National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Other Cardiovascular Conditions (GENTAC)

Specimen Collection Manual

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Project No. 0212746

March 2013

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1.0 Introduction to the Study

1.1 Study Summary

The purpose of the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Other Cardiovascular Conditions (GENTAC) is to enroll clinical patients who are receiving treatment for aortic aneurysms, aortic dilatation, aortic insufficiency, heart failure, or aortic valve repair and to collect clinical, laboratory, family pedigree data and biological specimens (e.g. blood, saliva, tissue). The collected information will facilitate research to determine the best medical practices that will advance the clinical management of genetic thoracic aortic aneurysms, and other cardiovascular complications

1.2 Data Coordinating Center

This study is being coordinated by RTI International (RTI), which maintains full responsibility for all data collection tasks performed for the GenTAC Study. The GenTAC Data Coordinating Center (DCC) will be managed out of RTI's Rockville, MD, office under the direction of Dr. Barbara Kroner (refer to Section 1.4 for RTI contact information).

1.3 GenTAC Coordinating Center

The Registry will include the following eight GenTAC Coordinating Centers (GCCs): Cornell University (NYC, NY), University of Pennsylvania (Philadelphia, PA), Johns Hopkins University (Baltimore, MD), NIA at Harbor Hospital (Baltimore, MD), University of Texas at Houston (Houston, TX), Baylor College of Medicine, Oregon Health Sciences University (Portland, OR) and The Queen's Medical Center (Honolulu HI). The biospecimen operations of each clinical center will be overseen by designated local staff (see section 1.5 for RCC contact information and addresses).

1.4 Data Coordinating Center Contact Information

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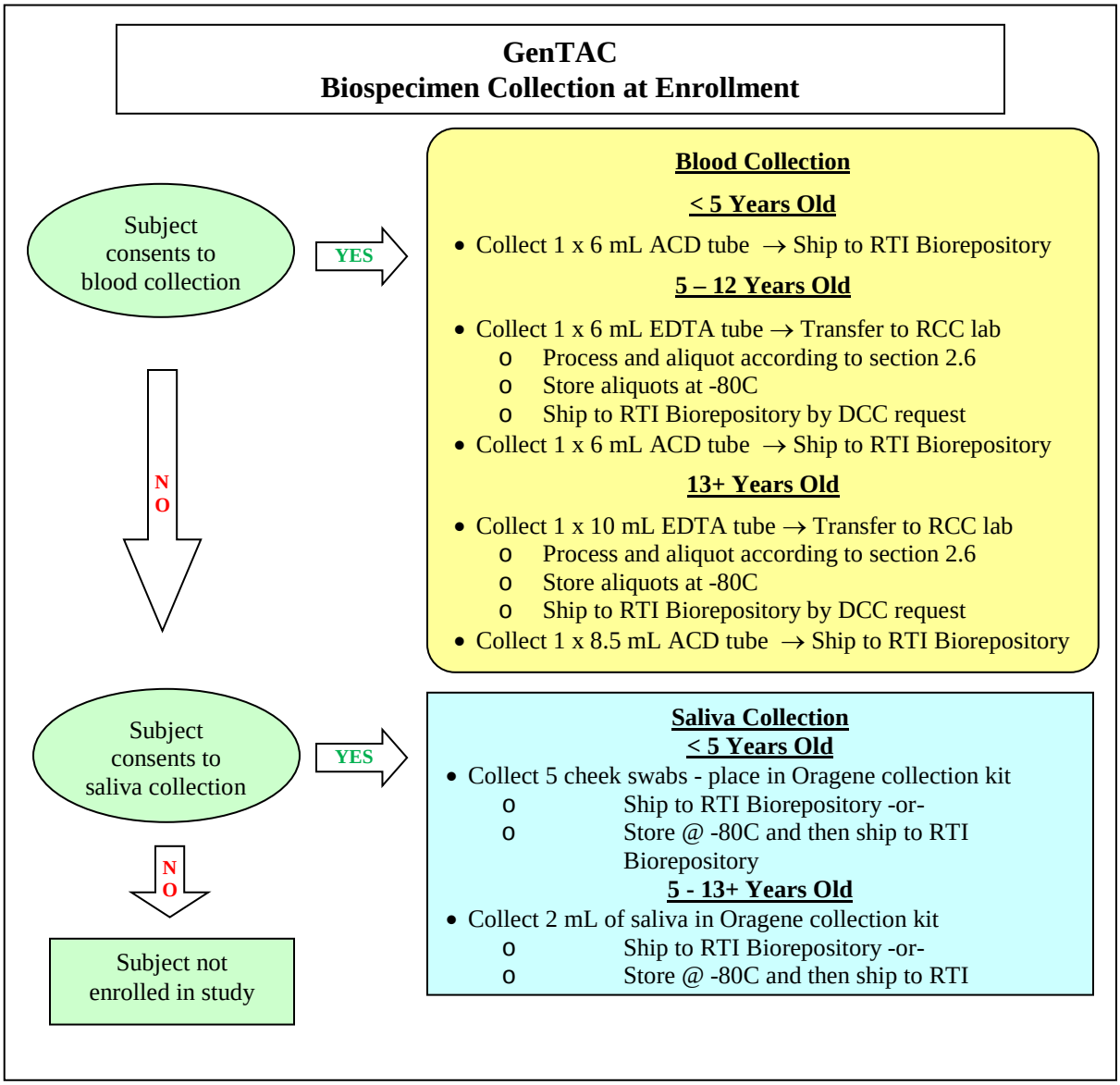
2.0 Specimen Collection

