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Research Compliance & Ethics / IBC
Standard Operating Procedure - Guidelines

Use of Fixed and Unfixed Human Central Nervous System (CNS) Tissue

Purpose:
This document provides standard procedures for the use of fixed and unfixed human central nervous system (CNS) tissue in research.

Scope:
This SOP was developed by the University of North Dakota Institutional Biosafety Committee (IBC) based on the current guidelines published by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO). Each site handling CNS tissues is required to conduct a site and protocol specific risk assessment. This document is to be used as a starting point to develop laboratory protocols and additional criteria for the safe handling of CNS tissues. In addition, all sites must comply with national and local requirements.

Hazards:
Hazards associated with human CNS tissue include exposure to bloodborne pathogens including HIV, Hepatitis B, Hepatitis C, and prions. Prion disease is a neurodegenerative and fatal disease affecting humans, wildlife, and domestic animals. Prions are proteins which may cause normal proteins to misfold, clump together, and damage the brain leading to dementia, difficulties in movement, personality changes and death. There is no cure for prion diseases.

All human brains and other potentially infectious CNS materials must be handled using the procedures outlined below, even if the material shows no evidence of infection. All CNS materials should be handled in an appropriately designated laboratory. There is no evidence of contact or aerosol transmission of prions from one human to another. Routes of exposure may include cuts/punctures or ingestion due to poor hand hygiene.

Note: If material known or suspected of containing prions such as Creutzfeldt-Jakob Disease (CJD), then dispose of and use an alternative tissue (unless studying prions) if possible. Formalin (formaldehyde) fixing of tissue does not destroy prions and can actually stabilize them, allowing them to remain infective.

Training Requirements:

All lab personnel that come in contact with or could potentially come in contact with CNS tissue are required to complete the bloodborne pathogen training and the CITI Biosafety Training. In addition, all personnel handling CNS tissue must be trained in the hazards of CJD and specific hazards associated with handling CNS tissue.

Procedures:

1. **Handling Guidelines for Working with Human CNS Tissue**
    a. The Laboratory must be designated as a minimum of a Biosafety Level 2 (BSL2) area. A contact name, after-hours telephone number and the types of biological agents being used in laboratory must be clearly posted.
    b. All laboratory staff should be documented as having their Hepatitis B vaccination, or a record of a declination form being signed and being in compliance with local requirements (e.g. titer)
    c. Treat all CNS tissues as potentially infectious.
    d. All fixed or unfixed CNS tissues, including wax blocks, must be stored within a watertight container labeled with the universal biohazard symbol. Sections secured under a mounted cover glass may be stored in a suitable container, labeled with the Biohazard symbol.
    e. All samples must be properly labeled and, if ethically appropriate, be traceable to their original source. Because the outside of a tube may be contaminated, tubes or containers should be handled with care and while wearing gloves.
    f. All work with CNS tissue is to be conducted in a proper containment device to contain aerosols.
        i. All vacuum lines shall be fitted with a HEPA or 0.2um filter.
        ii. Centrifugation must be done in closed containers and using sealed rotors/ or sealed buckets.
        iii. Sonication or homogenization of tissues must be performed in a properly certified Class II biosafety cabinet.
    g. All tissues, infectious waste and instruments must be decontaminated as described below.
    h. Microtome blades and knives used for cutting tissue must be cleaned with an instrument that does not put the hand or finger(s) of the operator in or near contact with the blade.

2. **Personal Protective Equipment (PPE):**
    a. Lab Coats: Lab coats are adequate for tissue manipulations. However, it is recommended that disposable arm sleeves be used during tissue manipulations.

b. Gloves: Double gloves are advisable for all procedures involving human CNS tissue. If gloves become contaminated they must be removed immediately and disposed in the biohazard bin.
c. Eye protection: Safety glasses or goggles for work involving splash, spray, or other eye hazards.
d. Respiratory protection: Use a properly maintained and inspected biological safety cabinet for procedures with a potential for creating infectious aerosols and splashes. If the procedure cannot take place in a safety cabinet then the use of a 'fit tested' respirator (N-95 or better) and enrollment in the UND respiratory protection program is mandatory.

***** All personnel must wash their hands thoroughly with appropriate disinfectant and warm water upon completion of laboratory activities, removal of protective clothing and prior to leaving the laboratory.

