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Version 2

HuBMAP MOSDAP (Multiple Organ Same Donor Authorization Project) Protocols V.2

 Version 1 is forked from [602.2 Donor Acceptance Criteria for URMHC HTC HuBMAP and LungMAP Inclusion](#)

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We use this protocol and it's working

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Abstract

Purpose and Scope of the Procedure

- Promote sharing, assessment and comparative analysis of cellular and molecular biology of multiple organs from single donors among HuBMAP components
- Standardize process and infrastructure for receiving multiple organ and tissue donations from healthy individuals along with detailed, relevant clinical history, and representing a diversity of race, ethnicity, sex.
- Use of a Donor Authorization Addendum, acceptable to the HuBMAP Consortium and to participating Organ Procurement Organizations (OPOs) and that directly addresses open-access genomic data sharing
- Scope: Coordination of screening, acceptance and receipt of tissue by subject matter expert research program components (TMCs) of the HuBMAP Consortium.

Principles

- Organ donations rejected for transplantation can be best used to decipher organ-specific cell types, gene and protein expression variation by modern molecular technologies, when studied as multiple organ, same donor samples such that comparison can be made across tissues with the same genetic and exposure background.
- Rapid, standardized and safe processing of human tissue requires coordination of multiples teams, materials and attention to protocols. This is achieved in this protocol by collaboration with organ procurement organization, research recovery organization, and tissue preservation laboratories, in order to prepare for state of the art molecular analysis at the single cell and spatial level.
- Rapid processing is required to maintain the tissues in a state as close to normal as possible. Multiple studies have shown intact RNA, protein and cellular viability when recovered from organ donors as described, with handling and recovery expectations as close as possible to that required of transplant organs.
- The protocol requests that organs are recovered by methods typical for transplantation including minimal warm ischemic time (WIT), cold perfusion with transplant-organ solutions (i.e. HTK, UW, SPS), submersion for shipping generally as whole organs in cold-storage transplant solution, with as brief cold ischemic time (CIT) as possible, optimally < 24 hours. Each organ is sent to the HuBMAP Tissue Mapping Center that has demonstrated expertise in preservation and molecular analysis of that organ / tissue.

Guidelines

Safety and Regulatory Considerations:

- Needs and privacy of donating family should be respected at all times
- Consent for tissue donation for research should be freely given
- Proper consent process will be assured.
- Organs and biospecimens should be shipped safely and expeditiously
- Shipping will meet the minimum requirement for dangerous goods under 49 CFR 172.700 and IATA 1.5.
- Care should be taken in preservation methods and storage so as to maintain quality of tissue. Tissue surfaces should never become dry, maintenance on wet ice in cold transplant buffer is preferred until properly preserved.



Materials

Contact Information for Referral Groups

NDRI - <https://ndriresource.org/for-researchers>

For each receiving tissue expert preservation lab.

Safety warnings

- ⚠ These are human tissue with potential to carry transmissible infectious agents. They should be handled as BSL level 2 or 3. They are screened for typical infections as for transplant organs but care and personal protective equipment should always be used when handling the samples.

Ethics statement

Human donor tissue is utilized following approved consent/authorization process, with authorization given for the organ and tissue donations to be used for research and published in a de-identified manner. Sharing of tissues and data between HuBMAP Consortium members is by signed material transfer and data sharing agreements. The privacy and confidentiality of donors and next of kin will be protected to the greatest extent possible in agreement with the authorization for research.

