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Stanford FAP Colectomy SOP - HTAN

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We use this protocol and it's working

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Abstract

Stanford FAP Colectomy SOP

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 colectomy.

Purpose:

To establish guidelines on:

- 1)Preparation for colectomy procedures at hospitals (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 in gross room

+rbchiu@stanford.edu I've highlighted a few spots in this document that I would like edits and additions made to. let me know if you have any questions or comments. Thank you for your work on this document Roxanne. If possible, I'd like to share this with the group on Monday to get more feedback.

Assigned to Roxanne Chiu

Attachments



Stanford FAP Colecto...

4.7MB

Materials

Supplies:

Supplies will be prepared by Roxanne to be ready for pick up by Aaron 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 and Carnoy's. Depending on time of procedure, Roxanne will either meet Aaron at the hospital or leave Porter with Aaron and supplies.

2 tissue collection sheets (1 for SBM and 1 for PBM)

Gloves

Cryovials labeled with sample names

- (A00X-C-###): 001-056, 101-156, 201-256

- Blood: 5 plasma, 2 buffy

- M+CS samples (prepare 10 to start)

- 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 50ml Falcon Tubes

- For Carnoy's media

- For freezing large samples

- For preserving large Carnoy's samples

1 pack 15ml Falcon Tubes

- For Carnoy's samples

2 Markers (freezer safe)

2 Pens

1 Tape

1 Ethanol spray bottle

Kim Wipes

2 Forceps

Ruler

3 Tube racks

100-150 numbered push pins on a Styrofoam box lid

Timer or stop watch



24-well plates (clear ones)

- Labeled 001-050, 101-150, 201-250

6-well plates (clear ones)

- Labeled 051-056, 151-156, 251-256 (for fresh tissue experiments)

Troubleshooting

Gross Room Set Up

- 1 The gross room area will be set up with 2 main stations.
 - 1.1 Specimen Bench. One bench for the colon
 - a. The supplies needed at the Specimen Bench are the forceps, push pins, petri dishes, and well plates
 - 1.2 Processing Bench - Another bench for processing samples
 - a. All other supplies (listed below) will be placed ergonomically around the Processing Bench.
 - b. Supplies should be set up in "mini stations" for:
 - i. Flash freezing with liquid nitrogen canister
 - ii. Cryostor with 200ul pipette, tips, Mr. Frosty, dry ice box,
 - iii. Carnoy's media and tube rack.

Description of roles

2 **Roles and typical persons involved (SWAT team):**

- Cutter : The Cutter will clean the colon and cut out the samples. The Cutter is responsible for laying the colon out with the descending colon on the left and ascending on the right, cutting it open lengthwise and resecting as much tissue of interest (i.e. polyps, adenocarcinomas and normal tissue sections) as possible while maintaining order with the SBM.
- Specimen Bench Manager : The SBM will work with the Cutter to record the Collection time, Stage, Phenotype, Location, and Neighboring samples for the tissues. The SBM is responsible also for keeping track of which tissue number has been collected by the The Cutter by ensuring 2 things:
 - 1) The Cutter has placed the tissue of interest into the well which corresponds with the tissue number.
 - 2) The SBM places the push pin with the corresponding tissue number into the colon where the tissue of interest was taken.
 - Once all the polyps have been collected and push pins are in place, the SBM and The Cutter are responsible for taking pictures of the colon for photographic evidence of each push pin's location.
- Tissue Handlers : The Tissue Handlers will hand the well plates/petri dishes full of ~6-10 tissues to The Processor at the Processing Bench. They also serve as assistants to The Processor.



- **Processor** : The Processor will measure the size of the tissues 3-dimensions and determine (with consultation with Aaron and Stephanie) the appropriate storage method.
 - Tissue Handlers will assist with labeling of extra tubes, storing of tissue into cryostor, and storing of tissue into Carnoy's. To avoid confusion, The Processor **should be the only person measuring sample sizes and relaying that information to the PBM**. After measuring and cutting the samples, the Processor will preserve all samples and divy up any samples that are to be stored in multiple ways to the Tissue Handlers to aid in preservations.
- **Clinical Research Coordinator/Processing Bench Manager** : The CRC/PBM will work with The Processor to record the Size, Rough fraction x Preservation method, Storage time, and any Notes for each of the tissues.