2.1 Collection Summary

A designated coordinator at each GCC will be responsible for directly handling or overseeing the collection, processing, storage and shipment of biospecimens. It is anticipated that one to two tubes of blood will be collected from each subject (see figure 2.1) with at least one tube being shipped directly to the RTI Biorepository for processing. Any additional tube(s) will be processed and stored by the research coordinator or local laboratory staff at the GCC. In some instances a subject may decline to consent to blood collection, on these occasions a saliva sample should be requested to obtain specimen material (white blood cells/buccal epithelial cells) for DNA analysis. In addition to blood and saliva the Registry will also attempt to collect tissue samples from enrolled subjects who undergo clinical surgical procedures.

Figure 2.1



2.2 Informed Consent

Prior to specimen collection, the GCC coordinator must verify that the subject has read, understood and signed the GenTAC study consent form. The consent document contains specific information that fully explains the purpose of this study, the type of specimens to be collected, the method of collection, use and analysis of the collected specimens.

2.3 Specimen Collection Supplies

The following specimen collection kits and associated supplies will be provided and shipped by the DCC to each GCC location. To replenish any of the kits or supplies please contact the RTI Biospecimen Manager at least two weeks in advance so the replacement items can forwarded in a timely manner. During the specimen collection phase of the study it may also be necessary for the DCC to recall unused kits due to a procedure modification or expiration of a kit component (i.e. blood collection tubes). The DCC will contact the GCC sites to make the necessary arrangements to have the unused kit(s) returned to the DCC to undergo the replacement or refurbishment of the kits and then have them returned to the sites

2.3.1 Blood Collection Kits

< 5 years old

- BD Yellow Top tube (364816) BD - w/ACD Solution B (1.0 mL) 6.0 mL collection capacity
- Specimen ID labels (one set of ten labels)
- Exempt Human Specimen label
- Absorbent tube pouch
- Self-sealing specimen transport bag

5 – 12 years old

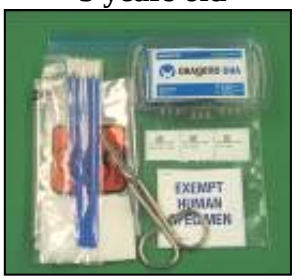
- BD Lavender Top tube (BD 367863) - w/spray dried K_2EDTA (10.8mg) 6.0 mL collection capacity
- BD Yellow Top tube (364816) BD - w/ACD Solution B (1.0 ml) 6.0 mLs collection capacity
- Specimen ID labels for ACD tube -10 labels per set)
- Specimen ID labels for EDTA tube -20 labels per set)
- Exempt Human Specimen label
- Absorbent tube pouch
- Self-sealing specimen transport bag
- 18 - 2.0 mL Sarstedt self-standing v-bottom cryovials

13+ years old

- BD Lavender Top tube (BD 366643) - w/spray dried K_2EDTA (10.8mg) 10.0 mLs collection capacity
- BD Yellow Top tube (BD 364606) - w/ACD Solution A (1.5 mLs) 8.5 mL collection capacity
- Specimen ID labels for ACD tube -10 labels per set)
- Specimen ID labels for EDTA tube -20 labels per set)
- Exempt Human Specimen label
- Absorbent tube pouch
- Self-sealing specimen transport bag
- 18 - 2.0 mL Sarstedt self-standing v-bottom cryovials

2.3.2 Saliva Collection Kits

<5 years old



- Oragene saliva collection kit (OG-250)
- Oragene swab kit (5 sponge swabs)
- Scissors
- Multi-language instruction sheet
- Specimen ID labels (one set of three labels)
- Exempt Human Specimen label
- Absorbent strip
- Self-sealing specimen transport bag

5 – 13+ years old

- Oragene saliva collection kit (OG-250)
- Multi-language instruction sheet
- Specimen ID labels (one set of three labels)
- Exempt Human Specimen label
- Absorbent strip
- Self-sealing specimen transport bag

2.3.3 Fresh Tissue Collection Kit

- 2.0 mLs Sarstedt cryovials pre-filled w/RNA Later (6 cryovials)
- 2.0 mLs Round bottom cryovials for snap frozen tissue (6 cryovials)
- Cryosettes for paraffin block (2 cryosettes)
- Specimen ID labels (two sets of ten labels)
- Exempt Human Specimen label

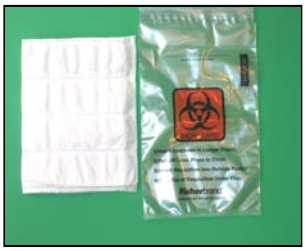
2.3.4 Paraffin Block Collection Kit

- Cryosettes for paraffin block (2 cryosettes)
- Specimen ID labels (two sets of ten labels)
- Exempt Human Specimen label

2.4 Specimen Shipping Supplies

The following specimen shipping supplies and associated materials will be provided by the DCC to each GCC location. Individuals packing and offering any GenTAC specimen shipments (ambient or frozen) to Federal Express or another air carrier must meet the training requirements as outlined in Section 1.5 of the IATA Dangerous Goods Regulations (IATA DGR) manual. The supplies listed below in Sections 2.4.1 and 2.4.2 are only to be used for "Exempt Human Specimens" according to section 3.6.2.2.3.6 of the IATA DGR. To ship specimens classified as Biological Substances, Category B (UN3373) see Section 2.4.3 of this manual. To replenish any of the shipping supplies please notify the RTI Biospecimen Manager at least two weeks in advance so that the replacement items can arrive in a timely manner.

