

# **UNC HEALTH REGISTRY/ CANCER SURVIVORSHIP COHORT**

**BIOSPECIMENS  
COLLECTION, PROCESSING and STORAGE  
PROTOCOL**



**UNC**

**LINEBERGER COMPREHENSIVE  
CANCER CENTER**

**N.C. CANCER HOSPITAL**

**University Cancer  
Research Fund**

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## **Section I: Blood specimen collection, processing and storage**

### **a. Overview**

At the North Carolina Cancer Hospital (NCCH) clinic visit when informed consent for the UNC Health Registry/Cancer Survivorship Cohort (HR/CSC) is obtained, recruitment specialists provide eligible participants with a blood collection kit (see Appendix I). The patient provides the blood collection kit to the phlebotomist at the time clinically ordered blood work is collected. The two additional research blood tubes collected include one 10mL lavender top tube collected for plasma and DNA and one 10mL red top tube collected for serum. To maximize the utility of the serum (e.g., for proteomics), the study staff allow the red top tube to sit at room temperature (RT) for 30 minutes immediately after collection to facilitate coagulation. Following the RT incubation the red top tube is placed directly on ice for transport to the Tissue Procurement and Biospecimens Processing facility (TPBP). The study staff places the lavender top tube on ice immediately after it is drawn. Blood tubes are transported on ice in secure containers within 2 hours of collection to the TPBP for processing and storage. Please refer to the Enrollment Protocol- Biospecimens for a more detailed explanation of the pre-clinic blood kit preparation, bar code label assignment, and blood collection and tracking process followed by UNC Health Registry/Cancer Survivorship Cohort staff members.

Once the blood samples arrive in the TPBP facility, the study staff logs the specimens into the Laboratory Information Management System (LIMS) and places the tubes in a designated rack in the TPBP refrigerator. Processing of all blood specimens to serum, plasma and DNA occurs promptly after TPBP sample receipt. Serum, plasma and extracted DNA are aliquoted according to standard protocols and stored long term at -80°C. All data stored in the LIMS system is transferred to the Lineberger Data Warehouse and Biospecimens Repository (LDBR) on a weekly schedule. Data and specimen requests are made centrally through the LDBR Governance and Data Sharing Committee.

### **b. Blood processing**

Once the tubes are drawn and transported to the TPBP the study staff log the specimens into the LIMS by scanning the specimen bar code label on each tube and entering the time of collection and the quantity of blood collected into the LIMS. The study staff then places the tubes in a rack in the TPBP refrigerator where they are stored at 4°C until processing. Lab processing is begun within 30 minutes of sample receipt at the TPBP. At the time that processing begins, TPBP staff generate a specimen-specific TPBP processing bar code label for each tube that is linked via the LIMS to the specimen collection ID. The TPBP bar code label encodes study specific information and includes a human readable portion to facilitate lab processing procedures.

#### **i. Plasma and DNA**

The 10mL lavender top vacutainer tube is centrifuged at 3250g for 10 minutes using a swinging bucket rotor to allow separation of plasma and red blood cells. After centrifugation the plasma fraction is removed with a sterile disposable pipette and aliquoted in equal volumes into 8 cryovials. The lab then processes the remaining contents of this tube to a white blood cell lysate using Puregene chemistry. This is a two step process: first after a short incubation in a red blood cell lysis solution, the sample is spun at 3250g for 10 minutes to pellet the lysed red blood cells; after centrifugation and removal of the supernatant, white blood cell (WBC) lysis solution is then added to the WBC cell pellet, which is then thoroughly vortexed and stored at room temperature (RT). The samples are maintained in this state for 1-7 days until DNA is extracted using the Qiagen Autopure extraction robot (see protocol below). DNA is quantitated using optical density and aliquoted into 4 equal aliquots at stock concentration. All optical density readings and stock concentrations are entered into the LIMS and routine biorepository reports are created using the LDBR Pentaho System.

## ii. Serum

Concurrently, also within 30 minutes of sample receipt and LIMS log in at the TPBP Facility, the technician extracts serum from the 10mL red top tube using the following process. The red top vacutainer tube is centrifuged for 10 minutes at 3250g in a swinging bucket rotor to separate serum from coagulated blood. With a sterile pipette, the serum fraction is removed and aliquoted equally into 4 cryovials.