Summary of Order of Events for the HuBMAP Multiple Organ Same Donor Project

- 1 OPO (Organ Procurement Organization of national Organ Procurement for Transplantation Network, OPTN) made aware of potential appropriate donor and notifies RRO (Research Recovery Organization) to screen.
- 2 RRO then screens with the Coordinating HuBMAP TMC (Tissue Mapping Center), sending the Donor Summary for review and decision on donor eligibility.
- 3 Case is accepted or declined based on Inclusion/Exclusion Criteria
 - Coordinating TMC contacts receiving tissue / subject matter experts if organ acceptability questioned.
- 4 OPO approaches NOK (next of kin) with authorization for recovery of organs for transplant followed by the HuBMAP research specific authorization process.
- 5 If NOK gives authorization and donor meets MOSDAP criteria, organ recovery is scheduled. Each organ/tissue goes through transplant allocation process first prior to release for MOSDAP research. Once organ accepted for MOSDAP research, the Coordinating TMC alerts accepting sites with anticipated timing of recovery and delivery.
- 6 RRO/OPO will be in contact with each confirmed accepting site to provide updates on courier and arrival timing until tissues are delivered.
- 7 MOSDAP Organ Recovery Protocols, provided by the Coordinating TMC after review by tissue subject experts, are followed by the recovery team.
 - 7.1 DBD (donation after declared brain death) preferred. Cold flush with transplant buffer, UW/HTK, and packing begins within minutes from asystoli as required by organ recovery regulations.
 - 7.2 DCD (donation after declared cardiac cessation)- cold flush with transplant buffer UW/HTK and packing on wet ice within 1 hour of asystoli preferred, 2 hours max.
- 8 Standard order and timing of Donor organ / tissue recoveries:
 - 8.1 1) Organs for transplant
 - 8.2 2) Organs for HuBMAP research (heart, lungs, spleen, kidney, pancreas, intestine, lymph nodes, thymus, fallopian tube, ovary, uterus, eyes, vertebral bodies, blood) recovered

sequentially within 4 hrs from asystoli

8.3 3) Potentially, a small biopsy of each organ can be flash freeze upon recovery and sent as fresh frozen to a designated lab for processing for specific assays

8.4 4) Tissues for transplant

8.5 5) Tissues for HuBMAP research - skin, knee, bone marrow (vertebral bodies) - up to 16 hrs post-mortem interval (PMI)

9 Each whole organ or tissue is shipped, as rapidly as possible, to one of up to 10 receiving sites on wet ice. Frozen biopsies may be sent to single site.

Step 1: Review Case: accept or decline based on eligibility

10 Coordinating TMC takes referral call or email for potential MOSDAP donor and starts Case Record in MOSDAP Screening Log.

11 Review Eligibility Criteria, Donor Demographics, Exposures, Inclusion and Exclusion criteria

11.1 Age Range: 18 – 70 (prefer < 50) years of age

Race: Any

Sex: No Preference

BMI: ≤ 35 Ch

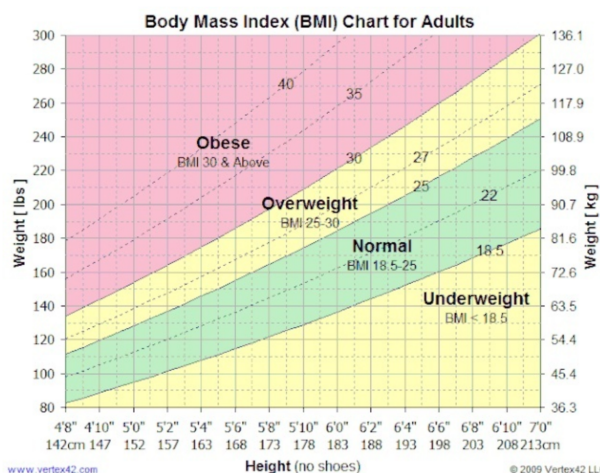


Figure. Chart indicating basis for choice of acceptable BMI.

- 11.2 Required Disease(s): Normal / Healthy
Required Medication(s): None
Required Surgeries(s): None
- 11.3 Infectious Disease Testing Required:
Yes – standard pre-transplantation serologies plus SARS-CoV2 PCR.
- 11.4 Sepsis Syndrome: None
- 11.5 Increased Risk (ex. blood transfusion): Always Acceptable but to review on screening
- 11.6 DCD: Some acceptable, Prefer DBD
- 11.7 Acceptable Warm Ischemic Time (WIT): No Restrictions but to review on screening, prefer < 15 min, may accept up to 3 hours depending on case
- 11.8 Downtime (in cardiac arrest prior to hospital admission): Some Acceptable, <= 60 min.
- 11.9 Time on mechanical ventilation: Some Acceptable, less than 5 days preferred.
- 11.10 Cancer: Never Acceptable, unless new diagnosis in organ not being sampled (ex. brain) and no indication of metastases
- 11.11 Chemotherapy history: Never Acceptable
Radiation history: Never Acceptable
- 11.12 Acceptable Diseases: None
Exceptions: some mild hypertension, some moderate hypertension if compliant with medication and/or some mild pre-diabetes with HgbA1c < 6.5
- 11.13 Unacceptable Diseases (if disease affects organs to be recovered):
Drug overdose.
Significant scarring, disease, trauma, hematoma or hemorrhage in organs and tissues to be recovered. Any disease > mild-mod and/or non-compliant with treatment.
Insulin dependent Diabetes, of any form, with severe complications and/or with HgbA1c > 6.5.