2.4.1 Exempt Human Specimen - Ambient Temperature Shipments



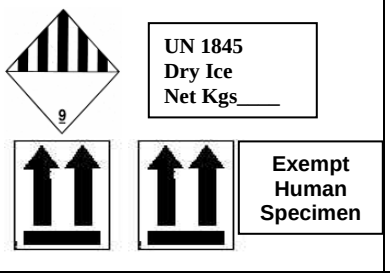
- Aqui-Pak absorbent pouch (hold, protect, and absorb tube contents)
- Self-sealing leakproof specimen bag w/document pouch

- White flat fold corrugated box, (8x8x2.75) w/side lock tabs
- Exempt Human Specimen label
- Pre-printed To/From address label
- Self-sealing FedEx Clinical Pack (large)
- FedEx Billable Stamp

- FedEx Billable Stamp – **Deliver to RTI**
- Priority overnight delivery
- RTI Street Address –**3040 Cornwallis Rd., Herbert Bld. So. Lab Door Room 106**
- Use for fresh specimen shipments only (Mon-Fri)

2.4.2 Exempt Human Specimen - Dry Ice Shipments

- Cardboard cryovial storage box
  (9 x9 space divider – holds a total of 81- 2ml cryovials)
- Dimensions 2" x 5.25"x 5.25"

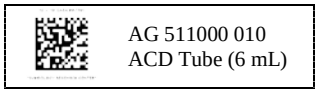
	• Leak proof self-sealing secondary container • Holds one 2” cryovial storage box or multiple saliva containers • Absorbent strip to absorb entire contents of all cryovials or saliva containers
	• Molded one piece polystyrene insulated shipper w/1.5 inch thick walls and tight fitting lid • Holds 4 cryovial storage boxes, 2 bags of saliva containers plus up to 15 lbs of dry ice
	• 200 lb. test corrugated carton
	• Hard copy Airbill – **Deliver to RTI** • Priority overnight delivery only • RTI address – **3040 Cornwallis Rd., Herbert Bld. So. Lab Door Room 106** • Ship only on a Monday or Tuesday
	• Miscellaneous Hazard Label Class 9 (Diamond shape) • Dry Ice text label w/net weight in kilograms • Package Orientation Arrows x 2 • Exempt Human Specimen

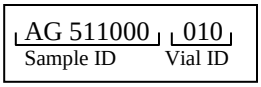
2.4.3 Biological Substances, Category B Shipments (UN3373)

All specimen shipments (e.g. ambient or frozen) that are expected to be classified as Biological Substances, Category B (UN3373) must be identified and packed according to IATA requirement 3.6.2.2.2.2 and IATA Packing Instruction (PI) 650. Shipments of this type must use secondary packagings and outer containers that are pre-tested as a complete set by the manufacturer to meet IATA packing requirements. If you are planning to make a Category B shipment, please contact the RTI Specimen Manager at least 7 business days in advance so that the required packaging supplies and instructions can be sent to your site.

2.5 Biospecimen ID Labels

Each collection kit (blood, saliva or tissue) will include a complete set of self-adhesive biospecimen ID labels. The labels are chemical and solvent resistant and have the ability to survive exposure to common laboratory environments such as liquid nitrogen (-196°C), autoclaving and 100°F water baths. The label print format will include; a 2D matrix barcode that encodes the specimen ID; and readable text that describes the specimen ID and sample material type (e.g. plasma, serum etc.). The sample ID is designed and printed according to prescribed GenTAC specifications (i.e. double alpha/six digit identifier followed by a three digit vial identifier.





2.5.1 Blood Collection Labels

The following ID labels will be included in each blood collection kit for each age group (see Section 2.3.1).

< 5 Years Old

- Blood collection labels for subjects less than 5 years of age will consist of a single panel of 10 labels (see below).
- Prior to collection, affix the designated sample ID label (AG xxxxxx 010) to the ACD blood collection tube.
- Affix the three labels (AG xxxxxx 007, 008 and 009) in the designated space of the biospecimen collection Form 10 (original and copies).
- Following collection, pack the unused labels with the collected ACD blood tube for shipment back to the RTI Biorepository.

<5

5 – 12 /13+ Years Old

- Blood collection labels for subjects between the ages of 5 – 12 and 13+ years old will consist of a double panel of 30 labels (see below).
- Prior to blood collection, split the double panel along the middle perforation.

- Affix the designated ACD tube label (AG xxxxxx 010) to the ACD blood collection tube and the designated EDTA tube label (AG xxxxxx 020) to the EDTA blood collection tube.
- Affix the three labels (AG xxxxxx 007, 008 and 009) in the designated space of the biospecimen collection Form 10 (original and copies).
- Append the remaining EDTA labels with the collected EDTA blood tube that will be transferred for processing to the GCC lab.
- Pack the remaining ACD labels with the collected ACD blood tube for shipment to the RTI Biorepository.