## iii. Automated DNA isolation from WBC lysate

### a. Materials

Gloves  
50 ml Autopure input qubes (pink caps)  
50 ml Autopure output qubes (blue caps)  
Autopure reagents from Qiagen  
Screw top 1.5-2.0ml microcentrifuge tube  
-80° C freezer

### b. Procedure

- After cell lysate incubation at RT for 1-7 days, prepare Autopure run.
- Enter rack set-up menu.
- Choose the "Cell Lysate XSpin XPour"\* protocol.
- Choose number of tubes (8 or 16).
- Choose re-hydration volume (1000µl).
- Enter run name
- Enter rack number
- Scan input and output qubes

- Place rack on machine
- Press start run
- After run has finished remove output qubes and incubate at 65°C for 1 hour or overnight at 55°C followed by overnight incubation at room temperature (RT) on the nutator at a speed of 25-30.
- Evaluate DNA solution after overnight rotating incubation, if DNA is not in solution more rehydration buffer may be added followed by additional incubations at 65°C and/or RT
- After hydration combine any samples that had been placed into multiple qubes prior to Autopure run.
- For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube.
- Place DNA at 4°C prior to quantitation and for short term storage. For long-term storage, store in protocol defined aliquots at -80°C.

\*Custom protocol developed for our lab with an increased spin time after the 70% ethanol addition and a two-step pour off of the ethanol wash.

### c. Blood product storage

All patients' blood aliquots including: plasma (8 equal aliquots), DNA (4 equal aliquots) and serum (4 equal aliquots) are placed in standard freezer boxes (81 aliquots/box) and stored in -80°C freezers. Off-site redundant biospecimen storage occurs at a secure facility. The University of North Carolina currently has a contract with the off-site facility, Kryosphere, located at 4222 Emperor Blvd. Suite 300, Durham, N.C.27703. This facility is located in the nearby Research Triangle Park (RTP) within 10 miles of the University of NC at Chapel Hill and the NC Cancer Hospital. Kryosphere is the region's first independent bio-repository with headquarters in RTP and space for over 15 million samples. Their state-of-the-art GMP (Good Manufacturing Process) facility provides custom bio-repository services, cold-chain-of-custody logistics and management of clinical trial samples. The facility is equipped with redundant utilities, monitoring systems, and 24/7 emergency response teams that will assure all research samples are safe and secure.

- i. **Patient Withdraw** – Blood products from study subjects requesting withdrawal should be removed from the repository and destroyed. UNC Health Registry processes for withdrawal (including laboratory notification and action) can be found in the UNC HR Protocol for Withdrawn Patients
- ii. **Orphaned lab barcode** (cannot be linked to research subject) – Blood products corresponding to orphaned barcodes that cannot be linked to a study subject (but were drawn from a consented study subject) are to be quarantined in the biorepository and flagged in the BSP LIMS. These samples are not to be distributed for research use. In the event an analysis becomes available that can

subsequently re-link the samples with the study subject – these samples may become available for future analysis.

## **Section II. Mouth rinse collection, processing and storage**

### **a. Overview**

UNC is a tertiary referral center and hence, a portion of patients have a central venous catheter in place and thus have initiated the first course of treatment at the time of their enrollment visit. Since it is not possible to collect pre-treatment blood samples for plasma and serum on patients with central venous catheters, a mouth rinse collection procedure to isolate buccal cell DNA is performed to enable germline DNA isolation from these patients.

### **b. Mouth rinse collection**

Immediately following consent, and prior to the clinic visit, the HR/CSC enrollment specialist identifies patients with existing central venous catheters eligible for mouth rinse collection. The enrollment specialist instructs the patient not to eat food and/or candy nor chew gum or drink or brush teeth 1 hour prior to the collection of the sample (e.g., during the clinic visit). Sample collection most often occurs following the clinic appointment in the phlebotomy area. HR/CSC staff administers the mouth rinse collection protocol using the mouth rinse collection kit (see Appendix II) and the following instructions. (This protocol is also adaptable to self-administered mouth rinse collection via mailed collection kits.)

#### *Instructions to the HR/CSC study staff member*

1. Read the instructions to the subject (steps 4-6 below) to familiarize the subject with collection process.
2. Remove the lid from the collection cup.
3. Open the bottle of Scope mouthwash

#### *Instructions to the subject*

4. Please pour half of the Scope mouthwash into your mouth. Please swish the mouthwash around in your mouth vigorously for 45 seconds. I will use my timer/wrist watch to time you. I will let you know when 45 seconds is over.
5. Holding the screw top collection cup close to your mouth, expectorate/spit all of the Scope mouthwash into the container. Close the collection cup briefly to prevent a spill.
6. Repeat this procedure 1 more time with the remainder of the Scope left in the bottle (swishing vigorously for 45 seconds while I time you). Open the screw

top collection cup from step 6 and expectorate/spit again into the same container.

