BICAN UM1 Rapid Autopsy Protocol – University of California, Irvine

Dr. Eizabeth Head's Lab & Dr. Xiangmin Xu's Lab

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Core Brain Procurement and Processing Team:

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When we have an incoming brain donation, please contact the following people and ask if they are available to help with brain processing:

List of contact personnel

- Dr. Preston Cartagena, pcartage@hs.uci.edu
- Dr. Adolfo Sequeira, psequeir@hs.uci.edu
- Dr. Firoza Mamdani, fmamdani@hs.uci.edu
- Gabriel Hui, MPH – Head Lab Manager (only available from 8-5pm), huig@uci.edu

If the above personnel are not available, then contact the Repository Team:

- Sierra Wright, stwright@hs.uci.edu
- Brianna Gawronski, bgawrons@uci.edu
- Kevin Wood, woodka@uci.edu
- Lourdes Gonzalez, lourdeg3@uci.edu

If they are unavailable, reach out to Dr. Elizabeth Head (heade@uci.edu) and ask if she can find Head Lab members who are available to help.

Use this Check list link to find a list of things that need to be done during prior to, during, and at the end of the Rapid Autopsy Protocol.

Scope

- This procedure will be applicable for all brains received by University of California, Irvine's Multiomic Brain Cell Atlas Network Brain Donation Program

Purpose

- This SOP provides all of the necessary steps to perform a rapid brain autospy and brain processing procedure.

Recruitment

- Please refer to the in-person recruitment script and instructions when approaching a patient of their NOK
- Provide the patient/NOK with the brochure, FAQ sheet, and contact card

Notification of Brain Donor

- When a recruiter notifies the lead coordinator of a decedent's interest in brain donation or of a pre-consented donors' death, they obtain all of the necessary information to complete the P**ost-Mortem Case Summary Form**.
    - Patient Name
    - Race, Ethnicity, and Sex
    - Date of Birth
    - Date and Time of Death
    - NOK name and contact information
    - Cause of Death
    - Other known medical history
    - Medications
- Contact Dr. Elizabeth Head (via Text, Call, or Email) of the donor. Provide her with the known information. Get her approval of the case prior to proceeding with the consenting process.

Consenting

- Once the Basic Donor information has been obtained, please fill out the post-mortem telephonic consent form and upload it to DocuSign.
- Once the document has been uploaded, please call the NOK to complete to telephonic consent process.
    - Record the call
    - Have an additional team member on the call as a witness
- Once verbal consent has been obtained, send over the docusign and walk the NOK through the process.
- End the call once the document has been signed.

Craniotomy and Brain Removal

- Follow the autopsy tech's crainotomy/brain removal procedure
- Assist the autopsy tech in performing the craniotomy
- Harvest the brain
- Place the brain in a red biohazard bag
- Fill the red biohazard bag with cooled saline
- Close bag and place in a bucket filled with ice
- Collect blood from the heart
- 5 mL in a gray tube for toxicology (label tube with time of collection and Patient ID)
- 5 mL in a red serum tube for infectious disease (label tube with time of collection and Patient ID)

Supplies/Tools:

- Protective Personal Equipment:
    - Gown
    - N95 mask
    - Protective glasses/goggles
    - Disposable face shield
    - Bouffant caps
    - Shoe covers
    - Regular nitrile gloves
    - Surgical gloves (or double nitrile gloves)

- Brain Processing:
    - Biohazard bag
    - Scale (weigh)
    - Notepad
    - Pen
    - Soak pads
    - Camera & memory card
    - Dissection blade
    - 4% paraformaldehyde
    - Brain bucket
    - 3D scanner
    - Computer
    - Slab labels
    - Blender
    - 4000mL Erlenmeyer flask
    - 4000mL ice water
    - Alginate (1 bag)
    - Embedder
        - Rods
        - Plexiglass
        - Ribbon
        - Large binder clips
        - WD 40 (for rail)
    - Brain sled
    - Teflon plates
    - Spatula (large & small)
    - Dye
    - Pipette tip
    - 4-degree mini fridge
    - Black mobile cart

- Flash Freezing Supplies & Equipment:
    - Cryo-gloves
    - Fume hood
    - Portable cart
    - Dry ice (~3 blocks)
    - Large sized bin (1)
    - Medium sized bin (1)
    - Freezer bags (~50 bags)
    - Bag sealer & cutter
    - -80 freezer boxes (~4 boxes)
    - Freezer box labels
    - 2-methylbutane
    - Tongs
    - Teflon plate (1)

