

Processing Human Temporal Bones

* Standard Operating Procedure (SOP): A document which describes the regularly recurring operations relevant to the quality of the investigation.

※ Researchers should follow written SOPs that describe the work to be conducted, and the safety measures to mitigate any hazards.

Principle Investigator:

Otolaryngology Laboratory

University of Minnesota – Head and Neck Surgery

Rm. 210 Lions Research Building, 2001 6th Street S.E. Minneapolis, MN 55455

SOP Prepared By: Grace Song

Approved By: _____ Date: _____

Signed copies of SOPs are in 3-ring SOP binders in Rm. 210 Lions Research Building.

Original Issue Date:

Revision Date:

Materials:

Supplies:

Glass jars (depends on how many bones are removed; usually 1 glass jar for each bone) with lids

Pipettes (1ml, 5ml, and 10ml)

Pipette controller

Scalpel

Tweezers

Metal toothpick

Paper towels

Chemicals:

10% buffered formalin

Phosphate buffer

Ethylenediaminetetraacetic acid (EDTA)

DI water and tap water

70% alcohol

35%, 50%, 70%, 80%, 90%, and 100% alcohol solutions

½ Ether and ½ Alcohol solution

1.5%, 3%, 6%, and 12% celloidin solutions

Chloroform

Procedure:

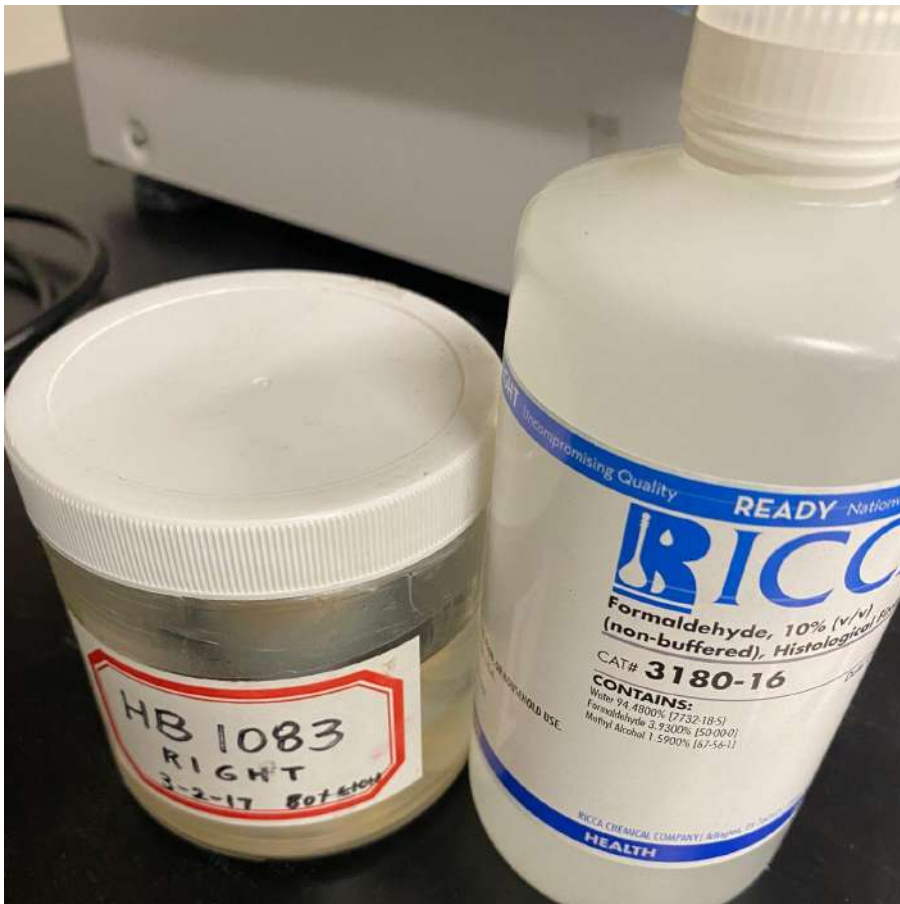
Preparation and removal of Temporal Bones

