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SOP for Endoscopy Collection

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We use this protocol and it's working

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Abstract

SOP for Endoscopy Collection

Purpose

1 Due to the time and temperature sensitive nature of tissue collection, we must be fully prepared with all supplies for the procedure and that all persons involved in the collection process are assigned a particular role. Here, we have provided detailed guidelines on exactly how to navigate through a endoscopy.

2 To establish guidelines on:

1. Preparation for endoscopy procedures at hospitals/clinics (e.g. supplies, gross room set up)
2. Responsibilities of different roles to ensure collection during procedure runs smoothly and efficiently
3. Storage methods of samples

Set Up

3 The tissue processing set up:

1. Option 1: Rack

a. Set up the necessary supplies for tissue handling and processing (forceps, petri dishes, well plates, razor blades, tubes, etc.) onto a wheeled rack to take into the procedure room. Include mediums of preservation as mentioned below.

i. Flash freezing with liquid nitrogen canister

ii. Cryostor with 200ul pipette, tips, Mr. Frosty, dry ice box

2. Option 2: Room

a. Set up the necessary supplies for tissue handling and processing (forceps, petri dishes, well plates, razor blades, tubes, etc.) in an adjacent empty procedure room. Include mediums of preservation as mentioned below.

i. Flash freezing with liquid nitrogen canister

ii. Cryostor with 200ul pipette, tips, Mr. Frosty, dry ice box,

Roles and typical persons involved (SWAT team)

4

- Tissue Handler: The Tissue Handler will collect the tissue from Dr. Ladabaum to place into the well plates and hand off to the Processor. They are also in communication with the Recorder regarding the stage and phenotype of each tissue.

- Processor: The Processor will measure the size of the tissues 3-dimensions and determine the appropriate storage method.

- Recorder: The Recorder will write down the time collected, stage, phenotype, measurements, the preservation method and any other relevant notes relavent to each tissue.

- If there are multiple Processors, they can help with labeling tubes, tissue preservation and storage. However there should be only one Processor **who is measuring sample sizes and relaying that information to the Recorder**. After measuring and cutting the samples, the Processors will preserve all samples and divide up any samples that are to be stored in multiple ways.

Consenting the Patient (CRC)

5 Clinical research coordinator (CRC) will call the patient before procedure (1 to 2 weeks before) to go over research and research protocols (IRB protocol/consent forms). Once the patient verbally agrees, a copy of the consent forms will be sent to them via email to look over before the day of the procedure/collection.

Procedure Set Up (CRC)

6 Once the patient has been verbally consented and agreed to participate in research, the clinical research coordinator will contact clinic/hospital staff to coordinate.

1-2 weeks before procedure:

a) Notify (email) surgeon performing endoscopy that their patient has consented to participate in research and that the research team will be collecting the colon once excised.

Day before procedure: Re-confirm with clinic and hospital staff of research group's attendance

Same day as procedure:

a) **Before patient arrival**

i Notify front desk that CRC will need to talk to the patient before being brought back to pre-op.

ii) Notify pre-op nurses that the patient is participating in research and give blood vials (2 purple tops and 2 streck tubes) to corresponding nurses to draw blood in pre-op.

iii) Go to main nursing station- notify nurses that CRC will be collecting colon polyps for research.

When patient arrives (pre-op):

a) Find private space in the waiting room to go over and sign research consent forms.

b) Go into pre-op (with a box of wet ice) and wait for blood samples to be collected from the patient.

Once blood samples are collected: notify research team members that purple tops have been collected, on ice, and are ready to be processed

When patient is in the procedure:

a) Once a patient is brought into the operating room- the research team can set up for collection in the procedure room or adjacent empty procedure room.

Procedure

7 **Arrival and preparation:**

SWAT team will arrive at the clinic at minimum an hour before patient arrival to account for collecting blood and setting up equipment. PPE must be worn at all times. Stanford Endoscopy Clinic PPE is located in the hallway across from procedure rooms and can be accessed with a working badge.

7.1 **Dissection of colon and Tissue Collection (Fig. 1):**

1. Once we receive the colon, the Cutter will place it on the Specimen Bench with the descending colon on the left and the ascending colon on the right.

a) This is done solely for consistency in taking pictures with how the images from our first colon were collected.