Note: In some cases the collected EDTA tube will also be shipped with the collected ACD tube to the RTI repository for processing. In those circumstances pack both sets of labels (EDTA and ACD) with the collected blood tubes for shipment to the RTI Biorepository.

5 - 12

AG 511000 001 Cryo – Lymphs ACD	AG 511000 011 Plasma EDTA 0.25 mL	AG 511000 021 Plasma EDTA 0.25 mL
AG 511000 002 Cryo – Lymphs ACD	AG 511000 012 Plasma EDTA 0.25 mL	AG 511000 022 Plasma EDTA 0.25 mL
AG 511000 003 Cryo – Lymphs ACD	AG 511000 013 Plasma EDTA 0.25 mL	AG 511000 023 Plasma EDTA 0.25 mL
AG 511000 004 PMNC – RBC Pellet	AG 511000 014 Plasma EDTA 0.25 mL	AG 511000 024 Plasma EDTA 0.25 mL
AG 511000 005 PMNC – RBC Pellet	AG 511000 015 Plasma EDTA 0.25 mL	AG 511000 025 Plasma EDTA 0.25 mL
AG 511000 006 PMNC – RBC Pellet	AG 511000 016 Plasma EDTA 0.25 mL	AG 511000 026 Plasma EDTA 0.25 mL
AG 511000 007 Form Label	AG 511000 017 Plasma EDTA 0.25 mL	AG 511000 027 Plasma EDTA 0.25 mL
AG 511000 008 Form Label	AG 511000 018 Plasma EDTA 0.25 mL	AG 511000 028 Buffy Ct EDTA 1.5 mL
AG 511000 009 Form Label	AG 511000 019 Plasma EDTA 0.25 mL	AG 511000 029 Form Label
AG 511000 010 ACD Tube (6 mL)	AG 511000 020 Plasma EDTA 0.25 mL	AG 511000 030 EDTA Tube (6 mL)

13+

AG 511000 001 Cryo – Lymphs ACD	AG 511000 011 Plasma EDTA 0.25 mL	AG 511000 021 Plasma EDTA 0.25 mL
AG 511000 002 Cryo – Lymphs ACD	AG 511000 012 Plasma EDTA 0.25 mL	AG 511000 022 Plasma EDTA 0.25 mL
AG 511000 003 Cryo – Lymphs ACD	AG 511000 013 Plasma EDTA 0.25 mL	AG 511000 023 Plasma EDTA 0.25 mL
AG 511000 004 PMNC – RBC Pellet	AG 511000 014 Plasma EDTA 0.25 mL	AG 511000 024 Plasma EDTA 0.25 mL
AG 511000 005 PMNC – RBC Pellet	AG 511000 015 Plasma EDTA 0.25 mL	AG 511000 025 Plasma EDTA 0.25 mL
AG 511000 006 PMNC – RBC Pellet	AG 511000 016 Plasma EDTA 0.25 mL	AG 511000 026 Plasma EDTA 0.25 mL
AG 511000 007 Form Label	AG 511000 017 Plasma EDTA 0.25 mL	AG 511000 027 Plasma EDTA 0.25 mL
AG 511000 008 Form Label	AG 511000 018 Plasma EDTA 0.25 mL	AG 511000 028 Buffy Ct EDTA 1.5 mL
AG 511000 009 Form Label	AG 511000 019 Plasma EDTA 0.25 mL	AG 511000 029 Form Label
AG 511000 010 ACD Tube (8.5 mL)	AG 511000 020 Plasma EDTA 0.25 mL	AG 511000 030 EDTA Tube (10 mL)

2.5.2 Saliva Collection Labels

- Labels for the saliva collection kit (Section 2.3.2) are a single 3 label panel (see below).
- Affix the label marked (AG xxxxxx 001) to the underside of the Oragene OG-250.
- Affix the two remaining labels (AG xxxxxx 002 and 003) in the designated space of the saliva collection form (original and copies).

2.5.3 Fresh Tissue Collection Labels

- Labels to identify freshly collected surgical tissue (see Section 2.3.3) from a single subject will consist of a double panel of 20 labels (see below).

- Affix the Snap Frozen (SF) labels (AG xxxxxx 001 to 006) to 6 empty 2 ml cryovials, and the RNA *later* (RNA) labels (AG xxxxxx 011 to 016) to the 6 pre-filled RNA later cryovials.
- Affix the three labels (AG xxxxxx 018/019/020) to each page of the tissue collection form (Form 9).
- Retain the remaining paraffin block (PB) labels and affix to the flat front edge of each cassette when the processed paraffin block cassettes are retuned from histology.

2.5.4 Archived Paraffin Block Tissue Labels

- Labels to identify archived paraffin block (PB) tissue (see Section 2.3.4) will consist of a single panel of 10 labels (see below).
- Affix the three labels (AG xxxxxx 001, 002, and 003) to each page of the SSIMS tissue collection form (Form 9) and affix (004) to the Tissue Form (Form 8).
- Labels to identify paraffin tissue blocks (AG xxxxxx 008, to 010) should be affixed to the flat front raised edge of each cryosette or tissue cassette.

2.6 Blood Collection

The most important GenTAC task as well as the most challenging is to standardize the collection and processing of blood samples across all GCC sites. A blood sample that is not collected, labeled, and processed correctly will most likely have a negative impact on the current study and on future downstream applications and assay results. Therefore, it is important that blood samples be collected and processed according to established procedures to ensure that cellular and molecular change reflects biological and not collection or process variability.