****** All personal protective equipment must remain within the laboratory.

3. Cleaning and Disinfecting Laboratory Areas and Equipment

a. All tissues, wastes, and instruments (e.g. specimen containers, knives, microtome blades, bench tops, etc.) can be disinfected by one of the options below:
    i. Immerse in 1.0N Sodium Hydroxide (NaOH)* or 2% sodium hypochlorite** for 1 hour. Transfer into water or rinse and autoclave*** (gravity displacement) for 1 hour at 121^{o}C.
    ii. Heat sensitive material can be treated with 2.0N NaOH or 2% sodium hypochlorite for 1 hour. Surfaces must remain wet for the entire hour then rinse well with water.
    iii. Immerse in 1.0N NaOH and autoclave using gravity displacement for 30 min at 121^{o}C. Clean rinse in water and sterilize by conventional means.
    iv. Disposable items may be sent for incineration.

*** 1N NaOH = 40 g NaOH per Liter water. Prepare daily. Alternatively, a stock solution of 10 N NaOH may be prepared with working 1N solutions made daily.**

**** 2% (20,000 ppm) sodium hypochlorite = 1 part bleach (6.15% stock) + 2 parts water. Prepare daily. 1:3 v/v dilution of commercial household EPA bleach.**

***** NaOH spills or gas may damage an autoclave. Containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended.**

Practice extreme caution for handling hot NaOH solutions and preventing gaseous exposure post-autoclave. Allow to cool before removal from autoclave. It is recommended to neutralize bleach with thiosulfate prior to autoclaving to prevent the release of chlorine gas.

b. Wherever possible cover work surfaces with a disposable material such as bench guard or use a spill tray with a liner. These can then be disposed of appropriately at the end of the procedure. Alternatively, wipe the bench with 2N sodium hydroxide (NaOH) for one hour or 2% sodium hypochlorite (NaOCI) and then rinse off. Be sure to ensure the bench is resistant to these chemicals and appropriate signage is used to prevent injury.
c. It is the responsibility of the users to ensure that all equipment is properly decontaminated before any service personnel are requested to repair equipment. The equipment must be tagged stating the method of decontamination.
d. Equipment leaving the area must be properly disinfected and tagged.

Disposal:

1. Solid Waste: All potentially contaminated materials must be either disinfected and then disposed of as infectious waste in the proper biohazardous containers (except for sharps) or incinerated on-site.
2. All contaminated sharps, including broken glassware, must be disposed of in authorized sharps containers then placed in authorized biohazard disposal bins-do not autoclave or otherwise treat.
3. Liquid waste: Contaminated liquid waste <u>should</u> be decontaminated either by autoclaving or with an appropriate disinfectant prior to disposal (if chemically suitable). Alternatively, absorb and incinerate in an appropriate container.

Accidents:

1. **Spills**:
   In the event of an accidental spillage or breakage of a sample the following decontamination protocol should be utilized.
   a. Do not inspect the spill closely.
   b. If aerosols could have been generated, leave the area immediately and contact the Biological Safety Officer.
   c. If aerosols could have been generated, bar all potential entrances to the laboratory for 30 minutes (to allow aerosols to settle)
   d. Enter the lab with all appropriate PPE, or don the appropriate disposable PPE.

e. Use appropriate equipment (dustpan, brush, forceps, etc) to handle broken containers.
f. Place paper towels or other absorbent material over the spill to absorb and contain. Cover the absorbent paper towels with disinfectant and let sit for 2 hours for 2N NaOH or 2. % sodium hypochlorite.
g. Remove towels and dispose of contaminated materials in proper biohazardous containers.

2. Personal Contamination:

a. If skin becomes contaminated with potentially infectious material, rinse the area and wash thoroughly with soap and water. Contact Occupational Health Provider and your Biological Safety Officer.
b. If eyes become contaminated, they should be washed immediately with water using an eyewash station. Report to Occupational Health Provider and notify the Biological Safety Officer.
c. Should an injury occur, the individual and other lab occupants must seek help via established site procedures. The Biological Safety Officer should be notified.

Sources of Information:

Biosafety in Microbiological and Biomedical Laboratories 6th edition, USSH Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, June 2020

WHO Guiding Principles on Human Cell, Tissue, and Organ Transplantation, WHO, 2010.