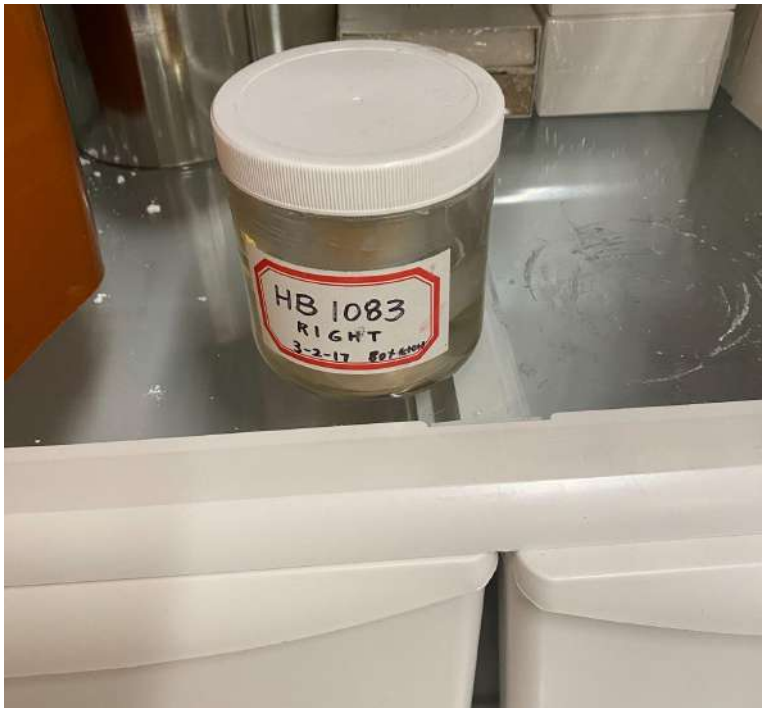
1. Before removal, check the removal kit to make sure we have enough gloves in appropriate sizes, the autopsy slips, and the tools are all there including a plastic bag for dirty tools
2. Get two jars filled with 10% buffered formalin
3. Label the jars with temporal bone number next in sequence and the sides of the ears (left and right)
4. Place the jars in the small cooler with towels
5. When going to the removal site, DO NOT take campus connector or public transportation because the formalin is toxic; can take personal car if weather is unfavorable
6. Once at the removal site, write down the information required on autopsy slip

- a. MOST IMPORTANT if no information is provided by the autopsy technicians, write down the hospital number and date of birth
 - b. The hospital number will help track the patient's information on the EPIC portal
 - c. For ABP, medical history will be provided by staff in a separate email
7. Double glove if you are the person touching everything that includes the jars and tool box
 8. After the removal, take off the dirty gloves and with the clean gloves put everything away and make sure we have all of our tools accounted for
 9. Back at the lab, put all the dirty tools in a silver bin (located under the second sink) with bleach and detergent and DI water to clean the tools
 10. Change the bloody formalin from removal with fresh solutions
 11. Waste the formalin solutions in a waste container labeled as 10% formalin (formaldehyde)
 12. Place the bones in the refrigerator for 1 week
 13. Wash all the tool including the drill
 14. Get a couple of gauze and wet it in the bleach and detergent solution
 15. Wipe down the drill; make sure to get the on and off switch
 16. DO NOT PLACE THE ENTIRE DRILL IN WATER
 17. Put the drill back in the tool box and leave the lid open to dry overnight
 18. The tools that are cleaned will be left on a towel to dry overnight
 19. After the overnight drying, put the tools back into the tool box

Fixation

1. After waiting for a week in the refrigerator incubation of the bones in formalin, change the formalin solution for fresh solution





