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Human Tissue Procurement from Lumbar Fusion Surgery for Chronic Low Back Pain

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We use this protocol and it's working

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Abstract

Purpose

Describe the human tissue removal, preservation, storage, and transportation procedures during tissue procurement from patients undergoing lumbar arthrodesis surgery (surgical immobilization of joint by fusion of the adjacent vertebrae) either via posterior or anterior approach.

Responsibility

The clinical research team at the University of Washington is responsible for maintaining this protocol, ensuring it is updated and controlled, and ensuring that users receive proper training prior to its implementation. The team operates under the guidance of the University of Texas at Dallas Human Tissue Procurement and Processing Core.

Definitions and Abbreviations

OR = operating room

PBS= Phosphate Buffered Saline

PPE = personal protective equipment

UTD = The University of Texas at Dallas

UW = University of Washington

Tissue Recovery Sites

Local hospitals

Tissue Recovery Teams

UW Surgical Staff

UW Research Coordinator

UW Scrub Nurse

Image Attribution

Illustration citation: DePuy Synthes. (n.d.). *Posterior lumbar fusion surgery* | *depuy synthes*. Johnson&Johnson Medtech. <https://www.jnjmedtech.com/en-US/treatment/spine/posterior-lumbar-fusion>



Materials

Materials provided by the hospital:

1. PPE including surgical scrubs gloves, hair nets, led vests and shoe covers
2. Waste disposal bins
3. Disinfectant wipe

Materials provided by UW Research Team

1. Powdered dry ice (see below)
2. Containers for dry ice (cooler, with biohazard label on the lid)
3. Sterile dissection tools (small forceps, scissors)
4. Sterile storage tubes (1/5mL epitubes, 5mL epitubes)
5. RNA*later* Stabilization Solution (RNA*later*, kept at room temperature) - ThermoFisher Scientific, catalog #: AM7024
6. Phosphate Buffer Solution, 1X (PBS)
7. Labels
8. Stickers
9. Tissue collection form
10. Shipping boxes
11. Sharpies

Powdered Dry Ice

- Dry ice (pellets or blocks) must be pulverized to a fine powder to ensure proper freezing of human tissues. Using a hammer, crush the dry ice in an ice bucket (Globe Scientific 2.5L Rectangular Ice buckets with Lid) or styrofoam container.
- To ensure that the dry ice particulates are small enough, run the pulverized dry ice through a mesh grate/netting.

Lumbar Tissue Recovery Notification

- 1 Patients scheduled for lumbar instrumentation and fusion will be identified through one of the following methods:

- EPIC Electronic Screening Report
- Clinic Visit Identification

Once identified, the study coordinator will review the patient's EMR to confirm eligibility based on study criteria. If eligible, the coordinator may initiate contact using a pre-approved script over the phone or discuss participation during the patient's pre-operative visit to obtain consent.

- 2 The research coordinator will approach potential participants and provide a detailed explanation of the study, including risks, benefits, and procedures. Consent will be obtained in accordance with ethical guidelines and institutional review board (IRB) standards. If the patient is identified through outpatient or inpatient settings, the coordinator will ensure the patient is fully informed and comfortable before consent.

- 2.1 Exclusion criteria include:

- patient being <18 years of age
- unable to consent due to language comprehension or non-English speaking, being unable to consent due to cognitive deficit
- undergoing cancer treatment

OR

- currently incarcerated.

- 3 Obtain baseline information regarding participants in accordance with study guidelines.

Surgical Procedure

- 4 The research coordinators prepare the materials/equipment and wait for the allotted time to join the surgeon in the OR.

- 5 The research coordinator dons PPE, enters the OR, and sets up their materials/equipment on a table within the OR.

- 5.1 **Incision and Exposure:**

A 4–8 cm vertical cut is made along the midline of the lower back, directly over the spinal area needing treatment. The **thoracolumbar fascia**, a thick connective tissue covering the muscles of the lower back, is carefully opened. Below this layer, the **paraspinal**

muscles—which support and move the spine—are gently separated to expose the bones of the spine.