Any previous gastrointestinal disease or bowel injury in mechanism of injury and cause of death.

Any cardiomyopathy or heart failure.

Any chronic obstructive pulmonary disease (COPD) or interstitial lung disease (ILD).

Any prior received transplant.

Any congenital anomaly felt to have affected organs to be recovered.

Any chronic kidney disease or pathology such as fibrin microthrombi, infection, necrosis, moderate to severe fibrosis, tubular atrophy, inflammation, arteriosclerosis, or greater than 10% glomerulosclerosis.

Any structural abnormality of uterus (fibroids or endometriosis) (ask if "still have periods").

Cataract surgery, glaucoma shunt, intravitreal injections, cornea donor, cornea with infiltrates, any condition of retina or choroid (i.e. AMD, macular edema, macular hole, retinitis pigmentosa, neurologic: Parkinson, dementia; Surgery: vitrectomy, pan-retinal coagulation, scleral buckle; major head trauma (falls, MVA, gunshot wounds, etc).

11.14 Acceptable Surgeries: None

Unacceptable Surgeries: None, unless negatively affects organs being recovered for research.

Prefer not recipient of massive transfusion protocol.

Kidney on pump while allocating remains acceptable.

11.15 Acceptable Medications: None, to be reviewed at screening

Unacceptable Medications: None

11.16 SOCIAL HISTORY:

Tobacco Use: $\leq 1/2$ ppd may be acceptable, > 1 ppd but discontinued > 5 years prior may be acceptable, vaping may be acceptable with estimate of quantity.

Alcohol Use: $<$ heavy use may be acceptable, heavy use but discontinued 5 years prior without sequelae may be acceptable.

Illicit Drug Use: No IV drug use; Any other to be reviewed case-by-case during screening.

11.17 LAB VALUES: inclusion/exclusion criteria to be reviewed on screening

Trends as important as most recent.

Required if related organ is available for research:

- Whole Pancreas - Amylase (23 - 851 IU/L), Most Recent Value: ≤ 250 ,
- Lipase (0 - 80 IU/L), Most Recent Value: ≤ 170
- HbA1c ≤ 6.5
- Whole Lung - Arterial Blood Gas - $pO_2 > 200$ on 100% (paO_2/FiO_2 ratio > 200 , preferred > 300)
- Chest Xray - no pneumonia or aspiration, no or minimal trauma.
- Whole Kidney - Creatinine ≤ 2.0 mg/dl - BUN ≤ 30
- Whole Liver - AST ≤ 200 - Alk Phos ≤ 400

- Female reproductive - estradiol, progesterone – typically not done for screening, use recovered plasma or serum after receipt.
- Whole Heart - Echocardiogram – Ejection Fraction $\geq 50\%$.
- Intestine - Abdominal CT or ultrasound – No history or imaging c/w trauma or chronic disease.
- Cultures - No positive blood or urine culture, No positive viral PCR.
- HLA typing required.

If available:

Urine protein – Dipstick $<2+$; Urine Protein/Creat Ratio <180.0 mg/g; Urine Protein, 24hr, <150 mg/24H.

Reports of most recent chest x-ray, echo, ultrasound and/or CT or MRI of abdomen.

Step 2: Requested protocols for organ/tissue recovery

- 12 If DBD: Organ Procurement Organization Recovery Team prepares for organ recovery as consistent with organ transplant protocol with cross clamp and in situ cold flush body cooling with minimal warm ischemic time. Includes flush with UW/SPS-1, HTK or similar organ transplant buffer, until solution runs clear
- 13 If DCD: Organ Procurement Organization / Recovery Team prepares for organ recovery as quickly following Cardiac Arrest as authorized by next of kin and allowed by regulations.
- 14 Remainder of organ / tissue recovery should be as consistent with organ transplant protocol as possible.
Warm ischemic time is to be minimized, tissues may be accepted up to 6 hours WIT.
- 15 Heart Procurement Instructions:
 1. Procure the whole heart.
 2. Flush with UW/SPS-1 or HTK until clear. Do not flush with saline.
 3. Packaging Instructions: Submerge the heart in chilled UW/SPS-1 or HTK – do not wrap in surgical towel.
Triple bag the tissue.
 4. Label the outer bag with project code, tissue, size, preservation, and donor number.
 5. Package on wet ice and maintain at 4°C until shipment.
- 16 Jejunum Procurement Instructions:
 1. Recover ~ 30 cm segment of jejunum just beyond the duodenum, distal to ligament of Treitz.
 2. Clearly mark the proximal end with a suture.
 3. Gently express fecal material, DO NOT flush lumen. Staple each end of segment closed.
 4. Submerge in chilled UW/SPS-1 or HTK - do not wrap in surgical towel. Triple bag the tissue.
 5. Label the Specimen Bag with project code, tissue, size, and donor number.

