**Human Tissue Collection Protocol**

Last updated by Greg Milbrun on 24 April 2023

Overview

This document explains the necessary steps for prepping, obtaining, and storing human hearts for the biorepository. This is intended to be a guide to be read before beginning collections, and during collection, if necessary.

Need more help?

Check the resources, and then see Ken

Main content

**Part 1: Get Access**

* You need access to both the OR and the scrub machine
  + Contact the Biorepository Program Coordinator.
    - This position is currently held by: Mindy Thompson

**Part 2: Prepping the Tubes**

**Important Note: It is best to have this done prior to the collection, but if it is not make sure, that you do this before the heart is ready. The only exception to this would be LVADs. See below for more details.**

* Collect the following:
* Cryogenic vials
* Sharpie
* Plastic Bag
* Styrofoam container with dry ice
* Obtain a Hashcode from the Batphone Log for the appropriate collection type.
* Label each vial with the Hashcode and the region it was collected from.
* The numbers needed for each collection type are found below:
  + - Heart Transplant:
      * 4 Epi Fat
      * 8 LV Epi
      * 8 LV Mid
      * 8 LV Endo
      * 8 Septum
      * 8 RV
      * 2 RA
      * 2 LA
    - Organ Donor:
      * 4 Epi Fat
      * 20 LV Epi
      * 20 LV Mid
      * 20 LV Endo
      * 12 Septum
      * 20 RV
      * 6 RA
      * 6 LA
    - LVAD:
      * These are the only ones that are best to do the day of. You can go ahead and label two with the hashcode and LVAD Core on it. Have some additional ones ready just in case.
      * If you use the additional tubes, label those as well.
* Once you have made one subcategory (ex: 8 LV Epi) Use a piece of tape to tape them together in a circle.

**Part 3: Set Up the Lab**

1. Place an underpad on the designated lab bench
2. Collect:
   1. One pair of large scissors
   2. One Pair of Small Scissors
   3. One small forcep
   4. One medium forcep
   5. Two weigh boats
   6. A Facemask and gloves to wear while dissecting the heart.
   7. The cryogenic tubes with the correct hashcode written on them
      1. It would be best if you have them separated out ahead of time, in the order that you prefer to dissect.
3. Grab the cooler with the biohazard sticker on it and fill the bottom with ice from room MS518
   1. Note: The code for the door is 749518 (PGY 518)
4. Fill up the liquid nitrogen bucket (the tall tan bucket with the biohazard sticker) with liquid nitrogen
   1. Note: Be careful not to do this too early, as the liquid nitrogen will evaporate. Best to wait until you are sure that you are going to go collect the heart.
5. Go down to the OR and change into the OR scrubs.
   1. You can get scrubs from the machine using your ID badge.
   2. You can access the changing rooms using the code:13579# (All of the odd numbers and #)
6. Keep in mind that it takes a while for the specimen to be ready. You do not want to be late, but you do not want to wait in the OR, if you are in the way. The timing of this is a mixture of practice and luck. Generally, it will take AT LEAST an 2 hours from the time of first incision for heart transplants. This timing is highly variable. For example, heart transplants with prior sternotomy can add about 1 to 1.5 hours to the required time. To summarize be there earlier rather than later, ask nicely for an update when possible, and most importantly stay out of the OR staff’s way.

**Part 4: Collecting the Specimen**

IMPORTANT NOTE: While in the operating room, DO NOT go within 2 feet of any sterile area or personnel. (Basically, if it is blue, stay at least 2 feet away from it.) You should not ever reach over the blue sterile area. Stay in the corner and wait for the circulating nurse or other OR staff to get the heart from the scrub nurse and place it into the cooler.

1. Ensure that you do not take any biohazard materials on the normal elevators. Please use the service elevators. (This includes the cooler with or without the heart)
2. Before passing a designated red line, ensure that you have a hair net, and a face mask on. Please make sure that you learn these designated areas while you are training.
3. Find the designated OR room
4. Sanitize hands before you go in, as well as once you are in the room.
5. Locate the circulating nurse, tell him or her who you are, and ask for a patient ID sticker, the pathology sticker, and the pathology papers
6. Sometimes they will send on the pathology papers, which is fine, just make sure that if they have not been taken to path that you do so, they will sometimes leave the pathology papers at the front desk, so if the papers are there, make sure you collect them no or on the way down to pathology.
7. Once ready, someone from the OR staff will provide you the sample in a dish (usually it is orange, but it might be blue.
8. Remember: DO NOT touch the sample if you are not wearing gloves. It is often easiest to just open the cooler and allow the staff member to place it in there.
9. Ask the nurse (or look to see if it is written on the OR board) for the times when cardiopulmonary bypass was turned on and/or when the crossclamp time was.
10. Note: If this is an organ donor, they will not let the failing heart out of the room until the new heart has arrived. So, if possible, try to find out when the heart was removed, so that we have an idea of how long the heart has been sitting on ice.
11. This can sometimes be a long time, and if it is, please notify Ken and Mindy.
12. Leave the OR, sanitizing upon exit.
13. Once you are passed the designated area, you may remove your mask.
14. Again, please use the service elevators on your way back to lab.

**Part 5a: Dissecting for Heart Transplants and Organ Donors**

Note: Try to do the following steps as quickly, but efficiently as possible. You know how fast you can or cannot go, but the faster the tissue is frozen, the better quality the sample will be. This being said, please do not compromise the quality of the samples that are collected, or your own safety.