2. The Cutter will then wash the colon and begin flaying it open lengthwise.
3. Before the cutter begins dissecting tissue of interest- pathology attending and PA staff will look at colon and collect/take notes/dye areas of interest for clinical diagnosis/practice. Attending MD should give approval to begin research collection.
4. The Cutter chooses the tissues of interest and marks them with a numbered pin. Those tissues are then ressected from the colon.

a) Tissues of interest include Adenocarcinomas, Polyps, and Normal tissues.

i) Polyps and adenocarcinomas: Identified by the surgeons, pathologists, and the Cutter.

ii) Normal tissues: Characterized by the Cutter as regions of the colon epithelium lacking polyps and adenocarcinomas.

5. The SBM shows The Cutter which well to place the tissue of interest into, making sure that all samples stay in order and that none of the wells are used more than once. Also, if certain tissues need to be reserved for fresh tissue dissociations, the SBM will make sure these are placed into specific wells and passed off to wet lab personnel.
6. All push pins, storage tubes, and well plates will be labelled with the same numbers (e.g. 001-056, 101-156, 201-256). Resected tissues will be marked with the push pins and placed into the corresponding wells, then stored in their respective storage tubes.
7. Repeat steps 3-5 until all tissues have been properly accounted for and stored.
8. Once all the available tissues are collected, or it has been ~2 hours since the colon was received, pictures of the entire colon will be taken, preferably from a dead-on top angle with the camera kept at the same height for each frame.

9. The SBM will be sure to record times and sample info and direct Cutter to place samples into appropriate well plates.

7.2 **Figure 1**

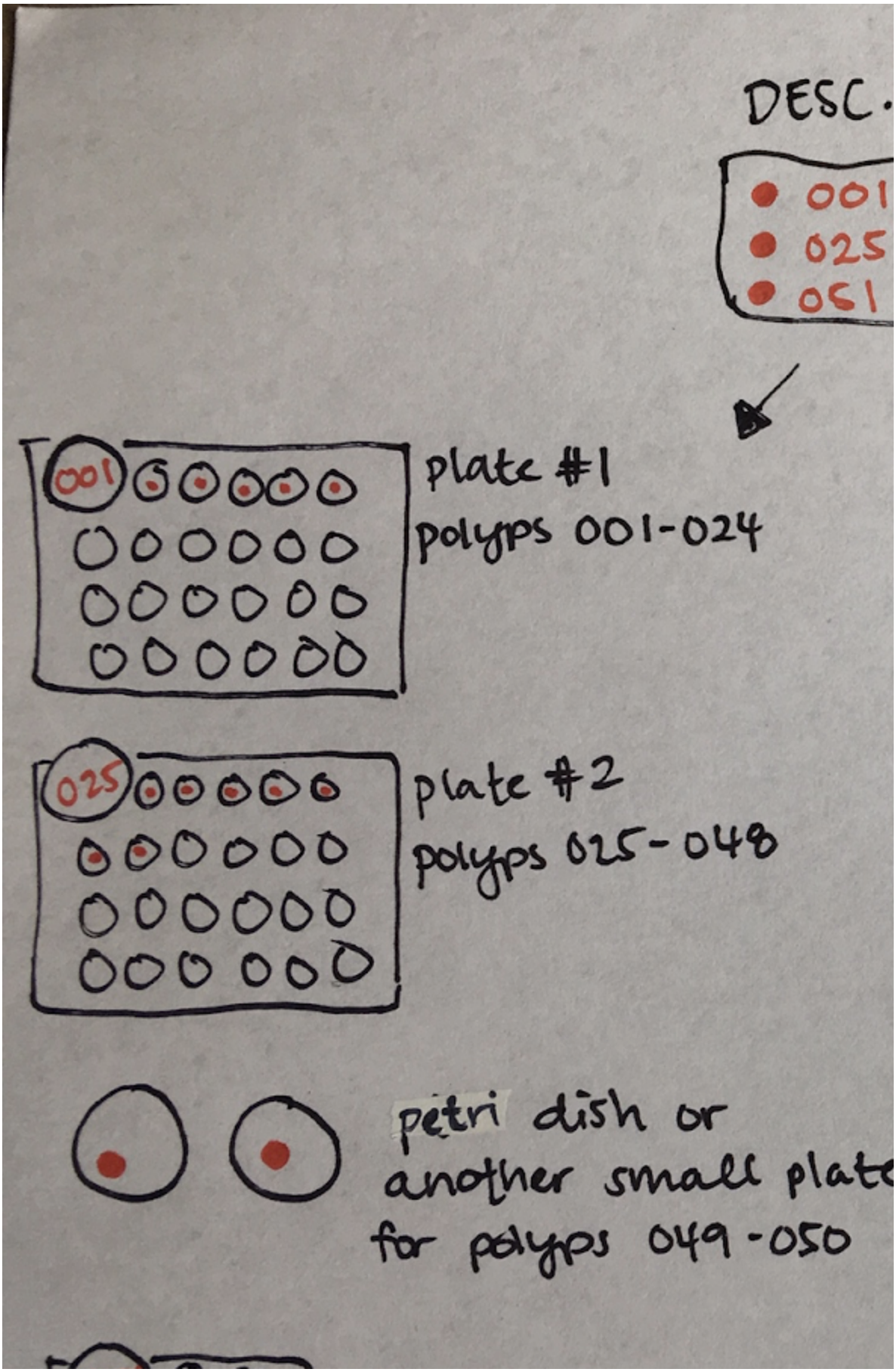
DESC.
001
025
051
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plate #1
polyps 001-024
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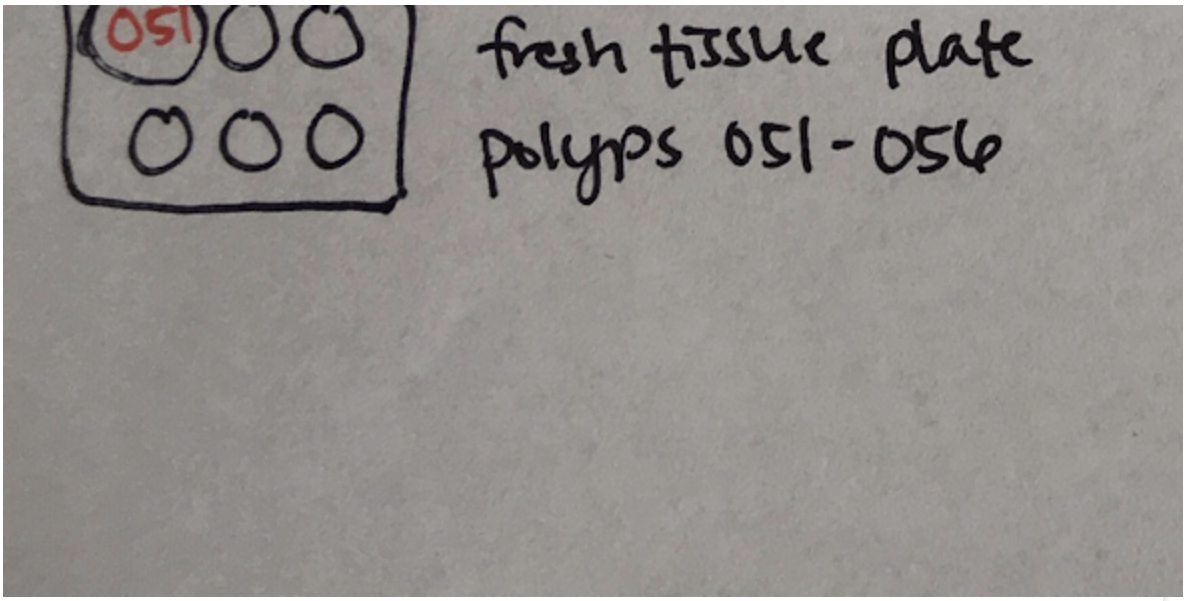
plate #2
polyps 025-048
petri dish or
another small plate
for polyps 049-050