6. Package on wet ice and maintain at 2-8°C until shipment.
- 17 Sigmoid colon procurement Instructions:
 1. Recover ~30 cm of the sigmoid colon.
 2. The proximal end must be clearly marked with a suture.
 3. Gently express fecal material, do not flush lumen. Staple each end of segment closed.
 4. Submerge in chilled UW/SPS-1 or HTK - do not wrap in surgical towel. Triple bag the tissue.
 5. Label the Specimen Bag with project code, tissue, size, and donor number.
 6. Package on wet ice and maintain at 2-8°C. Contact NDRI immediately following procurement.
 - 18 Kidney Procurement Instructions:
 1. Recover 1-2 Whole Kidney(s).
 2. Flush well with UW/SPS-1 or HTK until solution runs clear. Do not flush with saline.
 3. Store in chilled UW/SPS-1 or HTK – do not wrap in surgical towel. Triple bag the tissue.
 4. Label the outer bag with project code, tissue, size, preservation, which kidney was recovered (Left or Right) and donor number.
 5. Package on wet ice and maintain at (2-8)°C until shipment.
 - 19 Vertebral Bodies and Disc Procurement Instructions:
 1. Cut across vertebral bodies L3 and L5, not between, to recover 2 vertebral bodies with intact disc.
 2. Wrap specimen in sterile gauze. Triple bag in tightly sealed plastic bags.
 3. Label the Rigid Specimen Container with project code, tissue, size, location, and donor number.
 4. Package on wet ice and maintain at 2-8°C until shipment.
 - 20 Whole Knee Procurement Instructions:
 1. Recover 1 Whole Knee en bloc including surrounding skin and muscle 10cm above and 10cm below the joint line. The joint must not show signs of inflammation or arthritic disease.
 2. Wrap the knee specimen in gauze, place in tightly sealed plastic bags. Do not add buffer/fluid to bag.
 3. Label the Specimen Bag with project code, tissue, size, location (right or left), media and donor number.
 4. Package on wet ice and maintain at 2-8°C until shipment.
 - 21 Whole Liver Procurement Instructions:
 1. Recover 1 Whole Liver. (If Whole liver is not available, a minimum 5 cm x 5 cm sample is acceptable).
 2. Flush with UW/SPS-1 or HTK until clear. Do not flush with saline. Perform a final back-table flush with at least 1L of UW/SPS-1 or HTK.
 3. Remove gallbladder and flush the bile ducts. No bile should be present.
 4. Submerge the Liver in chilled UW/SPS-1 or HTK - do not wrap in surgical towel. Triple bag the tissue.