1. Once back in the lab, place the patient sticker in the batphone log, in the designated area.
2. Take a Picture of the Specimen
3. Double check your liquid nitrogen level
4. Place ice in one of the weigh boats, then place the other weigh boat on top.
   1. You want to ensure that while you are cutting that you are keeping the tissue as cold as possible.
   2. Refill this ice as needed
5. Grab the heart and feel inside the ventricles. Identify the left ventricle and the right ventricle.
   1. Note: The best way to do this once the heart has been expelled from the chest cavity is to evaluate the thickness of the ventricles. The left ventricle should be thicker, even in dilated hearts.
6. Cut out a decent sized circle from the left ventricle (you want this section to be towards the apex), and lay that piece in the weigh boat
7. Put the heart back in the cooler (remember you want to keep it cold)
8. Now you cut this sample, by layering it into 1/3. You can choose whether you start with epi or endo, but make sure to put the correct tissue into the correct tubes.
   1. The sample placed in the tube should be approximately:
9. After you place tissue in a tube, place the tube into the liquid nitrogen bucket.
   1. IMPORTANT: Keep track of how many samples that you take from each section to write in the bat phone log.
10. Everything else (the epi fat, RV,LA, RA, and septum) can be just cut into the small pieces and placed into the appropriate tubes.
    1. Aorta should be cut into thin rings and placed in tubes that are labelled accordingly.
    2. Note: Only take LA or RA if you are certain that it is LA or RA.
11. Once you have finished cutting, place the left-over pieces of the heart into the cooler.
12. Grab a transfer box and place the tubes in the transfer box. Place the box in the tank and write where you put the box in the bathphone log.

For Heart transplants and LVADS

1. Take the heart procured from heart transplants back down to pathology
   1. Ensure the dish the heart is in has a large, orange specimen sticker (99% of the time the circulating nurse will have placed this on the dish, only once has it not been added).
2. If you have not done so already, with the heart in the cooler, go to the OR front desk and grab a specimen sheet for pathology. This will be a single sheet of white paper located in a wire tray.
   1. If they specimen sheet is not in the tray NICELY ask someone behind the desk if there is a specimen sheet in the printer tray or if they could print the specimen sheet of the patient. Give them the patient name and/or the OR number of the surgery. They should be able to print you off a new specimen sheet.
   2. IMPORTANT: Double check that the name on the orange specimen sticker on the dish containing the heart matches the name on the specimen sheet.
3. Take the specimen sheet and cooler to surgical pathology (you should have been shown were this room is during shadowing. It is very close to the OR front desk).
   1. It is highly likely that there will be no one in pathology because it is the middle of the night. Just badge into the room and enter.
4. If the container is not in a clear bag, find a bag in the room and place the container into the bag.
5. Place the container with the heart into the refrigerator to the right of the entrance. The refrigerator will be in the front room to the right of the door (It is hard to miss).
6. To the right of the refrigerator there should be a wire tray. Place the specimen sheet into the tray.
7. If it during the day there may be a resident, tech, or other worker in pathology that can help you if you explain you are with the cardiovascular biobank can give them the specimen and specimen sheet.
   1. Take the empty cooler and return to lab.

For organ donor hearts procured from KODA there may not be any remaining tissue (depending on the amount of tissue given by KODA).

1. If there is a large amount of remaining tissue this can be taken down to pathology and disposed of in one of the biological waste bins. These are located in surgical pathology.
   1. Enter pathology, go straight into the second room, and enter the door at the other end of the second room. On the left there is a closet with various trash cans. Place the container with heart into the bin lined with an orange biohazard bag
   2. If you are unsure of where to dispose of the tissue you can store the heart in one of the refrigerators overnight and ask someone the next day.
2. Come back to the lab with the cooler
3. Place the underpad and weigh boats into the autoclave bag.
4. Use one of the biohazard Tupperware containers; fill it halfway with DiCon solution and the rest of the way with water.
   1. Let that sit for one day, but do not let it sit for too long because we do not want the tools to rust.
5. Dump the ice from the cooler into the sink.
6. Spray down the counter and the cooler with the Conflict spray.

**Part 5b: Dissecting for LVADs**

1. Like with the hearts, get a specimen sheet from the OR front desk

If it is during the day take the LVAD core and specimen sheet directly to pathology after getting it from the OR and have someone in pathology take approximately half of the tissue.

Give them the specimen sheet

Take the remaining tissue up to the lab

If it is after normal work hours go to lab first and return to pathology after taking specimen samples for the biobank

1. Once back in the lab, place the patient sticker in the batphone log, in the designated area.
2. The LVAD, unlike the other dissections does not get the LV split into layers. Simply cut into small pieces
3. Place into tubes with the proper hashcode that are labelled “LVAD Core”
4. Grab a transfer box and place the tubes in the transfer box. Place the box in the tank and write where you put the box in the bathphone log.
5. If it after normal work hours, there will likely not be anyone in pathology. Take the LVAD core up to lab and take half of the tissue for the biobank.

Take the remaining tissue down to pathology, store in their refrigerator, and place the specimen sheet in the same wire tray as with heart transplants

1. Place the underpad and weigh boats into the autoclave bag.
2. Use one of the biohazard Tupperware containers; fill it halfway with DiCon solution and the rest of the way with water.

Let that sit for one day, but do not let it sit for too long because we do not want the tools to rust.

1. Dump the ice from the cooler into the sink.
2. Spray down the counter and the cooler with the Conflict spray.

\*\*If it is not too late at night, take the biohazard bag up to the autoclave on the 6th floor and run the biobag40 option. Make sure to fill out the log in that room. If it is late at night, you can take it the next day, but try not to leave it for too long.