee requires a form be completed. The form is available at www.tnstate.edu/research. In Newark or Scotch Plains, please contact the Biosafety Officer listed above for advice.

Even if they are not select agents, microbial toxins with an LD_{50} less than 50 mg/kg are subject to the provisions of the TSU Lab Safety Plan's Particularly Hazardous Substances requirements. Written SOPs are required for chemicals with very low oral, inhalation or skin LD_{50}, and these SOPs must be reviewed by EOHSS.

If requested, EOHSS can provide assistance in the development of the written SOP. The SOP would include the key elements listed below:

A. Written safety protocols to cover the specific toxin(s) in use.
B. Security measures in place to protect against unauthorized access to toxin(s).
C. Inventory control system in place; all entries in a hardbound book, in ink.
D. Written plan for toxin-related emergencies (spill, exposure, etc.) posted.
E. BSL-2 or BSL-3 containment and practices in use.

REFERENCES

CDC Select Agent Program: http://www.cdc.gov/od/sap/index.htm
USDA Select Agent Program:
http://www.aphis.usda.gov/programs/ag_selectagent/
List of Select Agents: http://www.cdc.gov/od/sap/docs/salist.pdf
USA PATRIOT Act:
http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=107_cong_public_laws&docid=f:publ056.107.pdf

QUIZ QUESTIONS

1. A researcher who has 5.5mg of Staphylococcal enterotoxins is exempt from the select agent regulations.
   - True
   - False

2. Research with select agents such as Clostridium botulinum, Francisella tularensis, and exotic Vesicular Stomatitis Virus
   - must be registered with the CDC or USDA.
   - may only be conducted by individuals with FBI clearance.
   - is exempt from regulation based on the quantity being used.
   - d. Both A and B.

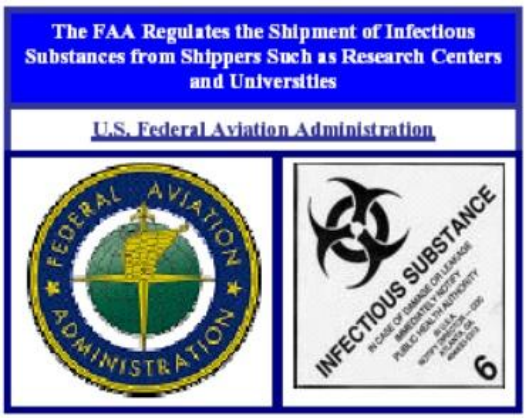
3. A researcher at UMDNJ is conducting studies using Cholera toxin, which has an LD_{50} of 250 ug/kg. Which of the following should be done before working with this biological toxin?
   a) Before possessing, using, or transferring this toxin, it must be registered with the CDC/USDA.
   b) There are no steps needed to be taken before conducting work with this toxin.
   c) This toxin should be registered with EOHSS, and a written SOP is needed before any work is conducted.

Biosafety Office

Tennessee State University

Section 8 of 8: Shipping Biological Materials and Dry Ice

(*You must answer each question correctly before proceeding to the next page*)



TRAINING REMINDER FOR PERSONNEL

All personnel who prepare shipping documentation, mark and label packages, fill packages or supervise transport of packages containing **patient specimens**, **infectious materials**, **dry ice** or **genetically modified microorganisms** via U.S. Postal Service, FedEx or other carriers must attend an IATA Shipping Training Seminar every two years. The training covers the critical issues listed below as well as changes and yearly updates:

- Are your shipping containers properly labeled according to IATA, DOT and/or USPS regulations?
- How long do you maintain files when shipping a hazardous material?
- Are you selecting the correct containers and labels for shipping hazardous materials?
- Do you have a current training certificate permitting you to ship hazardous materials?

EOHSS offers an IATA Shipping Training Seminar regularly.

2005/2006 SHIPPING DANGEROUS GOODS AND IATA UPDATE

Infectious substances are now classified as either Category A or Category B substances.

- **Category A Infectious Substances** are substances that can cause a permanent disability, or a life-threatening or fatal disease upon contact with a human or animal due to release

outside the packaging used in transport. They are assigned the following shipping names and UN numbers:

- **Infectious Substance affecting humans, UN 2814**, or
- **Infectious Substance affecting animals** *only*, **UN 2900**

Category B Infectious Substances are Infectious Substances that do not meet the criteria for inclusion in Category A. They are usually clinical, diagnostic or patient specimens which pose a low risk during transportation. They are assigned the following shipping names and UN numbers:

- **Biological Substance Category B, UN 3373**,
- **Diagnostic Specimen, UN 3373**, or
- **Clinical Specimen, UN 3373**.

"Biological Substance Category B" or "Diagnostic Specimen" packages are required to have a ***UN3373 diamond shaped label*** and the text "Biological Substance Category B" or "Diagnostic Specimen."