7. Thank you very much for donating your mouth rinse sample.

#### Instructions to the HR/CSC study staff member

8. Screw the lid tightly on the collection cup.
9. Record the date and time on the screw top collection cup.
10. Place the collection cup into the leak-proof zip-top biohazard specimen transport bag.
11. The sample are delivered to the TPBP lab within 24 hours of collection or at the time of the next clinic specimen batch transport, whichever comes first.
12. Samples are logged into the TPBP LIMS and placed in the designated TPBP location at room temperature until processing.

#### **c. Mouth rinse processing**

Total DNA is extracted from 30-50mls of mouth rinse using the Qiagen Autopure DNA extraction robot when a sufficient volume of samples are available (see automated DNA extraction protocol below). In some cases, reduced numbers of mouth rinse samples may require manual DNA extraction (see protocol below). Human-specific DNA is quantitated using the TaqMan® RNaseP assay. All DNA concentrations are entered into the LIMS and routine biorepository reports are created using the LDBR Pentaho System.

##### **i. Automated mouth rinse DNA isolation materials and procedure**

###### a. Materials

Gloves  
50 ml Autopure input qubes (pink caps)  
50 ml Autopure output qubes (blue caps)  
Autopure and all reagents including proteinase K from Qiagen  
Screw top 1.5-2.0ml microcentrifuge tube  
-80° C freezer

###### b. Procedure

- Within 24 hours, transfer supernatant into a 50 ml input qube. If samples are to be processed that day, scan qubes for Autopure run. If samples are not run that day, prepare a cell pellet by centrifugation, remove supernatant and store cell pellet at -80° C until processing. If volume is greater than 54 mls split sample equally into two qubes and process separately through the DNA hydration step.
- Before processing mouthwash specimens on the Autopure place proteinase K (PK) on machine if it is not already in place. After

placement of tube enter diagnostic screen and prime (*check name*) PK line (follow instructions on monitor). Note: Autopure LS door must be closed before proceeding. Repeat prime procedure 3 times.

- Confirm that RNase A has been added to the cell lysis reagent bottle.
- Enter rack set-up menu.
- Choose the “mouthwash extended spin”\*\* protocol.
- Choose number of tubes (8 or 16).
- Choose re-hydration volume (500-2000 µl for Scope rinses). Large-volume initial sample require larger hydration volumes.
- Enter run name
- Enter rack number
- Scan input and output qubes
- Place rack on machine
- Press start run
- After run has finished remove output qubes and incubate at 65°C for 1 hour or overnight at 55°C followed by overnight incubation at room temperature (RT) on the nutator at a speed of 25-30.
- Evaluate DNA solution after overnight incubation, if DNA is not in solution more rehydration buffer may be added followed by additional incubations at 65°C and/or RT
- After hydration combine any samples that had been placed into multiple qubes prior to Autopure run.
- For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube.
- Place DNA at 4°C prior to quantitation and for short term storage. For long-term storage, store in protocol defined aliquots at -80°C.

\*\*Custom protocol developed for our lab with an increased spin time after the 70% ethanol addition.

## ii. **Manual mouth rinse DNA isolation and procedure**

- Within 24 hours, transfer supernatant (if not received in a container appropriate for centrifugation) into a 50 ml conical centrifuge tube. If volume is greater than 54 mls split sample equally into two tubes. Process separately through the DNA hydration step.
- Centrifuge at 3000 x g for 8 minutes. Repeat centrifugation if pellet is loose.
- Discard supernatant into hazardous waste and freeze pellet at -80°C until cell lysis and DNA isolation.

### Lysis:

- Thaw cell pellets at 37°C.
- Add 1 ml Cell Lysis Solution (containing 5µl RNase A 4 mg/ml) to the cell pellet and invert gently 50 times. Knock tube if pellet is stuck. (Note

Cell Lysis Solution containing RNase A is made up fresh as a batch in the amount needed for the number of tubes to be processed that day.)