5.2 **Visualization and Confirmation:**

Once the area is exposed, metal retractors are used to hold the muscles aside, giving the surgeon a clear view of the back of the spine. An X-ray is taken to confirm the exact spinal levels being operated on.

5.3 **Bone and Joint Preparation:**

The **facet joints**, which connect the vertebrae and allow for movement, are carefully removed and their surfaces roughened (**decorticated**) to encourage new bone growth. This prepares the area for spinal fusion. The **transverse processes** (small bony projections on the vertebrae) are also prepared in this way. Bone graft material, often taken from the patient or a donor, is placed in these areas to promote the vertebrae to grow together and stabilize the spine.

5.4 **Placement of Hardware:**

Metal screws (**pedicle screws**) are placed on each side of the targeted vertebrae and connected with titanium rods to hold the spine in proper alignment as it heals (ref figure 1).

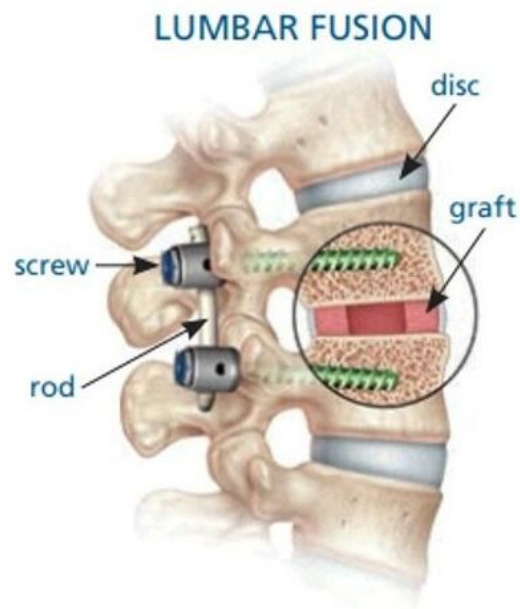


Figure 1. Illustration depicting the placement of pedicle screws, connecting rods, and bone graft material in relation to the adjacent, non-operative intervertebral disc

5.5 Nerve Decompression (if needed):

If nerves are compressed, part of the vertebra called the **lamina** may be removed (**laminectomy**) to create more space. Bone spurs or disc fragments pressing on the nerves are carefully removed using small surgical tools. The **ligamentum flavum**, a thick ligament in the spine, may also be trimmed or removed if it is causing pressure.

Tissues Collected for Research - Day 1

- 6 During the surgery, specific tissues normally cauterized or resected and discarded during the surgical procedure instead are carefully resected for research purposes to better understand spinal conditions:
 - **Thoracolumbar fascia:** A tough sheet of connective tissue covering the back muscles.
 - **Paraspinal Muscles:** The muscles that run alongside the spine, helping with posture and movement.

- **Facet Joint and Cartilage:** The small joints between vertebrae and their smooth cartilage surfaces that allow the spine to bend and twist.
- **Intervertebral Disc Annulus and Nucleus:** The disc between vertebrae consists of two parts, 1) the tough outer ring (annulus fibrosus) and 2) the soft, jelly-like center (nucleus pulposus) that cushions the spine.
- **Endplate:** A thin layer of cartilage and bone located at the top and bottom of each vertebra, adjacent to the intervertebral disc. The endplate separates the vertebral bone from the intervertebral disc and acts as a barrier to prevent the highly hydrated nucleus pulposus from bulging into the adjacent vertebral body.
- **Medial Branch of the Dorsal Ramus:** A small nerve branch that carries pain signals

6.1 Once removed, the surgeon transfers the tissue to the scrub nurse. During this transfer, the surgeon notifies the research coordinator of the tissue specimen type (e.g., thoracolumbar fascia, paraspinal muscle, intervertebral disc -- annulus), laterality, and any irregularities.