2.6.1 Pre-Collection Procedure

- Handle, collect, and process all blood specimens following your institutional policies and guidelines to prevent exposure to blood-borne pathogens or other potentially infectious materials.
- Confirm that the subject has signed the consent form.
- Verify blood collection tube(s) are at room temperature and labeled with the assigned GenTAC specimen ID.
- Confirm specimen label IDs affixed to blood collection tubes are the same as the label IDs on the collection form.
- Organize and prepare the blood collection work area and arrange the collection supplies.
- Place the labeled blood collection tubes in tube rack in the correct order of draw (see 2.6.2).

2.6.2 Order of Draw

The "order of the draw" refers to the sequence in which evacuated blood collection tubes should be filled to prevent the carryover of additives from one tube to the next. The GenTAC primary order of draw for blood collection kits with two tubes is; EDTA first and ACD second.

2.6.3 Blood Collection Procedure

- Perform the phlebotomy procedure following your institute's approved guidelines.
- When the first tube has filled to its capacity volume or blood flow ceases, remove it from the vacutainer tube holder and gently invert 6 to 8 times to mix blood and anticoagulant.
- **DO NOT SHAKE** the collected tube since vigorous mixing can cause hemolysis. (**Note** – If you are only collecting one tube then skip to section 2.6.4).
- Place the next tube in holder, following the prescribed order of draw (refer to 2.6.2) and repeat the collection step.

2.6.4 Post Collection Procedure

- Gently invert both collected tubes 8 to 10 times to ensure the anticoagulant additive and blood are thoroughly mixed.
- Place collected tubes in a tube rack in an upright position and maintain at room temperature until you are ready to process or pack the tube(s) for shipment.
- Discard the blood collection needle set according to your institute's waste policies.
- Before leaving the phlebotomy area verify that the specimen ID labels on the collected tube(s) are the same as the ID labels affixed to the blood collection forms.
- Complete the GenTAC blood specimen collection form by filling-in the required information (i.e. data and time of collection, type of collection tube(s) etc).
- Note and correct any discrepancies at this time. Maintain all collected blood tubes at room temperature throughout the collection process.

- Place the EDTA collected blood tube, a copy of the blood collection form and all remaining EDTA specimen ID labels in a specimen transfer bag.
- Transfer the collected EDTA tube(s) to the GCC processing laboratory as quickly as possible for processing.
- Prepare the ACD tube for shipment to the RTI Biorepository by inserting the collected ACD blood tube in the absorbent tube pouch. Place the tubes, a copy of the blood collection form and all remaining ACD labels into the provided specimen transport bag.
- Refer to Chapter 6 of the study manual for entering specimen information into SSIMS.
- Refer to section 4.0 to pack the ACD specimen(s) for shipment.

Note: In some cases the collected EDTA tube(s) will also accompany the collected ACD tube(s) to the RTI repository for processing. In those circumstances pack the EDTA tube and ACD tube in the same absorbent pouch. Place the tube pouch, a copy of the blood collection form, cyovials and both sets of specimen ID labels (EDTA/ACD) in a specimen transport bag for shipment to the RTI Biorepository.

2.7 Saliva Collection

In some cases the subject may refuse or be unable to participate in the blood collection procedure. For these occasions the GenTAC study will offer the subject a non-invasive collection procedure to obtain saliva. This method has become an extremely attractive and reliable method for the collection cellular material found in the mouth which can yield sufficient quantities of good quality DNA. For this type of collection the study will use the Oragene•DNA OG-250 Self-Collection Kit by DNA Genotek. The kit is an all-in-one system for the collection, preservation, transportation and purification of DNA from saliva. The kit can be used for all age groups that are being considered for study enrollment.

2.7.1 Pre- Collection Procedure

- Confirm that the subject has signed the consent form.
- Verify that the saliva collection device is at room temperature and labeled with a GenTAC specimen ID.
- Confirm that the specimen ID on the collection kit are the same as the label ID on the specimen collection form (see section 2.2.2 to view saliva collection kits).
- It is recommended by the kit manufacturer that individuals using the Oragene device **should not** eat, drink, smoke or chew gum for 30 minutes before collecting the saliva sample.
- Caution should always be used when inserting anything into a child's mouth. Do not leave the child unattended when using the sponges.
- Try not to rub the sponge directly on the child's teeth to minimize the amount of bacteria transferred to the sponge.

Saliva Collection Supplies

< 5 Years Old

- Oragene OG-250 saliva collection disc device
- Children's collection - 5 sponge swabs and one pair of scissors
- Saliva specimen ID labels

5 – 13+ Years Old

- Oragene OG-250 saliva collection disc device
- Saliva specimen ID labels

2.7.2 Saliva Collection Procedure

< 5 Years Old

- Begin procedure by removing the collection device and instructions from the packing material. Do Not puncture or remove the clear plastic film from the underside of the blue cap (this contains preservative fluid).
- Affix the assigned specimen ID label to the underside of the OG-250 saliva collection disc.
- Open a "saliva collection kit for young children" which contains 5 sponge swabs and a pair of scissors.
- Remove one sponge swab by the handle from the ziplock bag – take care not to touch the sponge tip to any surface.
- Hold the swab tightly and carefully place the sponge into the child's mouth in the *cheek pouch* (the space between the gums and the inner cheek).
- Gently move the saliva sponge around the upper and lower cheek pouches on both sides of the mouth to soak up as much saliva as possible.
- There is no need to 'scrape' the inner cheek with saliva sponges – simply collect as much saliva as possible from the cheek pouches.
- The sponge will absorb more saliva if it is left in the child's mouth for a longer time (up to 60 seconds).
- Before removing the swab - run the swab tip along the lower gum line between the gum and inner cheek to collect any saliva.
- Remove the swab and hold the tip over the open base of the collection device (see Figure 1).
- Using the scissors cut the swab tip from the thinnest part of the handle just below the tip (see Figure 2).