- Both category A and B specimens require adequate packaging to ship safely. This is referred to as a ***"Triple Packaging System,"*** which consists of:
  - (1) A watertight primary receptacle.
  - (2) A watertight secondary packaging.
  - (3) An outer packaging of adequate strength for its capacity, mass and intended use.
- New definitions:
  - A ***patient specimen*** is defined as a sample collected from humans/animals, and may include but is not limited to excreta, secreta, blood and its components, tissue and tissue fluids swabs and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment or disease prevention. Some patient specimens may be "exempt" but still require triple packaging.
  - A ***culture*** is defined as the result of a process by which pathogens are intentionally propagated.

TRANSPORTING CULTURES OR SPECIMENS BETWEEN LABS OR BUILDINGS

When transporting biohazardous substances between labs, buildings or campuses, it is important to make certain that the containers are sealed and that secondary containers are used. If you are transporting biohazardous materials between facilities, make sure you do the following:

- Specimen containers must be watertight and leak-proof. All materials must be transported and stored in a secondary container to prevent breakage. The secondary container must be capable of containing the materials if the primary container breaks or leaks.

- Absorbent materials must be included between the primary and the secondary container in a quantity sufficient to absorb all liquids and to cushion the materials to prevent container breakage.
- If the specimen container is a tube, ensure that it is tightly capped and placed in a rack to maintain an upright position.
- Place specimen containers and racks in robust, leak-proof plastic or metal transport boxes with secure, tight-fitting covers.
- Secure the transport boxes so that contents are not spilled or disrupted during transport.
- Label each transport box with its contents, a biohazard symbol, the name and telephone number of an emergency contact person and the receiver's name, address and telephone number.

QUIZ QUESTIONS

1. Indicate whether each of the following people need to attend an IATA Training Seminar every two years:
   a. John Wang, a secretary who makes an appointment with FedEx to pick up packages of biological materials.
      ○ Yes ○ No

   b. Casey McCarthy, an office secretary who prepares the required hazardous materials description on a shipping paper at the direction of another, item by item.
      ○ Yes ○ No

   c. The Christo labs send out a similar package containing a category A biological substances every month. Laura Chen prints out a Dangerous Goods Declaration form and signs it.
      ○ Yes ○ No

2. True or False: Transformed human cells meeting the definition of a Biological Substance Category B are being shipped. This package would need a UN3373 diamond shaped label and must be triple packed.

   ○ a. True

   ○ b. False

If you have any questions, please contact Dr. Mohammad Karim

BIOSAFETY TRAINING

TEST

TENNESSEE STATE UNIVERSITY
BIOSAFETY OFFICE

SECTION 1
QUIZ QUESTIONS

1. Which of the following is currently the most common chronic bloodborne infection in the United States?

☐ a. Hepatitis B virus
☐ b. Hepatitis C virus
☐ c. Human immunodeficiency virus (HIV)

2. True or False: The Hepatitis B vaccine is a series of three injections and is available at a reduced cost to TSU employees potentially exposed to blood or OPIM.
☐ True
☐ False

3. True or False: Human cell lines are treated as Bloodborne Pathogens by OSHA/TOSHA.
☐ True
☐ False

SECTION 2
QUIZ QUESTIONS

4. Which of the following statements is FALSE:

☐ a. Pipetting of BSL-2 materials should be done in a biosafety cabinet.

☐ b. In a BSL-2 lab, the biohazard symbol must be posted on the door and on potentially contaminated equipment.

☐ c. A biological safety cabinet must be present in the lab when using BSL-2 materials.

☐ d. All items potentially contaminated with BSL-2 materials must be autoclaved or chemically disinfected before they are disposed of

☐ e. Safety cups or sealed rotors must be used when centrifuging BSL-2 materials; otherwise, the centrifuge must be used inside a biosafety cabinet.

5. Material Safety Data Sheets for Infectious Substances are available from Canada's Health Protection Branch - Laboratory Centre for Disease Control. They can be accessed via the EOHSS website. Please review the MSDS for Vaccinia to determine which of the following statements is FALSE.

☐ a. The primary laboratory hazards are ingestion, parenteral inoculation, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues

☐ b. For spills it is best to use a 1% sodium hypochlorite solution, allowing a 30-minute contact time for decontamination, before cleanup.

☐ c. Vaccination is not recommended.

☐ d. The containment requirements for activities with cultures or potentially infectious clinical materials are Biosafety Level 2 practices, containment equipment and facilities.

SECTION 3:

QUIZ QUESTIONS

6. TSU's Bloodborne Pathogens Exposure Control Plan and Recombinant DNA requirements can be accessed through which of the following methods?

   ☐ a. Visiting the Biosafety website.

   ☐ b. Calling the Biosafety office.

   ☐ c. In the TSU Laboratory Safety Plan binder which should be in each laboratory.

   ☐ d. All of the above.

7. Human cell lines ordered from ATCC:

   ☐ a. Are considered other potentially infectious material (OPIM) by CDC and OSHA/TOSHA.

   ☐ b. May only be used in a BSL-2 laboratory that has registered with the campus IBC by completing a pathogens registration form.

   ☐ c. May be used with no restrictions.

   ☐ d. Are only to be handled by personnel who have proven immunity to Hepatitis B virus or who signed a Hepatitis B Vaccination declination form.

   ☐ e. a, b and d

8. A researcher at TSU is working with a retroviral vector purchased from Invitrogen to transfect human cell lines. Choose the best answer that describes the necessary steps before conducting this work.