- Incubate sample for 15 minutes at room temperature. (Note preparation can be kept for up to two years at this step). If after 15 minutes there are still clumps break them up if possible with a pipette, prior to adding the proteinase K.
- Add 10  $\mu$ l of Proteinase K Solution (20 mg/ml) to the cell lysate and invert 50 times to mix.
- Incubate lysate for 10 minutes at 65°C.

#### Protein Precipitation:

- Add 660  $\mu$ l Protein Precipitation Solution to the cell lysate.
- Vortex samples at high speed for 10 seconds to mix the Protein Precipitation Solution uniformly with the lysate.
- Place in an ice bath for 10 minutes to ensure a tight pellet in the following centrifugation step.
- Centrifuge at 3,000 x g for 10 minutes at 14°C. The precipitated proteins should form a tight, pellet. Repeat centrifugation if pellet is loose.
- Keep samples on ice while transferring supernatant into an output tube containing the Isopropanol to ensure the protein pellets remain tight?

#### DNA Precipitation:

- Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 15 or 50 ml output tube containing 2 ml 100% Isopropanol (2-propanol) and 5 $\mu$ l Glycogen Solution (20 mg/ml).
- Mix the sample by inverting gently 50 times.
- Centrifuge at 3,000 x g for 5 minutes at 14°C. The DNA may or may not be visible as a small white pellet, depending on yield.
- Pour off the supernatant and drain tube briefly on clean absorbent paper. Add 1 ml 70% Ethanol and shake the tube gently to wash the DNA pellet.
- Centrifuge at 3,000 x g for 2 minutes. Carefully pour off the Ethanol as DNA pellet may be loose.
- Invert and drain the tube on clean absorbent paper and allow to air dry 1 minute.

#### DNA Hydration:

- Add 500-2000  $\mu$ l of DNA Hydration Solution (based on the size of the initial cell pellet and the final DNA pellet).
- Rehydrate DNA by incubating at 65°C for 1 hour or overnight at 55°C followed by overnight incubation at RT on rotator set on a speed of 25-30.

- Evaluate DNA solution after overnight incubation, if DNA is not in solution more rehydration buffer may be added followed by additional incubations at 65°C and/or RT
- After hydration combine any samples that had been split do to large volume at the initial cell pelleting step.
- For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube.
- Place DNA at 4°C prior to quantitation and for short term storage. For long-term storage, store in protocol defined aliquots at -80°C.

#### **d. Mouth rinse storage**

The mouth rinse DNA samples are divided into 4 equal aliquots and placed in standard freezer boxes (81 aliquotes/box) and stored in -80°C freezers. Off-site redundant biospecimen storage occurs at a secure facility (see section c. above for details of off-site storage facility).

- Patient Withdraw** – Mouth rinse products from study subjects requesting withdrawal should be removed from the repository and destroyed. UNC Health Registry processes for withdrawal (including laboratory notification and action) can be found in the UNC HR Protocol for Withdrawn Patients
- Orphaned lab barcode** (cannot be linked to research subject) – Mouth rinse products corresponding to orphaned barcodes that cannot be linked to a study subject (but were collected from a consented study subject) are to be quarantined in the biorepository and flagged in the BSP LIMS. These samples are not to be distributed for research use. In the event an analysis becomes available that can subsequently re-link the samples with the study subject – these samples may become available for future analysis.

### **Section III: Tissue sample collection, process and storage**

#### **a. Overview**

The Tissue Procurement and Biospecimens Facility (TPBP) research staff collects excess tissue specimens from the operating room for patients consenting to the HR/CSC. For example, when a patient has a biopsy or surgery there may be some tissue or biologic specimen left over after the pathologist has completed their review. By agreeing to participate in the HR/CSC, participants are providing consent for their tissue to be processed and stored in the TPBP.

HR/CSC participants who appear on the operating room (OR) schedule are flagged for tissue collection by TPBP staff in the integrated HR/CSC tracking system. Tumor tissue collected in the OR is first evaluated clinically (e.g., for diagnosis) in Pathology then any remaining tissues (tumor and adjacent normal tissue) are taken to TPBP facility and

processed and archived for research. Processing samples includes snap freezing a portion ( $\geq 3$ mm tissue/cryovial) of the sample in liquid nitrogen within 30 minutes of collection. Snap frozen tissue is suitable for later extraction of RNA, DNA and protein. In addition, a portion of the tissue is formalin fixed and paraffin embedded (FFPE) within 1 hour of collection. For every 10 snap frozen vials of tissue, 1 aliquot (block) is banked as FFPE. In the case of a limited sample, the entire specimen is snap frozen. FFPE specimens are stored long term at room temperature and are suitable for histochemical techniques. For all specimens, corresponding data is collected and stored in the TPBP LIMS database including; time of biopsy/resection, time of devascularization, time of TPBP tissue pick up, size of sample, number of aliquots, anatomical site and time of processing completion.