7.3 **Sample processing (occurring at the same time as the steps above):**

1. Tissue Handlers will move between Specimen and Processing Benches, passing tissue samples along for processing and returning well plates and petri dishes for more samples.
2. To speed up the process, The Cutter will place several (~5-6) tissues into a single plate (e.g. the plate for descending tissues) and pass these off for processing.
3. While the first plate is being processed, the Cutter can start filling 5-6 tissues in a second plate (e.g. the plate for ascending tissues).
4. When the Processor is finished with the first plate, they will start on the second plate, and the Tissue Handlers will bring the first plate back to the Specimen Bench.
5. This rotation of plates will eliminate any downtime while tissues are being dissected and stored.
6. If there are more samples than anticipated (after well plates have been used), Cutter will place samples onto individual petri dishes. Tissue Handlers will place these petri dishes in sequential order on Processing Bench to ensure numbering stays in order.
7. If there is a back up in sample processing, the Processor will measure dimensions of the samples, then pass some of them off to the Tissue Handlers with direction on storage.

a) To keep duties organized in the event of a back up, the Processor will handle all flash freezing and pass off Cryostor and Carnoy's samples to the Tissue Handlers. The Tissue Handlers will inform the PBM the amount of Cryostor used for each sample.

Storage methods of samples in the gross room

8 **Storage Methods**

Flash Frozen

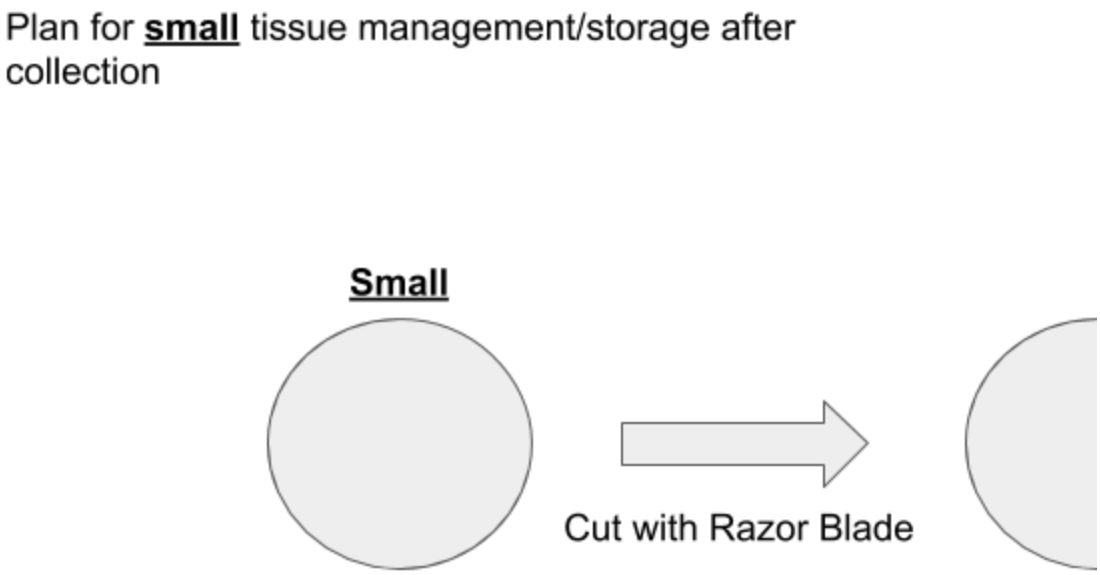
- Tissue preserved will be placed at the bottom of a Cryovial and immediately placed into the liquid nitrogen canister.

Cryostor
- If tissues are larger than 5mm in any dimension, tissue will be split, 50% FF and 50% Cryostor. The half that is stored in Cryostor will be placed in a Cryovial. The entire tissue will be covered with Cryostor, between 100-200ul depending on how large the section is.

Size and Tissue type criteria and their preservation methods

9 Small polyps
- Polyps less than 5mm in any orientation will be stored 100% FF (Fig 2).

Figure 2



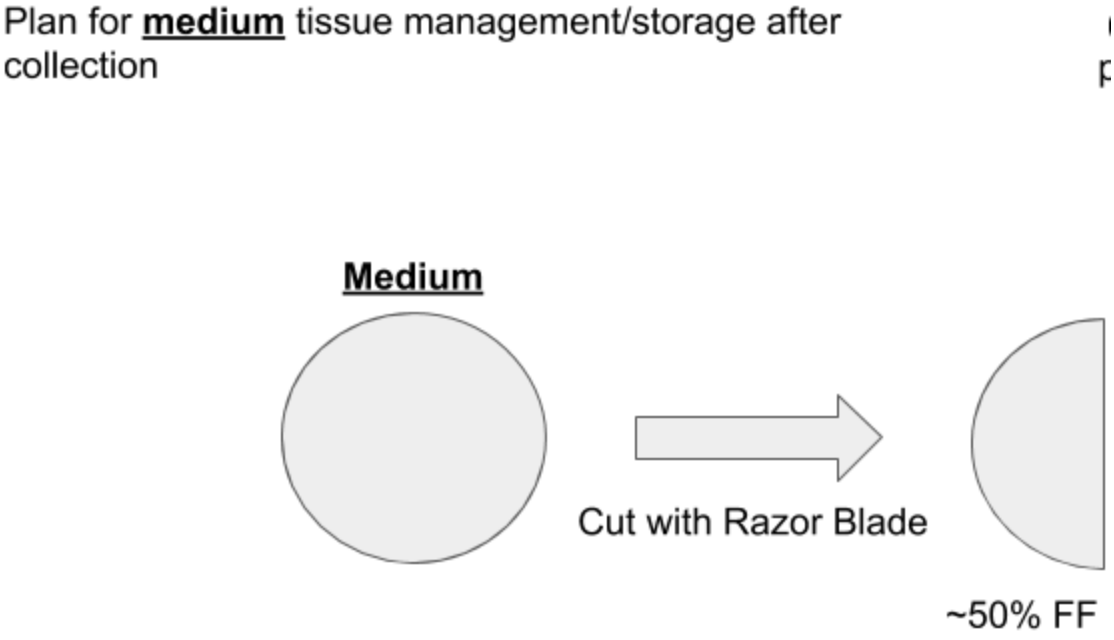
9.1 Medium and Large polyps

- Medium - Polyps between 5mm - 10mm in any dimension (Fig 3).
- Large - Polyps are greater than 10mm in any dimension (Fig 4).