5. Label the Specimen Bag (liver) with project code, tissue, size, and donor number.
 6. Package on wet ice and maintain at 2-8°C until shipment
- 22 4. Whole Lung and Blood Procurement Instructions:
1. Recover 1-2 Whole Lung(s) en bloc with at least the lower 1/3 of the trachea.
 2. Flush well with UW/SPS-1 or HTK until solution runs clear. Complete a minimum back-table flush of 500ml into the pulmonary artery and 500ml into the pulmonary vein. Do not flush with saline.
 3. Staple, clamp, or tape the trachea closed prior to shipping. Do not wrap in surgical towel.
 4. Place Lung(s) in a Specimen Bag and submerge with chilled UW/SPS-1 or HTK. Triple bag the tissue.
5. Recover at least ONE 10 CC sample of Blood in a Green Top (Heparin) Blood Tube.
If available, recover an additional total of SIX 10 CC samples of Blood in a Green Top (Heparin) Blood Tube and THREE 10 CC samples of Blood in a Red Top Blood Tube. Invert the tubes several times to mix,
Blood may be collected before or immediately following organ recoveries.
6. Label the outer bag(s) with project code, tissue, size, preservation, which lung was recovered (Left or Right) and donor number.
 7. Package lungs on wet ice and maintain at (2-8)°C until shipment.
 8. Package blood and store ambient until shipment.
 9. The Blood vial(s) should be placed, if possible (and for > 3 blood tubes), in a separate styrofoam container within the lung shipping container. Alternatively, the blood vials may be placed outside the organ box but within the shipping container within a biohazard bag (in this case, cut a small trough in the Styrofoam container for vial security). Blood sample(s) should not be immersed or placed in direct contact with wet ice to avoid freezing / cold damage.
- 23 Pancreas, Duodenum, and Spleen Procurement Instructions:
1. Pancreas, Duodenum, and Spleen: The pancreas and peripancreatic fat should be recovered intact. 10-20 cm of duodenum should be attached and stapled (NO silk ties) closed at both ends with excised pancreas. Surgical damage to the pancreas may be acceptable, but must be reported.
 - a. Recover the entire pancreas with duodenum (5-10cm), spleen (half to whole), and as much peripancreatic fat as possible (this will contain pancreatic lymph nodes).
 - b. The pancreas and peripancreatic fat must be excised with attached duodenum (10-20 cm) and spleen (whole spleen is preferred, but ½ is acceptable).
 - c. The duodenal bowel contents should be "milked" away from the pancreas as much as reasonably possible. Staple (NO silk ties) the duodenum with a length of ~5-10cm space between rows to avoid cross-contamination of the pancreas with the bowel contents.

d. Submerge all specimens (pancreas, duodenum, spleen, and peripancreatic fat) in an organ bag with chilled UW/SPS-1 or HTK. Do not wrap in a surgical towel. Triple bag the tissue.

2. Thymus: Difficult to distinguish from fat in adult, but do recover if can be identified. If available, recover the whole thymus. Place in a conical tube filled with chilled UW/SPS-1 or HTK.

3. Label the outer bags with project code, tissue, size, preservation and donor number.

4. Package on wet ice and maintain at (2-8)°C until shipment.

24 Abdominal Skin Procurement Instructions: 1. Recover two 3 cm x 3 cm samples of Skin - one Full-Thickness from the abdomen and the other Full-Thickness from the calf.

Samples must be free of any scarring or ulceration.

2. Divide each specimen in half and store in 2 specimen containers, one filled with DMEM & Antibiotics and a second filled with 10% formalin. There should be a total of 4 Specimen Containers, two with formalin and two with DMEM & Antibiotics.

3. Label the Rigid Specimen Containers with project code, tissue, size, location (abdomen or back), media and donor number.

4. Package on wet ice and maintain at 2-8°C until shipment.

25 Female Reproductive System (FRS) Procurement Instructions:

1. Recover uterus, ovaries, fallopian tubes and cervix en bloc. (See diagram, referenced below)

a. Divide right round ligament and broad ligament from Fig B to D. Repeat on left from Fig A to C.

b. Use fingers to push bladder apart from the uterus and cervix.

c. Clamp and cut right and left ovarian vessels running in the infundibulopelvic ligaments (Fig D and C)

d. Continue to divide the broad ligament medially on both sides (E on Figure).

e. Clamp and cut the uterine vessels and the uterosacral ligaments (F and G in Figure)

f. Push bladder apart from uterus and cervix.

g. Palpate the bottom of the cervix between bladder and rectum

h. Place clamps across vagina below cervix. Transect vagina with scalpel or scissors.

Remove the FRS en bloc.

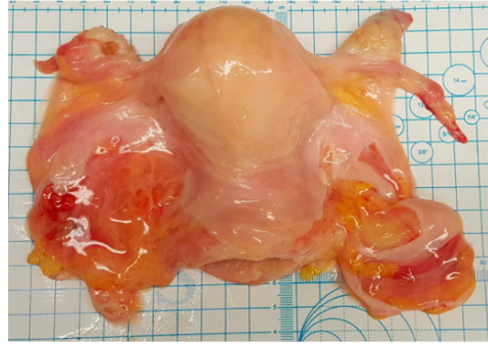
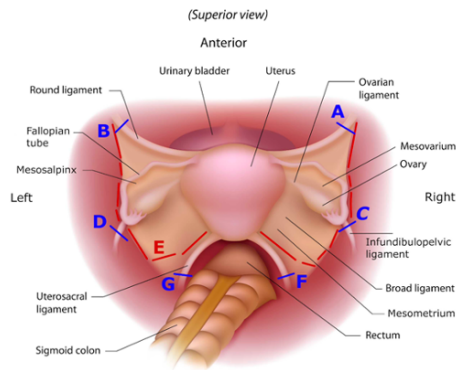
i. Close vagina with running stitch to reduce leakage