## **b. Participant identification**

To identify and ensure collection of tissue by the TPBP staff in the operating room (OR), the HR/CSC staff runs a report with the Pre Care Clinic (Pre-Operative clinic) schedule on a daily basis. This report highlights patients who have previously consented to the HR/CSC that have visits in the Pre Care clinic in preparation for their upcoming procedure. This electronic report can be found in the LDBR (<https://ldw.unc.edu:8543/pentaho/Login>) under the PRegistry tab. The Informed Consent and the HIPAA signature pages are printed out on a light blue paper for the HR participants that have attended Pre-Care visits. These copies are taken to the chart room in Pre Care by an HR/CSC staff member and inserted into the patient's chart. TPBP staff pull all charts that contain light blue consent/HIPAA forms from the Pre-Care chart room for the next day's surgeries... They compile a list of these patients and then compare this list with a final OR schedule that is supplied to TPBP staff as a "final" schedule after 2:00 p.m. on the day prior to the scheduled surgeries. The target list is then revised and finalized by 5:30 p.m. This target list includes the surgeon, OR number and the time of procedure. Current procedures for target list preparation will soon be revised as automated methods of identification of HR/CSC patient's eligible for research tissue sample collection are developed and implemented through the LDBR.

## **c. Tissue collection**

The OR nurse pages the TPBP technician to the operating room using the TPBP pager number. They provide the OR room number when paging. Once the technician has been paged to the operating room, tissue is to be retrieved within five minutes. This is necessary to ensure that the specimen is banked in a timely manner (within 30 minutes of tissue collection/ischemia), thus allowing for high quality tissue. When reporting to the OR it is the TPBP technician's responsibility to confirm that the consent and HIPAA forms are signed with corresponding dates marked on the consent form. The TPBP technician follows OR protocol and transports the specimen on ice from the OR to the pathology assistant (PA) in Surgical Pathology. The TPBP technician informs the PA of the presence of the HR/CSC research consent. It is up to the PA to determine if leftover tissue is available for TPBP research banking and if so to provide the appropriate specimen to the TPBP technician.

Once it has been determined that leftover tissue is available for research banking, the TPBP technician processes the tissue into the correctly sized samples for snap freezing and formalin fixation/paraffin embedding in the Surgical Pathology laboratory. It is here that the tissue is placed either on dry ice (snap frozen samples) or into cassettes and then into formalin for fixation. All tissue samples are transported either on dry ice or in formalin from the Surgical Pathology laboratory to the TPBP at the end of the day where they are either inventoried or placed in long term storage (e.g., snap frozen) or remain in formalin for 16-48 hours. After formalin fixation, tissues are further processed to FFPE via the Leica processor in the TPBP. The TPBP technician enters relevant data into the LIMS including medical record number, date of birth, race, gender, and full name. Other recorded information includes date banked, surgeon, time TPBP technician is paged to OR, time of tissue ischemia, time retrieved from OR, time received from Surgical Pathology to begin TPBP processing, time processing completed by TPBP, tissue type, and the total aliquots banked for each tissue type. The bar code labels are generated during processing and applied to all cryovials and FFPE cassettes. Once all aliquot types have been processed accordingly the pager time is noted and recorded and the case is officially banked.

#### **d. Tissue processing**

##### **i. Snap frozen**

Tissue aliquots designated for snap freezing are cut into sections that range anywhere from 2mm<sup>3</sup> to 5mm<sup>3</sup> (see Appendix III for supplies). This is highly dependent on the tissue type and total amount available for research use. Tissue is placed into the snap frozen cryovial tubes using sterile forceps and labeled. All samples are labeled with a bar code label with study encoded information and a human readable portion containing TPBP ID, patient number, specimen ID number, date, study ID, and tissue type. (TPBP ID number is patient and anatomical site specific while specimen ID is aliquot specific.) Once labeled, the tissue is frozen by dropping the cryovial into liquid nitrogen. At the end of the day all samples are transported to the TPBP liquid nitrogen freezers and placed into inventory by assigning them a physical location and scanning the barcode into the LIMS.