- Cleaning Supplies:
    - Bleach
    - Dish Soap
    - Medium sized bins (2)
    - Disinfecting wipes
    - Drying towels
    - Sponge
    - Strainer (for alginate)
    - Biohazard waste bin

Rapid Autopsy Preparation/Setup Protocol

***Things to prepare while waiting for brain to arrive to UCI brain lab**

1. Prepare slab labels (https://healthuci-my.sharepoint.com/:w:/g/personal/jvberry_hs_uci_edu/ERSIFHZd1xJGpBHzDRwcvGcBZ071cH6QqNlDnp3Jhew91A?email=emendoz7%40hs.uci.edu&e=4%3ATAmwwj&fromShare=true&at=9&CID=dd97fa31-dcac-e6f3-f55f-d1fac638a127)
2. Labels should go as follows – UCI.CASE AND YEAR.BRAIN REGION AND slab # (example: UCI.0124.CX12 → UCI CASE 01-24 CEREBRAL CORTEX slab 12)
3. Print labels on label paper
4. Cut out slab labels and make sure to keep them chronological. Attach small binder clip to keep them organized.
    a. Make sure to keep the non-adhesive back intact.
5. Cut freezer bags (need about 50 bags). *Check to see if there are enough precut freezer bags in cart first. *
    a. Cut them big enough to fit brain slabs and small enough to fit in freezer box.
    b. Place cut bags in cart.
6. Turn on the imaging camera and monitor.
    a. Check to make sure that the entire green box and fiducials are in view.

7. Prepare a 10% bleach bath (for the Teflon coated plates and cutting boards).
8. Prepare a soap bath (for rods).
9. Print Data Sheet (https://healthuci-my.sharepoint.com/:w:/r/personal/emendoz7_hs_uci_edu/_layouts/15/Doc.aspx?sourcedoc=%7B387C3FD3-A347-4BAB-B45F-7985078A6DBC%7D&file=BICAN%20UM1%20Brain%20Data%20Sheet.docx&action=default&mobileredirect=true).

Rapid Autopsy Protocol

Sampling

1. Begin by donning PPE
    a. The order for putting on PPE is apron/gown, N95 mask, protective glasses/googles, disposable face shield, bouffant caps, shoe covers, regular nitrile gloves, and surgical gloves (or double nitrile gloves)
2. Remove the brain from the bucket.
3. Drain saline solution and other fluids from brain bag into the biohazard waste bin.
4. Remove the brain from the bag and place it on a soak pad to remove any remaining fluids. Then, carefully place in pre-weighed biohazard bag.
5. Weigh brain and record weight.
6. Photograph all sides of the brain (dorsal, ventral, right and left hemisphere). Make sure to include a ruler for reference in all photos.
7. Document & photograph any pathology (stroke, contusions, hemorrhage, lesions, trauma, etc.).

Half-Hemisphere Fixation

1. Determine which hemisphere to fix (alternate each brain)
2. Move the basilar artery to the side of fixation.
3. Bisect the brain (with the midbrain still intact).
4. Cut the cerebrum mid-sagittally by positioning the brain caudal side up.
5. Slice between the mammillary bodies and the center of the optic chiasm through the corpus callosum.
6. **be sure the vertebral-basilar artery remains intact
7. Photograph the medial and lateral sides of the hemisphere
8. Pat the blood off the brain.
9. Place chosen hemisphere medial side down in 10% formalin.
10. Ensure that the hemisphere is laid flat with the cerebellum tucked under the occipital lobe.
11. Place fixed hemisphere in the 4^{o}C fridge.
12. To avoid contamination of the fresh tissue with the fixative remove top pair of gloves and replace them with fresh pair.

