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🌐 Human Dorsal Root Ganglion Tissue Procurement from Thoracic Vertebrectomy Patients at the University of Texas MD Anderson Cancer Center

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PRECISION Human Pain ...



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We use this protocol and it's working

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Abstract

Purpose

Describe the procedures involved in removal, transport, processing, and storage of human dorsal root ganglion tissue from thoracic vertebrectomy patients at MD Anderson Cancer Center.

Responsibility

The Human Tissue Procurement Core at MD Anderson Cancer Center is responsible for maintaining this protocol, ensuring it is updated and controlled, and ensuring that users receive proper training prior to its instrumentation.

Definitions and Abbreviations

DOB = date of birth

DRG = dorsal root ganglion

EBSS = Earle's Balanced Salt Solution

ID = identification number, the unique number assigned to the patient by the Human Tissue Procurement Team

MDACC = University of Texas MD Anderson Cancer Center

OR = Operating Room

PPE = personal protective equipment

Tissue Recovery Sites

Main ORs, University of Texas MD
Anderson Cancer Center

Tissue Recovery Teams

MD Anderson Surgical Staff: Spinal Surgeons, OR Surgical Technicians, and OR Nurses

MD Anderson Tissue Procurement Team: Research Assistants, Graduate Students, Post-Doctoral Research Fellows, or Faculty



Materials

Collection

1. Scrubs/surgical wear
2. EBSS, gibco 14155-063
3. Centrifuge tubes (50 mL,
4. Parafilm
5. Ice
6. Cooler with biohazard sticker
7. high-speed drill (Medtronic MidasRex) or ultrasonic bone cutting tool (Misonix Bone scalpel)
8. ronguers or curettes
9. right-angle clamp
10. 2-0 silk ligature
11. a tenotomy scissor

Processing

1. 70% ethanol (decontamination before and after procedures)
2. EBSS, gibco 14155-063
3. 100 mm Petri dishes (sterile disposable)
4. Freezer block
5. Aluminum foil
6. scalpels (sterile disposable)
7. forceps (autoclaved)
8. scissors (autoclaved)
9. 1.5 mL or 2 mL centrifuge tubes (sterile)
10. Dry ice or liquid nitrogen for freezing
11. Bleach (decontamination after procedures)



Safety warnings



General Safety Procedures:

1. Laboratory personnel must complete training in laboratory safety and refresh every three years. Personnel must also complete Human Subject Protection Training and Hazard Communication and Bloodborne Pathogen Training. Individuals involved in shipment of tissue samples must also complete Shipping Infectious and Biological Materials Training and refresh every three years.
2. After completion of introductory training modules, laboratory staff are added to the IRB protocol for human DRG tissue studies. No staff may transport specimens without being added to this protocol.
3. Laboratory personnel must be screened and cleared by Employee Health before work with potentially infectious materials. Personnel entering the laboratory will receive specific training related to these hazards and safety precautions prior to approval of work with human tissue specimens. Doors should remain locked when not in use and access to the lab limited to approved staff or those with Primary Investigator permission.
4. All human specimens will be treated as if infectious with Universal Precautions.

Ethics statement

All human subjects research is performed in accordance with approved IRB protocols by personnel who have completed Human Subject Protection training.

DRG Recovery Notification

- 1 MDACC spine surgeon notifies the MDACC Tissue Procurement Team of a patient who is to undergo a neurosurgical procedure that will involve the sacrifice of one or more spinal nerves as standard of care.
Surgeon provides the following information:
 - 1) Patient identification
 - 2) OR time and date
 - 3) Expected number of DRG
- 2 The MDACC Tissue Procurement Team reviews the information and accepts or declines the donor for DRG Study Enrollment. Accepted donors are consented and registered.

Tissue Procurement

- 3 At the start of surgery, the MDACC Tissue Procurement Team prepares the materials/equipment to receive and process tissue in the laboratory at MDACC.
- 3.1 In a culture hood, 25 mL of chilled (4C) media (EBSS, gibco 14155-063) is added to 50 mL centrifuge tubes which are labeled, dated, and sealed with parafilm.
- 3.2 Specimen collection tubes are then placed on ice in a leak-proof cooler with a biohazard sticker and lab contact information.
- 4 MDACC Tissue Procurement Team personnel responsible for specimen transport will don scrubs and transport the cooler to the OR.
- 4.1 Prior to entering the restricted access area, personnel apply hand sanitizer and don appropriate PPE (a surgical mask and surgical cap or head cover).
- 4.2 The cooler is brought into the OR and MDACC Tissue Procurement Team personnel verify with OR staff that the surgeon is aware of the protocol procedure and the patient consent forms and tissue bank request have been completed and confirm contact information for specimens or additional specimen collection tubes.
- 4.3 The labeled cooler with storage tubes and sterile media is placed outside of the sterile surgical field.
- 5 All portions of the surgical procedure and tissue collection are performed by the MDACC Surgical Staff.