Figure 1

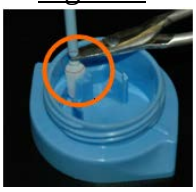


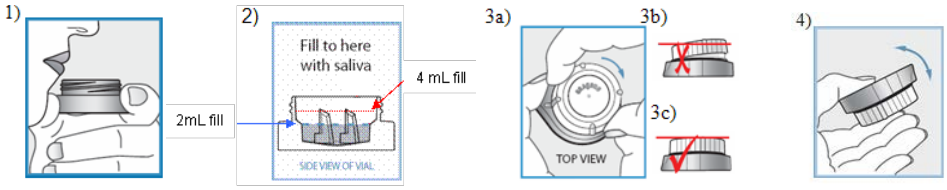
Figure 2

- For the collection of up to 5 saliva sponges from the same child repeat this procedure with the remaining four swabs.
- A rest period of about 5 min between each collection of every 2 sponges is helpful. Follow the sequence shown in the diagram below.
- To prevent the saliva samples from drying out, cap the vial (see step 4) within 15 min of the first collection. If you have not had a chance to collect all 5 sponges within 15 minutes, you may carefully re-open the kit.
- If you remove the cap make sure that the interior face of the cap is facing upwards when putting it on any surface. Do not spill the contents.

- Once all 5 sponge swabs are collected – cap the collection device and gently invert the capped device and mix the fluid with the swab tips for 10 seconds.
- If you have trouble closing the cap all the way, turn the cap slightly counter-clockwise then try to close it again.

5-13+ Years Old

- Start the collection procedure by removing the collection device and instructions from the packing material. Do Not remove the clear plastic film from the underside of the blue cap (this contains preservative fluid).
- Affix the assigned specimen ID label to the underside of the OG-250 saliva collection disc device.
- Request the subject to spit saliva until the volume of saliva fluid (not bubbles) is approximately 2 mLs as shown below in figures 1 and 2.
- To increase saliva flow, have the subject close their mouth, wiggle their tongue and rub their cheeks. Some people also find it easier to produce more saliva if they place a pinch of plain white sugar on their tongue.
- The collection should be completed within 30 minutes.
- After collection, screw the blue cap tightly onto the container base (this will cause the plastic film to be pierced and release the preservative fluid as shown in figures 3a, 3b). If you have trouble closing the cap all the way, turn the cap slightly counter-clockwise then try to close it again.
- Gently invert the capped collection device for 10 seconds as shown in figure 4 to mix the preservative fluid and saliva.



2.7.3 Post Collection Procedure

- Complete the GenTAC specimen collection form (Form 10) by filling-in the required information (i.e. data and time of collection, type of collection (s) etc).
- Confirm and validate the labels affixed to the collected saliva specimens against the specimen labels attached to the blood collection forms.
- Note and correct any discrepancies at this time.
- Temporarily hold all collected saliva devices at room temperature.
  Note: If you are shipping blood samples on that day to the RTI repository you can include the saliva sample in that shipment.
- Refer to Chapter 6 for entering specimen information into SSIMS.
- Pack the specimen(s) for shipment according to the procedures described in Section 4.0 of this manual.
- If there are no fresh blood shipments scheduled then store the collected saliva samples in the sealed specimen pouch at -80^{0}C.
- You can include the frozen saliva samples in dry ice shipments of frozen plasma and buffy coat samples to the RTI Biorepository.

2.8 Fresh Surgical Tissue Collection

In some cases fresh tissue samples will be obtained from study subjects who require surgical

repair of their aortic aneurysm, dissection, and/or arterial occlusive disease. Once a study subject is identified as a surgical candidate and the appropriate consents are obtained, tissue may be collected. Protocol-based tissue collection techniques will be used to procure aorta tissue as soon as possible after removal from the subject (<30 min from time of removal at surgery to tissue preservation or freezing) while adequately preserving the specimen for molecular and pathological characterization. It is anticipated that the tissue will be preserved using the following methods;

- Snap Frozen/Flash Freezing – fresh tissue is placed in a labeled cryovial which is then partially submerged in liquid nitrogen.
- RNA *Later* – fresh tissue is submerged in RNA Later preservation solution, stored at $+4^0$C for a minimum of 12 hours, removed from the preservative and then transferred to -80^0C storage.
- Paraffin Block – fresh tissue is treated and fixed following an in-house procedure and embedded in IHC-grade paraffin.