Consenting Patient: (CRC)

- 3 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)

- 4 Once the patient has been verbally consented and agreed to participate in research, the clinical research coordinator will contact operating room (OR) staff to coordinate specimen pick up, as well as gross room staff to reserve space for specimen collection.
- 5 **●1-2 weeks before procedure:**
 - Notify (email) surgeon performing colectomy that their patient has consented to participate in research and that the research team will be collecting the colon once excised.
 - Notify (email) gross room PAs that we will be collecting colon tissue for research and will need gross room space. Ask the PA who their attending pathologist will be so that they are aware.
- 6 **●Day before procedure:** Re-confirm with the gross room PAs that we will need space in the gross room.
- 7 **●Same day as procedure:**

7.1 ○ **Before patient arrives:**

- Check in with gross room staff and reserve a bench for collection. Go over the timeline for the day to ensure that the research team will have the space reserved.
- Notify front desk that CRC will need to talk to the patient before being brought back to pre-op. They will usually call the CRC once the patient checks-in.
- 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.
- Go to OR main nursing station- notify nurses that CRC will be collecting a colon for research. The nurses will let you know what operating room that the patient will be in, as well as the operating room phone number.
- Call the operating room to notify nurses in the room to call the CRC as soon as the colon is removed and ready for pick up.

7.2 ○ **When patient arrives (pre-op):**

- Find private space in the waiting room to go over and sign research consent forms.
- 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 (usually Aaron)

7.3 ○ **When patient is in surgery:**

- Once a patient is brought into the operating room- the research team can set up for collection in the gross room.
- Check in with the operating room nurse periodically.
- Operating room nurse will call CRC once the colon is ready for pick up.
- CRC and Ed will go to OR (in full PPE) to collect the colon and walk it to the gross room to begin collection.

Processing Tissue Procedure

- 8 *Arrival and preparation:* SWAT team will arrive at the gross room at minimum an hour before the colon is removed from the body. PPE must be worn at all times. Stanford Hospital's Gross Room PPE is located on the metal rack next to the side entrance.
- 9 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.

- 9.1 This is done solely for consistency in taking pictures with how the images from our first colon were collected.
- 10 The Cutter will then wash the colon and begin flaying it open lengthwise.
- 11 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.
- 12 The Cutter chooses the tissues of interest and marks them with a numbered pin. Those tissues are then resected from the colon.
- 12.1 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.
- 13 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.
- 14 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.
- 15 Repeat steps 3-5 until all tissues have been properly accounted for and stored.
- 16 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.
- 17 The SBM will be sure to record times and sample info and direct Cutter to place samples into appropriate well plates.

Sample Processing (occurring at the same time as the steps above)

- 18 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.



- 19 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.
- 20 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).
- 21 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.
- 22 This rotation of plates will eliminate any downtime while tissues are being dissected and stored.
- 23 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.
- 24 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.
- 24.1 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

- 25 Flash Frozen - Tissue preserved will be placed at the bottom of a Cryovial and immediately placed into the liquid nitrogen canister.
- 26 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.
- 27 Carnoys - Carnoy's media will have been aliquoted into 15ml or 50ml Falcon tubes prior to procedure. The tissues to be stored in Carnoy's will be determined by Aaron.
- 27.1 Carnoy's Samples - Samples to be stored in Carnoy's will be specified by the PBM (Aaron).*

Adenocarcinoma (AdCa)
AdCa-adjacent Polyp
AdCa-adjacent Normal

AdCa-distant Large Polyp**
AdCa-distant Smaller Polyp**
AdCa-distant Normal section**

* Samples are described with the Adenocarcinoma as the reference point. So, either adjacent to or distant from the Adenocarcinoma.

**These samples should be from a different region of the colon than what the Adenocarcinoma came from. For example, if the AdCa is in the Descending colon, the AdCa-distant samples should be from the Transverse or Ascending colon. The Carnoy's tubes will be pre-labeled and will each contain ~5-10ml Carnoy's media. Sample will simply be dropped into the tube containing the media and stored at 4C on wet ice.

Size Criteria for Preservation methods

- 28 Small polyps - Polyps less than 5mm in any orientation will be stored 100% FF (Fig 2). Figure 2
- 29 Medium and Large polyps - Medium - Polyps between 5mm - 10mm in any dimension (Fig 3).
- 30 Large - Polyps are greater than 10mm in any dimension (Fig 4). - Split in half and store 50/50 FF and Cryostor. - 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.
- 31 Normal tissues - For ease of use in the future, normal tissue samples will be cut into manageable pieces (cut by the Processor) before being placed into a cryovial. When placing the normal tissue samples in the cryovial, be sure to lay it as flat and as un-twisted as possible so that its orientation can be easily determined when it is used for experiments in the future. Normal tissue samples do not need to be measured.