Chapter 6 Human Tissues and Cell Culture

Work with Human Tissues

Human blood, blood products, body fluids and tissues are listed as potentially Hazardous Biological Materials. Consult the Human Subjects Committee for specific policies regarding the collection and use of human tissues, blood and other products for research purposes.

Biosafety Level 2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues because of the infectious agents they may contain. Biosafety Level 2 practices and procedures are consistent with the concept of "Universal Precautions" which requires all specimens of human blood, blood products, body fluids and tissues to be treated as if they are infectious. The federal regulation, Occupational Exposure to Bloodborne Pathogens, mandates a combination of engineering and work practice controls, training and hepatitis B vaccination to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials which may contain human pathogens.

Hepatitis B vaccination is available to all occupationally at-risk university employees (refer to Chapter 8, Blood-borne Pathogens Standard) through the Department of Risk Management and Safety. Mandatory safety training, which provides information on protection from occupational exposure to blood-borne pathogens, is provided by Risk Management and must be attended by all laboratory personnel working with human tissues or fluids. The Principal Investigators of such labs must also complete the blood-borne pathogens course. For more information on training or registration for hepatitis B vaccine, call Risk Management at 621-1790.

Laboratory personnel (faculty and staff) in research laboratories working with Human Immunodeficiency Virus (HIV) or Hepatitis B Virus (HBV) or materials suspected of harboring these viruses must fulfill additional requirements as follows:

1. The employee must attend a one time general laboratory safety training offered several times a year by Risk Management.

2. The employee must have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

3. Before being allowed to work with HIV or HBV, the employee must demonstrate to the PI a proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory.

4. An employee with no prior experience in handling human pathogens must be trained in the laboratory prior to handling infectious materials. Initial work activities shall not include handling of infectious agents. A progression of work activities will be assigned as techniques are learned and proficiency is developed. Participation in work activities involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the Principal Investigator/laboratory supervisor.

Cell Culture

When cell cultures are known to contain an etiologic agent, an oncogenic virus or amphotropic packaging system the cell line must be classified at the same level as that recommended for the agent.

The following must be handled at Biosafety Level 2 (including a Class II Biosafety Cabinet) or higher containment level:

All cell lines (primary and established) of human/primate origin

All cell lines derived from lymphoid or tumor tissue

All cell lines exposed to or transformed by any oncogenic virus

All cell lines exposed to or transformed by amphotropic packaging systems

All human clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy)

All cell lines new to the laboratory (until verified to be free of all adventitious agents)

All mycoplasma-containing cell lines

All cell lines (primary or established) of animal origin

NOTE: It is not acceptable to handle any of the above cell lines in a clean bench or horizontal laminar flow hood. Refer to Chapter 16 for more information on the types of biosafety cabinets. Researchers handling cells outside of a biosafety cabinet must wear personal protective equipment (gloves, lab coats and eye protection).

Handling frozen cell cultures

Storage and retrieval of frozen cell cultures from liquid nitrogen requires appropriate Personal Protection Equipment (PPE). The three major risks associated with liquid nitrogen (-196° C) are: frostbite, asphyxiation, and exposure. Gloves thick enough to act as insulation but flexible enough to allow manipulation of ampoules should be worn. When liquid nitrogen boils off during routine use of the freezer, regular ventilation is sufficient to remove excess nitrogen, but when nitrogen is being dispensed, or a lot of material is being inserted in the freezer, extra ventilation will be necessary.

When ampoules are submerged in nitrogen, a high-pressure difference results between the outside and the inside of the ampoule. If it is not perfectly sealed, this results in inspiration of liquid nitrogen which will cause the ampoule to explode violently when thawed. Wear eye protection and a face shield. This can be avoided by storing the ampoules in the gas phase or by ensuring that the ampoules are perfectly sealed. Thawing from storage under liquid nitrogen must always be performed in a container with a lid, such as a plastic bucket and eye protection and face shields must be worn.

Quiz

1. Human blood, blood products, bodily fluids and tissues are listed as Potentially Infectious Material (PIM) according to the OSHA Bloodborne Pathogen Standard. Accordingly, what level of biosafety level practices and procedures must be adhered to.

- ❑ Biosafety Level (BSL-1).
- ❑ Biosafety Level (BSL-2).
- ❑ Biosafety Level (BSL-3).
- ❑ All of the above.

2. The main reason why BSL-2 level practices and procedures are used for PIM is because:

- ❑ Blood spills are messy.
- ❑ PIM must be put into double red bags for disposal.
- ❑ Bloodborne infection prevention
- ❑ None of the above

3. From a risk standpoint, contracting Human Immunodeficiency Virus (HIV) is much more common than Hepatitis.

- ❑ True.
- ❑ False.

4. The OSHA Bloodborne pathogen standard mandates the following controls be implemented to reduce the risk of occupational exposure to bloodborne infection:

- ❑ Engineering including ventilation.
- ❑ Work practices.
- ❑ Employee training.
- ❑ All of the above.

5. The Risk Management and Safety Department (RM&S) conducts mandatory safety training for all University of Arizona employees working with bloodborne pathogens.

- ❑ True.
- ❑ False.

6. Laboratory personnel (faculty and staff) who work with Human Immunodeficiency Virus (HIV) or Hepatitis B Virus (HBV) or materials suspected of harboring these viruses must fulfill additional requirements as follows:

- ❑ Must attend one time general laboratory safety training offered by Risk Management.
- ❑ Laboratory personnel must have prior experience working with HIV or HBV..

- ❑ Must demonstrate proficiency in working with HIV or HBV to Principal Investigator (PI) or the PI must develop progression of work activities as techniques are learned and proficiency developed .
- ❑ All of the above.

7. The risk of acquiring a laboratory acquired infection (LAI) from human or primate cell culture sources is considered low, and therefore exempt from special biosafety precautions.

- ❑ True.
- ❑ False.

8. Cell cultures that contain the following must be classified at the same level as the agent:

- ❑ Etiologic agents.
- ❑ Oncogenic virus.
- ❑ Amphotropic packaging agent.
- ❑ All of the above.

9. Horizontal laminar flow benches (Clean Benches) are acceptable for all tissue culture work.

- ❑ True.
- ❑ False.

10. Storage and retrieval of frozen cell cultures from liquid nitrogen involve all of the following hazards except:

- ❑ Electrocution.
- ❑ Frostbite.
- ❑ Asphyxiation.
- ❑ Explosion of ampoules.