2. Leave the bones in the hood at room temperature for another week



Decalcification

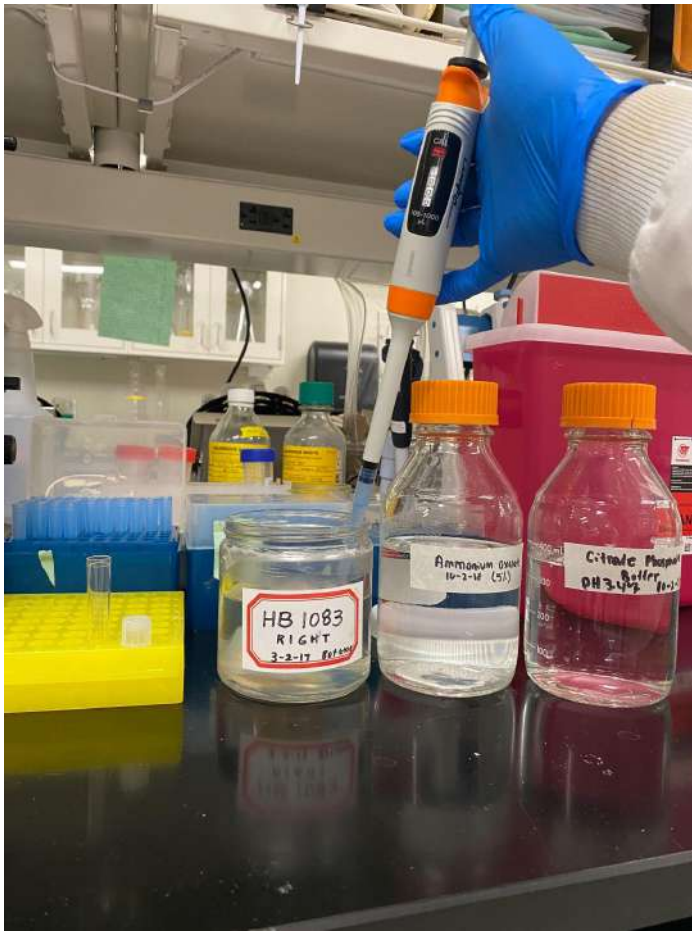
1. After the phosphate buffer stage, the bones will go into EDTA solutions for decalcification
2. Change the EDTA solutions for the bones every 3 to 4 weeks



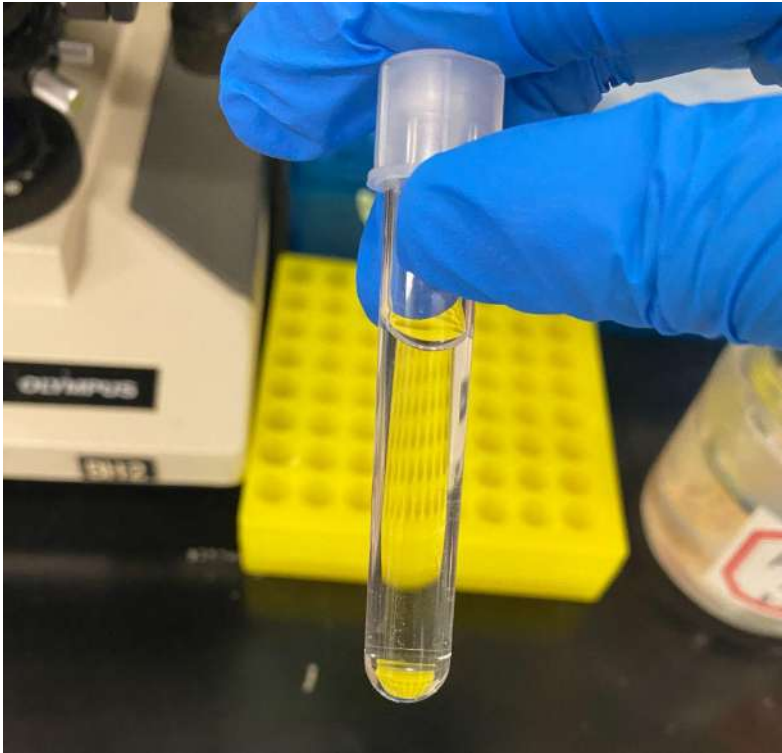
3. For new bones, trimming and calcium tests will not be done until the 5th change of EDTA solutions



4. For each change of solution, it can be wasted down the sink with running water
5. After the 5th change of EDTA solution, calcium tests and some trimming can be done (on separate SOP for calcium tests and trimming)
6. For calcium tests, the old solution of EDTA is needed so do not throw away solutions



7. Leave the calcium tests overnight and check the next day for results
8. If the calcium tests are positive (white particles seen), then the bones need to be changed to new EDTA solutions
9. If the calcium tests are negative (clear solution, no white particles), then the bones need to be changed for new EDTA solutions