Half-Hemisphere Flash Freeze

Detach Posterior Fossa

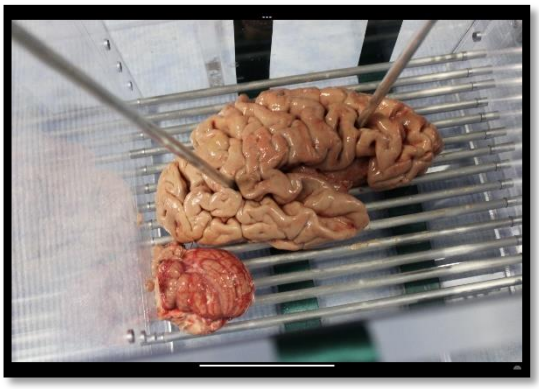
1. With the remaining, unfixed (fresh) hemisphere remove the brainstem and cerebellum.
    a. First, make a cut between the mammillary bodies and quadrigeminal plate.

    b. Then, (making sure not to damage the pons) make a lateral cut in the fourth ventricle to separate the cerebellum from the brain stem.
2. Place brainstem in a Ziploc bag and place in fridge until sectioning.
3. Place **cerebellum** in the embedder.

3D Scanning

for detailed, step-by-step instructions, please refer to Jillian Berry's - 3D Surface Brain Scan Procedure

4. Prior to scanning, remove the meninges from the fresh hemisphere.
5. Pat off excess blood and saline from the brain.
6. Photograph the medial and lateral sides of the hemisphere.
7. Place the hemisphere on the plexiglass scanning table. Making sure not to cover any of the black and white dots.
8. Turn off the lights.
9. Reorient computer monitor so that it faces scanning table.
10. Follow the 3D Surface Brain Scan Procedure.



Embedding

1. Place cerebellum in the embedder with vermis up against wall of the embedder (in the correct anatomical position)
2. Place the fresh hemisphere medial side down with the frontal lobe facing away from the cerebellum (in the correct anatomical position)
3. Take a picture of the brain for reference.
4. Pour 500g of dental alginate and 4L of ice water into the blender.
5. Blend the alginate and the ice water together for 30 sec to 1 min @ speed setting #6
6. While waiting for the alginate mixture to finish blending, place two poles lightly in the sulci of the brain to hold it in place. Place one pole in the center of the occipital lobe and the other in the center of the frontal lobe. Also, place one pole on the cerebellum to hold it down.
7. Immediately after blending, pour the alginate mixture into the embedder mold while having a helper hold the poles steady in place (if not poured immediately, the alginate will solidify)
    a. Ensure that the occipital and frontal poles are on the same axis
    b. Start pouring from one corner then pour over the top
8. Keep poles in place until alginate has completely solidified (approx. 5-10 min)
9. While the alginate solidifies, use the Terazyme to clean out the blender before the residual alginate solidifies.
10. Remove the metal rods from the alginate block.

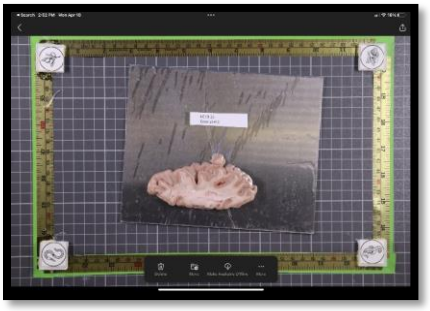
11. Once alginate has solidified, use the tow straps (green ribbons) to lift the alginate block out of the embedder.
12. Place alginate block in brain sled with the frontal lobe oriented toward knife guides.
13. Place the plexiglass sides and green ribbon from embedder in bleach bath.
14. Place metal rods in soap bath.

Brain/Alginate Slicing

1. Slice the alginate at **8mm** increments until you reach brain matter
2. Once you find the brain, slice every **4mm** (one notch)
    a. Use a very large binder clip at the back end of the sled to hold the alginate cube in place, while you cut
        i. Make sure you move the binder clips forward as you move the brain forward
    b. Make sure to place the Teflon plates in front of the sled right before you begin slicing, so that the slab will fall right onto the plate
    c. Use the spatula to gently separate the newly sliced slab from the rest of the brain, so that it doesn't stick together when you remove the plate.
3. Once each slab is cut and plated, remove the alginate and place the corresponding label (sticker) on the plate next to the slab (do not stick the sticker to the plate).
    a. Make sure the labels go in chronological order
4. Once you reach the cerebellum (i.e. you have two brain slabs per slab of alginate), place the corresponding label for each brain slabs on the plate next to their corresponding slab.
    a. the first cerebellum slab will be labeled with sticker number 70 (even) or 69 (odd), depending on whether the occipital tissue slab on the same plate has an even or odd sticker number
    b. from there, each cerebellum slab will be labeled with a sticker number in descending chronological order
        i. the vermis should have the lowest sticker number
5. Once you have finished slicing the alginate, remove the brainstem from the fridge
6. Mark posterior side with India ink by drawing a line going from the most superior aspect to the most inferior aspect. Allow the ink to dry.
7. Embed brainstem in alginate.
8. Slice the brainstem into 4mm slabs.
    a. Mark the caudal face with India ink (use a different color if available).
    b. Label each slice with its corresponding sticker number
        i. #1 – Substantia Nigra
        ii. #2 – Pons
        iii. #3 – Medulla