2. Add a suture to the anterior surface of the uterus for orientation purposes.

3. Submerge en bloc in chilled UW/SPS-1 or HTK – do not wrap in a surgical towel. Triple bag the tissue.

4. Label the outer bag with project code, tissue, size, preservation, and donor number.

5. Package on wet ice and maintain at 4C until shipment.



Preparation for Shipping

- 26 Recovery Team to package and ship such as to meet requirements of 49 CFR 172.700 and IATA 1.5.

Shipment is to reach each organ and tissue receiving preservation site within 24 hours of recovery.

Metadata

- 27 Receiving Lab to Record:
- UNOS #
 - Courier and Tracking Numbers
 - Project Code: MOSDAP
 - Use labelling schema: HuBMAP Lab ID with tissue code and location:
HAAA-BBB-CCC
 - HA: Donor ID will be provided (H indicates HuBMAP MOSDAP Project)
 - B = 3 letter to identify organ: HRT, KDN, PNC, SIT, LIT, EYE, SKN, KNE, LIV, UTE, OVA, FAL, BMO, LNG, PLA, SER, PBM
 - C = additional location information as needed
 - Condition of Shipping materials (eg. Ice sufficient, +/- leakage, appropriate layered packaging)
 - Gross Condition of Organ Received (eg. Pink and healthy vs evidence of hemorrhage or trauma, +/- submerged, +/- localizing suture in place, identities if multiple tissues received)
 - Weight of each organ or tissue received
 - Linear Dimensions (Approx volume): Ht, Width, Thickness
 - Photographs of intact organ or tissue as received on board with rulers in anatomical positions

- Photograph each dissection step to maintain record of origin of blocks to be available for assay.
- Record dates and times of arrival, beginning of processing (unpacking), dates and times of each transition of tissue through preservation, storage and assay preparation, storage and preservation medium and methods (eg. -80, 4deg, room temp) – will need to report time intervals as metadata
- Record histological reports and quality criteria

28 Receiving Lab Preservation Requested:

- Remove excess tissues around organ of interest
 - Keep photographic record of processing to aid in relating each preserved sample back to the CCF (common coordinate framework) of the organ.
 - Consider collecting samples of lymph nodes, excess large vessels, nerves, other tissues

Best to place these back in storage buffer and Tend to organ of interest First)
 - Sample or section organ into pieces representing major anatomical features of the organ (eg lobes, proximal/distal) • (eg. 8 segments in liver)
 - From each anatomical segment, prepare at least 5 sets of tissue samples be preserved, neighboring tissue (alternating) preserved in multiple ways as needed for down-stream assay.
 - Divide these pieces serially into approximately 0.5 – 1 cm³ portions (and photograph)
 - Further preserve these sequential tissue pieces in:
 - 10% formalin, processed then to paraffin (FFPE) (ovary 4% PFA (sucrose?) in PE)
 - Fresh Frozen, without fixative in cryovial over liquid nitrogen and stored at -80deg (Skin – sc prep on fresh without freezing, as well as a sample frozen over Liz N2)
 - Fresh Frozen embedded in OCT over dry ice/ethanol or dry ice isopentane bath (liver isopentane)

(Kidney prefers - metal block (aluminum ¾-1 in) over liquid nitrogen)
 - Fresh Frozen embedded in CMC over dry ice/ethanol or dry ice isopentane bath
 - 4% PFA, cryoprotected in 10% sucrose, embedded in OCT over dry ice/ethanol or dry ice isopentane bath (not typical for skin).
 - Mark and record orientation of tissue when embedded in OCT/CMC/paraffin
- Complete Metadata Ingest Sheet for
 - 1) Organ pieces ("block"),
 - 2) Tissue blocks (both organ pieces and blocks for sectioning),
 - 3) Sections or Suspensions
- These tissues, aside from what the receiving TMC uses them for, are to be bio-banked and shared with other members of the HuBMAP Consortium
- Assay of multiple tissues will be done at one or more locations with expertise in that assay, or if TMC with expertise in the tissue will run their own version of that assay.