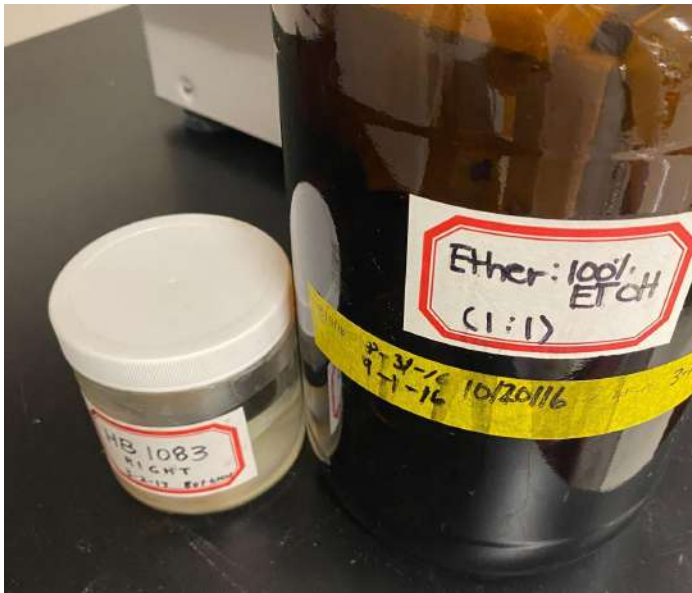
10. The bones will only pass to the next stage in processing if the calcium tests show **3 negative results in a row**
11. For trimming, try to open the superior semi-circular canal (SSC) with a scalpel (trimming will be on a separate SOP)
12. Trim away excess tissue not needed on the bottom of the bone
13. Make sure the bones can stand evenly on a flat surface
14. Once the bones pass the decalcification stage, the bones can move on through the process

Neutralization and Water run

1. After bones are decalcified, they are put in phosphate buffer solutions for 2 days
2. Each time change the solutions for new buffer
3. Following two days, the bones are put in running DI and tap waters that run the entire lab day
4. For the DI water running, place an empty glass jar with a lid that's located near the second sink and put that jar under the jar with the bone
5. No jar is needed for the tap water run
6. The water running is for two days
7. On the second day of water running, switch the bones, so that the bone that was in DI water on the first day is now in tap water, vice versa
8. At the end of each water run, dump out the water and fill both jars with DI water and leave it overnight
 - a. This step is done before leaving work
9. Running the bones in water overnight is not recommended because the sinks might overflow during the night

Dehydration

1. Dump the second day waters in the sink
2. The bones will be now taken through the alcohol stages
3. Each change of alcohol is a day each following this order: 35%, 50%, 70%, 80%, 90%, 90%, 100%, 100%, 100%, $\frac{1}{2}$ ether and $\frac{1}{2}$ alcohol, and $\frac{1}{2}$ ether and $\frac{1}{2}$ alcohol



4. Since we know that at the end of this stage is the celloidin embedding, we want to make sure that the first day of embedding does not fall on a Monday
5. The celloidin solutions have been sitting all weekend and not shaken
6. Calculate when the first day of embedding is and if the day does fall on a Monday, repeat the 80% alcohol change
7. Sometimes the 35% and 50% alcohol solutions will be repeated because the solutions were too cloudy
8. Just make to calculate the days including if there were repeats in alcohol solutions and adjust the schedule to match the embedding day

Embedding

1. Once the dehydration stage is done, the bones are ready for embedding
2. The first embedding medium is the 1.5% celloidin

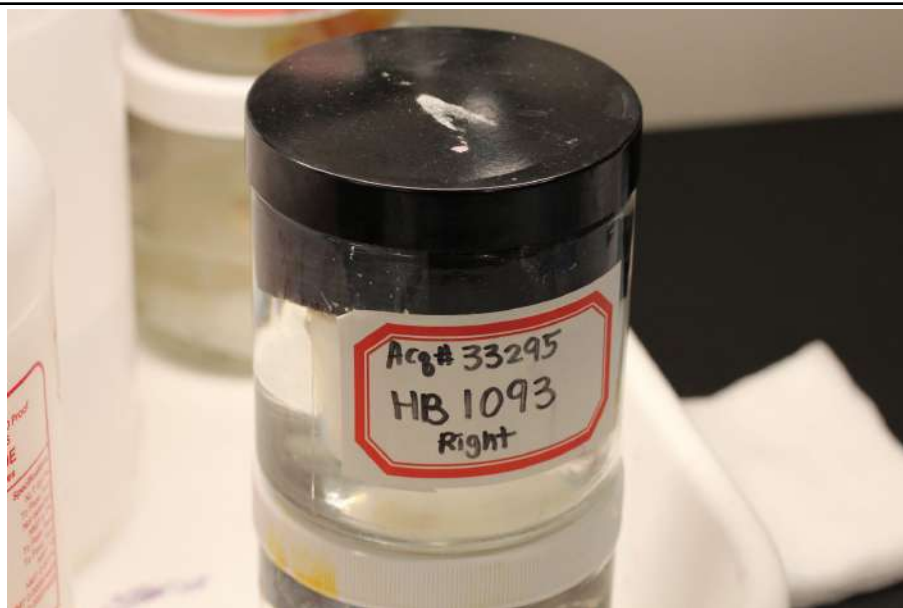


3. Pour the solutions in the jars and turn the bones every hour with a metal toothpick all throughout the day
4. Turning the bones will help the air bubbles come out
5. The bones will sit in 1.5% for 2 weeks