2.8.1 Pre Collection Procedure

- Confirm that the subject has signed the consent form.
- Prepare the work space for receiving specimens from the OR. Ensure that you have the appropriate supplies to section and preserve the excised tissue (liquid nitrogen, dry ice, storage vials, specimen ID labels, cutting instruments etc.)
- RTI will provide each RCC site with fresh tissue collection kits (see section 2.2.3), each kit (one per subject) will include the following items;
    - Six (6) Sarstedt 2 mL cryovials that are pre-filled with 1.5 mLs of RNA*later* preservation fluid.
    - Six (6) NUNC 2 mL cryovials for snap frozen tissue specimens
    - Two (2) cryosettes to store and transport paraffin embedded tissue specimens, if needed. Sites can also choose to use the embedding cassette.
    - Twenty (20) pre-printed cryolabels that display sample ID, readable text and 2D matrix barcodes (see section 2.4.3).
- Affix and confirm that the sample ID labels on the specimen collection form (use form label 018, 019 or 020 in the space designated for the specimen ID) and on the vials are the same specimen ID.

2.8.2 Fresh Tissue Collection Procedure

A. RNA*later* Protocol

- Label the pre-filled RNA*later* Sarstedt cryogenic vials (2.0 mL) with a pre-assigned sample ID number.
- Aortic samples excised during the operation are made available for preservation.
- Make every effort to expedite the transfer of harvested tissue to the sterile pre-filled containers of RNA*later* following harvest. Transient exposure of the specimens to air can result in RNA degradation.
- Rinse tissue specimens with saline. Using sterile instruments, remove blood clots and adipose tissue from the aortic sample.
- Section the tissue into pieces no larger in height and width than 0.5 x 0.5 cm. However, tissue pieces can exceed 0.5 cm in length.
- Using sterile forceps, place a sectioned piece of tissue into the labeled cryovial containing RNAlater, and ensure that the specimen is completely submerged. Repeat this process for all tissue pieces designated for preservation in RNA*later*.
- Place the vials of RNA*later* and tissue in a cryovial rack in an upright position into

order to keep specimens submerged. Tissue will float at first – leave at room temperature for 30 – 60 minutes and the tissue will eventually submerge.
- Do not freeze samples in RNA*later* immediately; store at +4°C overnight (minimum of 12 hours of refrigeration) this will allow RNA*later* to thoroughly penetrate the tissue.
- After storing at +4°C overnight, sterilely remove the RNA*later* supernatant with a pipette and discard according to in-house procedures.
- Transfer and store the RNA*later* treated tissue at -80°C.
- Complete the tissue collection form for all items related to the processing of RNA*later* treated tissue.

B. Snap Frozen Tissue Protocol

- Label the NUNC cryogenic vials (2.0 mL) with a pre-assigned sample ID number for the snap frozen (SF) specimens.
- Aortic samples are excised during the operation. Make every effort to expedite the transfer of harvested tissue.
- Rinse tissue specimens with saline. Using sterile instruments, remove blood clots and adipose tissue from the aortic sample.
- The tissue sample is sectioned into pieces (approximately 1.0 x 0.5 cm).
- The specimens are placed into labeled cryogenic vials.
- The samples are snap frozen in liquid nitrogen for approximately 1 minute by submerging the cryogenic vial with tissue in liquid nitrogen.
  (**Note:** Do not allow liquid nitrogen to come in direct contact where the vial O-ring and screw cap meet. There is a potential for the O-ring seal to leak and allow liquid nitrogen to become entrapped inside the vial. When the vial is exposed to warmer temperatures this may the liquid to change to gas causing pressure buildup and resulting in a possible explosion or biohazard release).
- Carefully transfer the frozen tissue vial(s) to an insulated container filled with dry ice (pellets or rice). Immerse the snap frozen tissue vial(s) in the dry ice to prevent exposure to ambient air temperatures and possible thawing.
- Transfer the frozen samples in dry ice to a -80°C freezer for storage.
- Complete the tissue collection form for all items related to the processing of snap frozen tissue.

C. Paraffin Embedded Tissue Protocol

- Specimens intended for paraffin embedding should be processed after the completion of other fresh tissue procedures, such as snap freezing, and submersion in RNA stabilizing reagent.
- Following in-house procedures identify and transfer the tissue to a histology lab for formalin preservation and paraffin embedding.
- Once the tissue has been embedded - retrieve the paraffin embedded tissue block(s).
- Affix a label on the flat face front of the tissue cassette using a sample ID label that ends with 008, use 009 and 010 for additional blocks.
- Place the paraffin block(s) into a ziplock bag and store at room temperature in a secure area that is air conditioned (to prevent the paraffin from becoming soft).
- Complete the tissue collection form for all items related to the processing of paraffin embedded tissue.

2.8.3 Post Tissue Collection Procedure

- Verify that the sample ID labels affixed on the tissue form are correct.
- Complete the specimen form and refer to Chapter 6.0 of the GenTAC Operations Manual on how to enter specimen information into SSIMS.

2.9 Archived Tissue Collection

In some cases there may be archived paraffin tissue samples that are available for collection. Once archived tissue for a study subject has been identified ensure that the appropriate consents are obtained before the tissue is retrieved for collection.

2.9.1 Pre Collection Procedure

- Confirm that the subject has signed the consent form.
- Make the necessary inquiries to obtain the archived tissue
- RTI will provide each GCC site with archived tissue collection kits (see section 2.2.4), each kit (one per subject) will include the following items;
    - Two (2) cryosettes to store and transport paraffin embedded tissue specimens
    - Twenty (20) pre-printed cryolabels that display sample ID, specimen type and 2D matrix barcodes (see section 2.4.4).
- Affix the designated sample ID labels on the specimen collection form (use form label 018, 019 or 020 in the space designated for the specimen ID).