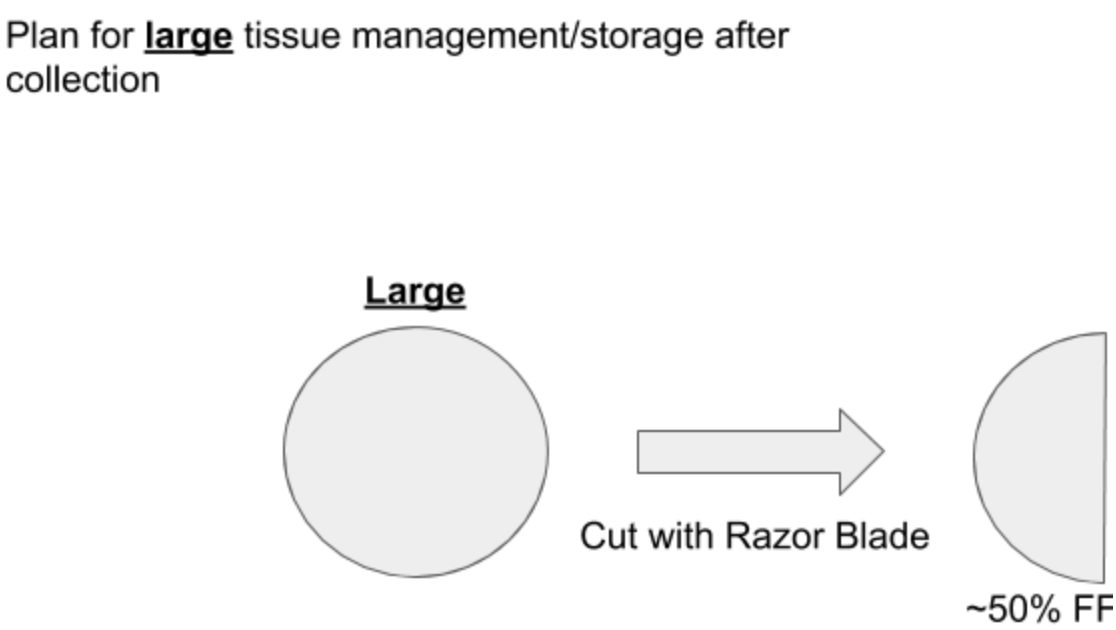
i) Split in half and store 50/50 FF and Cryostor.

ii) Tissue can be split further, with additional pieces stored in 100-200 ul Cryostor, if needed (Fig 4). Tissues should be minced with razor blade before storage in Cryostor. Only cut the tissue a few times, enough to break it up but not so much that tissue is heavily fragmented. Afterwards, these Cryostor samples will be placed on wet ice, followed by a Mr Frosty container in -80 once the procedure is complete. Be sure to communicate with the PBM the exact amount of Cryostor used for each sample.

Figure 3



9.2 **Figure 4**



Supplies

10 Supplies will be prepared the day before to be ready for pick up and transport to hospital on the day of the procedure. The day of, we will need to fill all wet and dry ice boxes and the liquid nitrogen canister. We will also need to make fresh aliquots of Cryostor.

1 tissue collection sheet
Gloves
Cryovials labeled with sample names

- (A00X-C-###)
- Blood: 5 plasma, 2 buffy
- M+CS samples
- Extra cryovials

Wet ice boxes and wet ice

- Small: for blood
- Large: for intermediate cryostor + samples

Dry ice boxes and dry ice
- Small: for freezing blood
- Large: for Mr. Frosty cryostor samples

Petri dishes
Razor blades
1mL Pipette + tips
200ul Pipette + tips
Cryovial freezing container (Mr Frosty)
LIquid nitrogen canister and liquid nitrogen
1 pack 15ml Falcon Tubes
- For blood processing
- Large polyps

2 Markers (freezer safe)
2 Pens
1 Tape
1 Ethanol spray bottle
Kim Wipes
2 Forceps
Ruler
3 Tube racks
Well plates (6, 12 or 24) (clear ones)
- Labeled starting at 1