Imaging

*****make sure to turn on, set up, and test the imaging system, prior to photographing your slab*****



In chronological order, move the plates over to the imaging station

   a. Make sure the slab and label are in the camera's frame of view.
   b. Make sure that the camera is focused (half press the capture button to focus the image)
2. Once all plates have been photographed, place them on the cart and move them over to the Biosafety hood for flash freezing
   a. Make sure to bring dry ice cart and gallon zip lock bags with you

Flash Freezing

PPE Modifications

1. Remove both pairs of gloves, and replace them with a pair of cryo gloves (underneath) and nitrile gloves (on top).
1. Bring vacuum sealer, ~50 pre-cut baggies, Styrofoam box, two autoclave bins, ~3 blocks of dry ice, and pre-labeled slab storage boxes to the fume hood.
2. In a large autoclave bin break ~3 blocks of dry ice by carefully smashing with a hammer or mallet.
3. Place some of the dry ice pieces into the Styrofoam box. This will be used to keep flash frozen slabs frozen while we freeze the rest of the brain.
4. Lift the sash of the fume hood to the optimal height (do not exceed the maximum height allotted).
5. Place an empty large autoclave bin in the fume hood.
6. Pour isopentane (2-methylbutane) into the bin. Add enough to cover the brain slabs when submerged (~7mm in height).
7. Add big pieces of dry ice to each corner of the bin to raise the temperature of the isopentane and replace dry ice pieces as they evaporate.
8. Once you're ready to start flash freezing, remove brain slabs from the 4C fridge and place them on the cart in chronological order and transport them to the fume hood.
9. Before moving the slab into the fume hood, remove the label and place it into a pre-cut bag.
10. Then, place a Teflon coated plate on top of the brain slab (as to make a slab sandwich) and carefully submerge it into the isopentane bath.
11. Keep the plates submerged until the brain slab has completely frozen.
12. Once frozen, use tongs to remove the slab sandwich from the bath. Remove brain slab and place into pre-cut bag with corresponding label. Ensure that the label does not touch the brain slab.
13. Once the brain slab and label are in the bag, vacuum seal the bag using the vacuum sealer.
14. Place the sealed brain slab in the in the pre-labeled slab storage box and place this inside the Styrofoam box. Once the slab box is full transfer it to the -80 freezer and start the next box.
15. Continue performing the same process (in chronological order), until all samples have been frozen.

Cleaning

Fume Hood Clean Up

1. Carefully remove dry ice chunks from large bin with 2-methylbutane.
2. Carefully pour used 2-methylbutane from large bin in labeled waste container. If the container is full, get another one and label it accordingly (i.e., copy information from the other container with 2-methylbutane).
3. Place bins and other dishes used on cart and remove all used absorbent pads. Used absorbent pads go in the red biohazard bin.
4. Wipe the fume hood and any surface touched with 10% bleach. Repeat the process with disinfectant wipes. Make sure to leave the fume hood in cleaner conditions than found.
5. Transport all used dishes to lab space and begin washing them.
    a. Soak the teflon coated plates and cutting boards in a 10% bleach bath (1 part bleach : 9 parts water)
6. Once clean, hand dry all dishes and store them where they belong.

Wet Lab Clean Up

1. Soak the cutting boards in a 10% bleach bath (1 part bleach:9 parts water)
2. Remove the used absorbent pads from the counter and toss them in the red biohazard waste bin. Wipe down every surface with Saniwipes and 10% bleach if it came in contact with fresh tissue.
3. Clean all dishes in the sink, hand dry them, and place everything back where it belongs.
4. Clean floors with Swiffer.
5. Remove PPE and toss it in the red biohazard waste bin.

Note: Alginate, wipes, and any other discardable item that potentially encountered fresh tissue goes in the red biohazard waste bin.