   ☐ a. This work must be carried out at BSL-2, but registration is not required with the IBC.

   ☐ b. This experiment is exempt from the NIH rDNA guidelines and registration with your campus IBC is not required.

   ☐ c. An rDNA and Pathogen Registry Form must be submitted to your IBC.

SECTION 4
QUIZ QUESTIONS

9. What type of gloves offer superior protection from blood and OPIM with good tactile sensation?
   ☐ a. Nitrile gloves.
   ☐ b. Powdered latex gloves.

☐c. Vinyl gloves.
☐d. Unpowdered latex.

10. Match the cause with the each of the three incidents below:
    ☐a. No use of sealed rotors or safety cups
    ☐b. No safety needles
    ☐c. Improper eye protection
    ☐d. Work practices (PPE, decontamination, use of BSC)

11. A Thomas Jefferson University researcher developed vaccinia infection in her left eye.
    ☐a
    ☐b
    ☐c
    ☐d

12. Yale researcher developed Sabia Virus.

    ☐a
    ☐b
    ☐c
    ☐d

13. A post-doc was viewing a gel on a UV-transilluminator and suffered serious burns to face and eyes.

    ☐a
    ☐b
    ☐c
    ☐d

14. Which of the following is FALSE when working in a Class II biological safety cabinet?
    ☐a. The person working at the hood is protected because materials used within the cabinet are contained by room air that is drawn into the cabinet.

    ☐b. Cultures in the cabinet are kept sterile because the air in the cabinet has passed through a high-efficiency particulate air (HEPA) filter.

    ☐c. Using a Bunsen burner within the cabinet will help keep cultures sterile.

    ☐d. Air exhausted from the cabinet is filtered by high-efficiency particulate air filters, so contaminants do not enter the lab.

SECTION 5:
QUIZ QUESTIONS

15. The materials listed below are going to be disposed. Next to each item, indicate the proper trash container for disposal.
    A. Normal Trash

B. Regulated Medical Waste Container

a. unused petrie dishes
- ☐ A.
- ☐ B.

b. used petrie dishes
- ☐ A.
- ☐ B.

c. gloves used for chemicals (not overtly contaminated)
- ☐ A.
- ☐ B.

d. gloves used for biological materials
- ☐ A.
- ☐ B.

e. paper towels used for general lab clean-up
- ☐ A.
- ☐ B.

f. agarose gels
- ☐ A.
- ☐ B.

g. acrylamide gels
- ☐ A.
- ☐ B.

16. True or False: Laboratory supplies that were in contact with human cell lines obtained from ATCC must be decontaminated before being disposed of in the regulated medical waste container.
- ☐ True
- ☐ False

17. True or False: Pound for pound, it costs approximately four times as much to dispose of Regulated Medical Waste as normal trash.
- ☐ True
- ☐ False

SECTION 6:
QUIZ QUESTIONS

18. If you are unable to clean a biological spill on your own, you can call EOHSS for assistance. What is the best way to reach them?

☐a. Call EOHSS directly at their office phone number.

☐b. Call Public Safety Dispatch and the dispatcher will call EOHSS.

☐c. Send an email to EOHSS requesting assistance.

☐d. None of the above.

19. What is meant by the term "exposure incident?"

☐a. An exposure to mucous membranes such as the eyes and nose.

☐b. An exposure to non-intact skin such as skin that has a rash or is chapped.

☐c. A stick from a contaminated needle or sharp.

☐d. Any skin exposure to BSL-2 materials, independent of whether the skin is intact or non- intact.

☐e. a, b and c.

SECTION 7:

QUIZ QUESTIONS

20. If you are unable to clean a biological spill on your own, you can call EOHSS for assistance. What is the best way to reach them?

☐a. Call EOHSS directly at their office phone number.
☐b. Call Public Safety Dispatch and the dispatcher will call EOHSS.
☐c. Send an email to EOHSS requesting assistance.
☐d. None of the above.

21. What is meant by the term "exposure incident?"

☐a. An exposure to mucous membranes such as the eyes and nose.
☐b. An exposure to non-intact skin such as skin that has a rash or is chapped.
☐c. A stick from a contaminated needle or sharp.
☐d. Any skin exposure to BSL-2 materials, independent of whether the skin is intact or non-intact.
☐e. a, b and c.

SECTION 8:

QUIZ QUESTIONS

22. Indicate whether each of the following people need to attend an IATA Training Seminar every two years:

a. John Wang, a secretary who makes an appointment with FedEx to pick up packages of biological materials.
☐Yes
☐No

b. Casey McCarthy, an office secretary who prepares the required hazardous materials description on a shipping paper at the direction of another, item by item.
☐Yes
☐No

c. The Christo labs send out a similar package containing a category A biological substances every month. Laura Chen prints out a Dangerous Goods Declaration form and signs it.
   - ☐ Yes
   - ☐ No

23. True or False: Transformed human cells meeting the definition of a Biological Substance Category B are being shipped. This package would need a UN3373 diamond shaped label and must be triple packed.
   - ☐ True
   - ☐ False