##### **ii. Formalin-fixed Paraffin Embedded (FFPE)**

Tissue is cut into sections no thicker than 4mm and must be of a size that easily fits into the histology cassette (see Appendix III for supplies). Each cassette is to be labeled with a unique TPBP number as well as the tissue type, date, consented study, and specimen ID. Protocol calls for 1 FFPE specimen per 10 snap frozen aliquots. If only a small piece of tissue is available (less than 2 snap frozen vials), only snap frozen aliquots will be banked. The specimens are first fixed in 10% Buffered Formalin for a time period of at least 16 hours but lasting no more than 48 hours. After the fixation process, the specimens are rinsed in water for 10 minutes and put into 70% Ethanol until processing begins. The remainder of the processing protocol is automated using

the Leica ASP300. The primary processing schedule for the Leica ASP300 is stated below. This will be entered into the automated processor by the TPBP manager who is responsible for any edits or modifications to the processing schedule program.

Each Solution is for 30 minutes:

- 1- 70% Ethanol
- 2- 80% Ethanol
- 3- 80% Ethanol
- 4- 95% Ethanol
- 5- 95% Ethanol
- 6- 100% Ethanol
- 7- 100% Ethanol
- 8- Empty
- 9- Xylene
- 10-Xylene
- 11-Cleaning Xylene
- 12-Cleaning 100% Ethanol
- 13-Cleaning Water

3 Paraffin Cycles-58 °C with 140 pressure and vacuum setting

After fixation is completed, the labeled cassettes are opened and tissue removed. The tissue is placed in an appropriate sized heated mould. The tissue specimen is held down with a dissecting needle while partially filling the mould with molten paraffin. The tissue is then secured by quickly cooling the base of the mould, the block label is placed appropriately and the mould filled to the top with paraffin. The blocks are cooled to set the paraffin for 30 minutes before being removed from the mould. The blocks are ready to be sectioned or stored.

#### **e. Tissue storage**

At the end of the day all banked tissue is brought from Surgical Pathology to the TPBP. Snap frozen tissue is transported on dry ice. Snap frozen tissue is arranged by TPBP ID number and specimen ID and then all cryovial bar code labels are scanned into the LIMS. The box and position are recorded into LIMS to indicate the specimen's exact location in the inventory. All snap frozen samples are placed in standard freezer boxes that contain 81 aliquots per box and stored in liquid nitrogen dewars at -180°C. The FFPE tissue is transported to the TPBP in an enclosed container at room temperature. All FFPE paraffin blocks are stored in moisture resistant cardboard boxes or plastic storage boxes in the TPBP lab storage room, until further processing. This storage room is kept at or below room temperature and has no windows to avoid exposure of samples to sunlight and/or extreme temperature changes.

- i. **Patient Withdraw** – Tissue products from study subjects requesting withdrawal should be removed from the repository and destroyed. UNC Health Registry processes for withdrawal (including

laboratory notification and action) can be found in the UNC HR Protocol for Withdrawn Patients

## **SECTION IV: Sample Distribution Process**

To be determined – place holder

## **SECTION V: Appendices**

### **Appendix I: Blood collection kit supplies:**

Gloves

10 ml red top vacutainer tube

10 ml lavender top vacutainer tube

6 x 7 zip-top biohazard specimen transport bag

Bar coded labels (4 /kit)

UNC Health registry orange phlebotomist instruction sheet (1/kit)

Rack

Timer

Wheeled thermal storage and transport bag

**Appendix II: Mouth rinse collection kit (staff-administered)**

Nitrile gloves  
Sterile blue absorbent pad, small  
Rack  
1 conical vial  
1 screw top collection cup  
Label  
Scope mouth rinse, 1.5 oz. (44ml) travel size  
Collection instructions  
Timer  
6 x 7 zip-top biohazard specimen transport bag  
Wheeled thermal storage and transport bag

### **Appendix III: Tissue collection supplies**

Nitrile Gloves  
Sterile Scalpel blades and blade holder  
Sterile forceps  
2 ml Cryovial for storing snap frozen tissue  
Cassettes to store FFPE tissue  
Optional cassette engraver for labeling cassettes  
Containers for 10% formalin  
Sterile containers for transportation  
Table top LN2 container to store LN2  
Liquid nitrogen for freezing the snap frozen tissue  
10% formalin  
Statmark histology marker pen to label the cassettes  
Zebra labels for labeling cryovials and  
Sterile Saline  
Sterile biopsy gun and coring needles  
Wheeled thermal storage and transport bag