Blood testing for Infectious Disease:

1. Go to https://www.lifepoint18.com/UCI/lts/login/login.cfm?&refresh=no&js=1
2. Login in using your credentials.
3. Under '**Patient Management**' select '**Add Patient**'
4. In the '**Add Patient**' window, select '**Generate UCI MRN**'. This will generate a UCI MRN for the case (example – S87-10000005).
5. In the '**Add Patient**' window, fill in sections with an *. Then hit '**Next**'.
6. Verify information and hit '**Next**' if things look correct.
7. Under Insurance hit '**Next**' and then '**Save**'.
8. Under '**Order Tests**' **>** '**Patient Info**' hit '**Next**' on the top right.
9. Under '**Order Tests**' **>** '**Diagnosis**' hit '**Next**' on the top right.
10. Under '**Order Tests**' **>** '**Tests**', go to the box next to '**Search**' and type "**Serology**" and then click '**Search**'. Select the option titled, '**Basic Donor Serology Panel – ZDONOR**'. Then click '**Next**' on the top right.
11. Under '**Review Test Requisition**' check that you selected the correct test. Then fill in the fields marked with an *. Under '**Collection Date**' add the date of specimen collection and under '**Collection Time**' add time of specimen collection.
12. Under '**Other Identifier**' type UCI [case #].

13. Under '**Requesting Physician**' select '**Search**' and type '**Monuki**', and select '**1568542728 098947 Monuki M.D.,Edwin S - 101 THE CITY DR , ORANGE, CA 92868**'.
14. Check order request once more, and then hit the green '**Order**' button.
15. **Print** the Order Request and place it in the biohazard bag.
16. Every specimen tube must be labeled with the **(1) patient's first and last name and (2) secondary identifier (i.e., DOB, MRN, or Client ID)**. Once the tube is labeled, then place it biohazard bag with Order Request. Note: Make sure to label tube identical to that specified on Order Request. Also, we pre-printed tube labels – please use these if they are available (https://healthuci-my.sharepoint.com/:w:/r/personal/jvberry_hs_uci_edu/_layouts/15/Doc.aspx?sourcedoc=%7B5BDFCD9E-57D7-440D-A6C1-AD079DB7D6BC%7D&file=Tox%20and%20Infectious%20Disease%20Labels%20.docx&fromShare=true&action=default&mobileredirect=true).
17. Drop-off the biohazard bag with blood sample and order request in the '**Drop-off**' box on the 3rd floor of the UCI Medical Center room [add room #]. *Note you will need key card access to enter the room.

Blood Toxicology Testing:

1. Fill out 'Analysis Requisition and Chain of Custody' form (found in drawer next to computer in wet lab.
2. Under 'Work ID' add UCI [case #]
3. Under 'Sample ID' say where it came from and what number is it (example: Willed Body #3).
4. Add 'Date of Birth' and select 'Sex'.
5. In the table, fill in 'Collection Date', 'Collection Time', 'Specimen Type', and 'Specimen Source', and 'Container Labeled as'.
6. Under 'Tests Requested' check the box labeled '8052B Postmortem, Expanded, Blood (Forensic)'.
7. Under 'Brief Case History / Circumstances of Death' specify the cause of death by checking the box next to the appropriate answer and specifying in the space provided, if known. If unknown check the box next to 'Undetermined' and write 'Unknown' in the space provided.
8. Label blood sample tube with **Subject Name**, **Case ID** (UCI ##-##), **Sample Source/Matrix** (example: Blood from heart). To do this use the pre-printed labels and stick it on the tube https://healthuci-my.sharepoint.com/:w:/r/personal/jvberry_hs_uci_edu/_layouts/15/Doc.aspx?sourcedoc=%7B5BDFCD9E-57D7-440D-A6C1-AD079DB7D6BC%7D&file=Tox%20and%20Infectious%20Disease%20Labels%20.docx&fromShare=true&action=default&mobileredirect=true.
9. Grab NMS shipping box from shelf, biohazard bag, and biospecimen shipping labels (red sticker that reads "LAB SAMPLE DO NOT TAMPER") (2). Grab cooling packs from –20C fridge. Place blood specimen tube in biohazard bag. Then carefully fold the 'Analysis Requisition and Chain of Custody' form and place it in the outside pocket of the biohazard bag. Place the biohazard bag with the sample and form in the shipping box ensuring that you surround the sample with the cooling packs to ensure it does not go bad. Place one shipping label on each entry point of the box. This will ensure that the sample is not tampered with.