- 5.1 Each surgical procedure includes a removal of posterior bony elements including spinous process, lamina, facet joints, and possibly the vertebral body of the affected levels. A high-speed drill (Medtronic MidasRex) or ultrasonic bone cutting tool (Misonix Bone scalpel) are used for removal of the spinous process, lamina, facet joints, facets.
- 5.2 Additional bone removal may be necessary using ronguers or curettes in order to complete exposure of the spinal dura (Figure 1, white triangle) and proximal spine nerve and DRG (Figure 1, white arrow).



Figure 1.

After posterior bony elements have been removed, the spinal dura (white triangle) and proximal spine nerve and DRG (white arrow) are exposed.

- 5.3 After completion of the removal of posterior bony elements and exposure of the spinal dura a high-speed drill and curettes are used to remove the most dorsal portion of the pedicles of the involved vertebral levels.
- 5.4 A right-angle clamp is used to isolate the nerve roots. Each nerve root is ligated using a 2-0 silk ligature and sharply cut using a tenotomy scissor with care to divide the nerve proximal and distal to the dorsal root ganglion.
- 5.5 The dorsal root ganglion is then removed and placed directly into the specimen collection tubes containing sterile media (EBSS). The storage tubes are marked with the spinal level, laterality, and patient identification. The storage tubes are sealed and returned to ice filled cooler. The MDACC Tissue Procurement Team is then notified of the tissue specimen(s).
- 6 Upon receiving notification of availability for tissue specimens, MDACC Tissue Procurement Team personnel involved in transport don scrubs and PPE (surgical mask

and head cover) and return to the OR. In the OR:

- 6.1 Confirm that the specimens are in the collection tubes and have been placed back in the cooler and that the tubes have each been labeled by staff for the research protocol appropriately as per MDACC surgical biospecimen collection guidelines for research.
- 7 The cooler is taken to surgical pathology for specimens to be registered. Once processing is complete, the specimens are released to the transporter and brought to the laboratory.
- 8 Universal precautions are followed for handling specimens upon arrival in the laboratory:
 - 8.1 Wash hands thoroughly before procedures.
 - 8.2 Lab coat, gloves, and safety glasses must be worn during processing of specimens. Used gloves are to be discarded in biohazard waste bins, not reused.
 - 8.3 All sharp objects must be disposed in an approved sharps container within easy reach immediately after use. No sharps are reused or recapped.
 - 8.4 Gloves are removed and hands washed before leaving the specimen processing area.

Specimen Processing

- 9 Specimens are kept in labeled collection tubes on ice or at 4C until processed.
- 10 Specimen processing area is kept clear, and all surfaces wiped clean with 70% ethanol prior to any procedures. Tools are sterile disposables (i.e., Petri dishes, scalpels) or autoclaved (forceps, scissors) and changed out between specimens. Reusable tools are soaked in soap and water with 10% bleach for 30 min, cleaned of tissue debris, and dried fully before being autoclaved. Disposable tools are discarded in biohazard bins or sharps containers.
- 11 Specimens are transferred from collection tubes to a 100 mm Petri dish with chilled EBSS (4C) on a freezer block wrapped with aluminum foil.
- 12 Using sterile scalpel, forceps, and scissors, remove tissue surrounding ganglion, including connective tissue, nerve root, fat, and muscle.



- 12.1 You should be left with a small, round ganglion in its capsule that can be distinguished from the surrounding tissue as it feels much tougher when probed. Some larger nerve roots can be mistaken for ganglia, but the difference will be apparent when you dissect them apart. Ganglion sizes range from small (approx. 5 mm diameter thoracic) to large (over 15 mm diameter lumbar). Within the capsule, ganglion tissue is distinguishable from the nerve root by its color. The soma appears light to dark yellow and may have small dark brown dots visible (especially prevalent in older patient tissue), while nerves are pure white and will show up as thin white streaks.
- 12.2 Freezing: Label a sterile 1.5 mL or 2 mL centrifuge tube with the lab ID number for the patient and the level/side (i.e., AA111 T12L). Place the specimen inside the tube and place in a cooler on dry ice or submerge in a container of liquid nitrogen. Alternatively, pulverize dry ice with a mallet and bury the specimen in powdered dry ice for 3-5 min before moving to a labeled 15 mL tube. Store at -80C until shipped.

After completion of procedures

- 13 After dissection, any remaining fluid and tissue is treated with fresh 10% bleach and discarded in biohazard waste bins after 30 min. All work surfaces are decontaminated after the end of procedures. All tools and supplies are discarded or cleaned and all surfaces treated with disinfectant such as fresh 10% bleach for 30 min followed by wiping with 70% ethanol.
- 14 All disposable supplies and decontaminated tissue are disposed in biohazard bins.
- 15 Specimen storage:
 1. Refrigerators and freezers used for specimen storage are accessible only by approved personnel and marked with biohazard indicators
 2. Fresh frozen specimens are stored in -80C freezer in boxes indicating human samples on the label