6. Label each jar with labeling tape with the celloidin percentage and date (sometimes label if the solution will be discarded after use)
7. At the end of the day before leaving work, cover the jars with parafilm to prevent evaporation of the ether solution
8. Mark in the calendar on which day the solutions will be changed for next embedding medium
9. If the celloidin solutions are new solutions, save them in a jar labeled as used celloidin with corresponding percentage
10. After two weeks, change the solutions to 3% celloidin, repeat steps 3 to 8
11. The bones will sit in 3% for 3 weeks
12. After three weeks, the bones will be placed in 6% celloidin, repeat steps 3 to 8
13. For 12% celloidin change, use new jars for each bone
14. Pour in 12% and make sure to turn the bones
15. The solution is thicker, so make sure the air bubbles are guided out with the metal tooth pick
16. At the end of the day, center the bone with the SSC facing up in the middle of the jar
17. DO NOT TIP THE GLASS JAR AT ANY TIME BEFORE AND AFTER 12% IS POURED
18. Parafilm the jar and let it sit until embedding medium is firm (usually takes about a year)
19. About a month before the bones reach the year mark, air the 12% celloidin bones by taking off the parafilm
20. When the embedding material is firm, cut an arrow in the celloidin to indicate how the bone will be mounted on slides after staining
 - a. The arrow is cut by having the nerves on top
21. Cut the bone out of the jar and take excess celloidin and save them for later use



22. Pour in chloroform in the glass jars and let the bones embedded in celloidin sink in the chloroform solutions for 1 day
 - a. Allows the bones to harden
23. Change chloroform solution to 80% alcohol solution, sits for 1 day
24. Another 80% alcohol solution change and goes into storage ready to be cut

**Hazard Identification and Risk of Exposure to the Hazards:**

10% formalin buffered solution (formaldehyde)

Ether Ethyl

Celloidin

Chloroform

Exposure Controls Specific to Above Risk of Exposure:

Change solutions in hood

Do not breath in fumes

Wear gloves and lab coat when handling these chemicals

Will differ by protocol. Please refer to the protocol.

(Lab- and procedure-specific personal protective equipment, biological safety cabinet, centrifuge secondary containment, work practices, immunizations if needed, sharps injury prevention, etc.)

Biological Waste Disposal Methods:

None

***For Pick Up:**

FM or call 624-2900 for a pick-up request if biohazardous material is not normally picked up in your area

Spill Response Procedures:

Always in secondary containers (white trays)

Use paper towels to soak up solutions

Record the amount and kind of liquid spilled. Response will vary according to the liquid spilled. *(Lab- and procedure-specific methods)*

May customize the spill template, <http://www.dehs.umn.edu/Docs/DecontaminationTemplate.doc>

Accident Response Procedures:

If Incident Results in a Hazard Exposure (i.e. face or eye splash, cut or puncture with sharps, contact with non-intact skin):

- 1; flush splashes to the nose, mouth, or skin with water; and flush eyes at the nearest eyewash station with clean water for 15 minutes.
- Call 911 or seek medical attention.

Version 1.

- For urgent care employees may go to [HealthPartners](#) Occupational and Environmental Medicine (M/F day time or Urgent Care after hours), or [UMMC-Fairview Hospital](#) (24 hrs). You may seek medical attention at the closest available medical facility or your own healthcare provider.
- Follow-up must be done by HealthPartners Occupational and Environmental Medicine.
- Report the incident to your supervisor as soon as possible, fill out the appropriate documentation.
 - [Employee First Report of Injury](#)
 - [Supervisor Incident Investigation Report](#)
- Send [Incident Report Form](#) to the IBC if exposure has occurred during work on an IBC protocol.
- Report all biohazard exposures to the Office of Occupational Health and Safety (612-626-5008) or uohs@umn.edu.

Note: It is important to fill out all of the appropriate documents to be eligible to collect workers compensation should any complications from the hazardous exposure arise in the future.

References: *(Images and Procedures from <http://www.animalcare.ubc.ca/sop/ACS-2012-TECH10.pdf>), (SOP definition from <http://www.fao.org/docrep/w7295e/w7295e04.htm>)*