The level and extent of tissue received can vary from patient-to-patient. As a result, tissue size and quality may vary.

7 The scrub nurse removes the tissue from the surgical instrument and places this on TELFA or a sample petri dish, which is then passed to the research coordinator.

8 The research coordinator flushes the tissues with 4-7 mL of PBS to remove excessive blood before placing the the majority of tissues into epitubes filled with RNAlater (see next step).

NOTE: It is the responsibility of the coordinator to communicate with the nurse concerning PBS use, as some nurses will do this by default prior to transferring the tissue.

9 The majority of each tissue type should be fully submerged in RNAlater. Depending on the tissue size, either a 5 mL or 1.5 mL Eppendorf tube will be used. If the total tissue volume exceeds what can be accommodated in two 5 mL tubes of RNAlater, any remaining tissue should be flash-frozen immediately without prior exposure to RNAlater to preserve it for alternative processing methods. Flash-frozen tissues must be placed on powdered dry ice for 2-3 minutes before being transferred to an Eppendorf tube.

Any tubes will be labeled with the research ID, date, biospecimen information, and if they were flushed with PBS (#) or processed in RNAlater solution (*).

- Label example: "01, MM/DD/YY, C.L #*, L4-5". In this instance, # indicates PBS was used, and * indicates RNAlater was used.

Packing, Clean-up, and Transport - Day 1



- 10 Excess biofluids, TELFA, and petri dishes are disposed of in OR waste bins.
- 11 Used tools and sharpies are placed into a large Ziplock bag and sealed, to be sterilized by research coordinators.
- 12 The dry ice cooler containing the biospecimen tubes, and Ziplock bag are taken with the research coordinator.
- 13 Upon exiting the OR, PPE research coordinator removes all PPE and take flash frozen biospecimens to the freezer, and any biospecimens placed in a refrigerator (4 Degrees C) for overnight treatment.

Tissues Collected for Research - Day 2-4

- 14 The research coordinators prepare the necessary materials and equipment, and retrieve the RNAlater-preserved samples from the refrigerator immediately before flash freezing.
- 15 To flash freeze samples:
 - 15.1 Remove tissue section from RNAlater using tweezers
 - 15.2 Place in powdered dry ice
 - 15.3 Cover to freeze for 2-3 minutes
 - 15.4 Flash frozen tissues are then transferred to a fresh Eppendorf tube, and the previous label (see Step 9) is re-written onto the new tube.

Packing, Clean-up, and Transport - Day 2

- 16 Excess biofluids, epitubes, and baggies are disposed of in hazardous waste bins.
- 17 Used tools and sharpies are placed into a large Ziploc bag and sealed, to be sterilized by research coordinators.



- 18 The dry ice cooler containing the biospecimen tubes, and Ziplock bag are taken with the research coordinator.
- 19 The research coordinator takes the flash frozen biospecimens from step 15.4 to the -80°C freezer.

Biospecimen Logging, Storage, and Shipment

- 20 All frozen biospecimen tubes collected from a research subject are placed into a cardboard cryo freezer box that is labeled on all sides with the study and PIs name. These boxes are stored in a secure -80°C freezer.
- 21 All biospecimen tubes are logged on a tissue storage log that resides within a protected data server. The following information is kept in the tissue log that resides within a protected data server. The following information is kept in the tissue log:
 - Research ID
 - Age
 - Sex
 - Race
 - Ethnicity
 - Medical History (both EMR retrieval and patient self-report during interview)
- 22 Images of the tissue within the OR (after flash freezing) are placed into designated folders on the protected data server for each subject.
- 23 Once 5 patient samples are stored, tissues to be shipped to UTD on dry ice, maintaining the standards outlined by the US Department of Transportation and Hazardous Material Regulations 49 CFR § 100-185. UTD team is notified of shipment and sent tracking information via email.
- 24 Tissue is logged and stored at -80°C upon arrival.