2.9.2 Archived Paraffin Tissue Protocol

- Once a tissue specimen has been identified – request that a whole block or section of a block be obtained from the custodial authority.
- Place the whole or partial paraffin embedded tissue block(s) into a cryosette container and label the flat front side of the container with a sample ID label that ends with 008, 009 or 010.
- Place the cryosette w/paraffin block(s) into a ziplock bag and store at room temperature in a secure area that is air conditioned (to prevent the paraffin from melting).
- Complete the tissue collection form for all items related to the processing of paraffin embedded tissue.

2.9.3 Post Tissue Collection Procedure

- Verify that the subject and sample ID label affixed on the form are correct.
- Check the box that identifies if the aorta tissue is normal or abnormal.
- Place a check in the box next to each used sequence number label for the corresponding preservation method.
- Enter the date and time of processing and the storage box number into which the sample was placed for storage/shipment to the RTI repository.
- In the far right column please indicate the name of the person processing the samples and note any relevant comments.
- Following tissue processing and storage, complete the tissue collection form (Form 9) and enter all subject, specimen and storage information into SSIMS.

3.0 Process and Store Blood Specimens

3.1 Introduction

Handle and process all EDTA whole blood specimens according to your institutional specimen safety guidelines and policies as well as the GenTAC collection, processing, storage and shipping procedures. All EDTA whole blood specimens collected at the GCC should be processed, aliquotted and stored within two hours of collection. The aliquot vials of plasma and buffy coat material should be stored at -80C and then shipped at a later on dry ice to the RTI biorepository. All ACD tubes (see section 2.6) will held at room temp, packed according the IATA DGR requirements (refer to section 2.4 and 2.4.3) and then shipped by overnight courier service (i.e. Federal Express) to the RTI Biorepository for processing and storage.

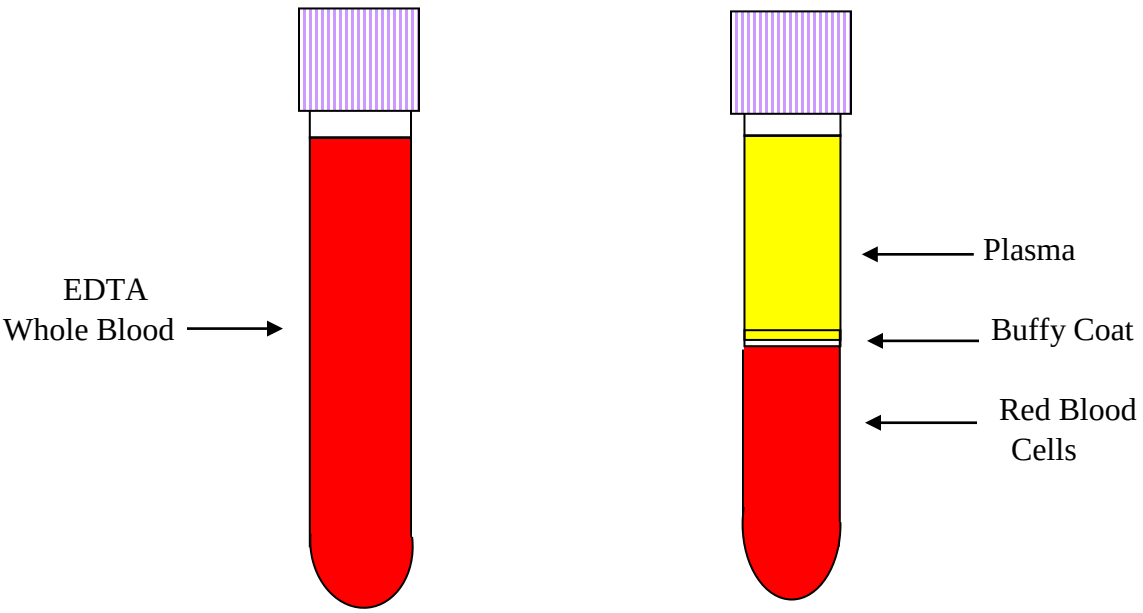
3.1.1 Process EDTA Blood Tube for Plasma and Buffy Coat

- All collected whole blood EDTA should be processed for plasma and buffy coat, fractions within 2 hours of collection, unless otherwise directed.
- Process specimens in a biosafety hood, if possible or on a lab bench in enclosed room.
- Centrifuge each collected EDTA tube of whole blood at room temperature (18-25^{0}C) for a minimum of 15 minutes at 1300g / Relative Centrifugal Force (RCF).
- The whole blood (Figure 1) will separate into three distinct layers (see Figure 2); 1) an upper layer (plasma), 2) a lower layer (red blood cells [RBC]), and 3) a thin interface layer between the plasma and red cells that contains the buffy coat (leukocytes and platelets).

  <u>WARNING:</u> Excessive centrifuge speed (over 1300g) may cause tube breakage and exposure to blood and possible injury. Use the following formula to calculate your centrifuge speed for a given *g* force:

x g / RCF = 0.00001118 x radius of rotation (cm) x rpm^2

Figure 1 **Figure 2**



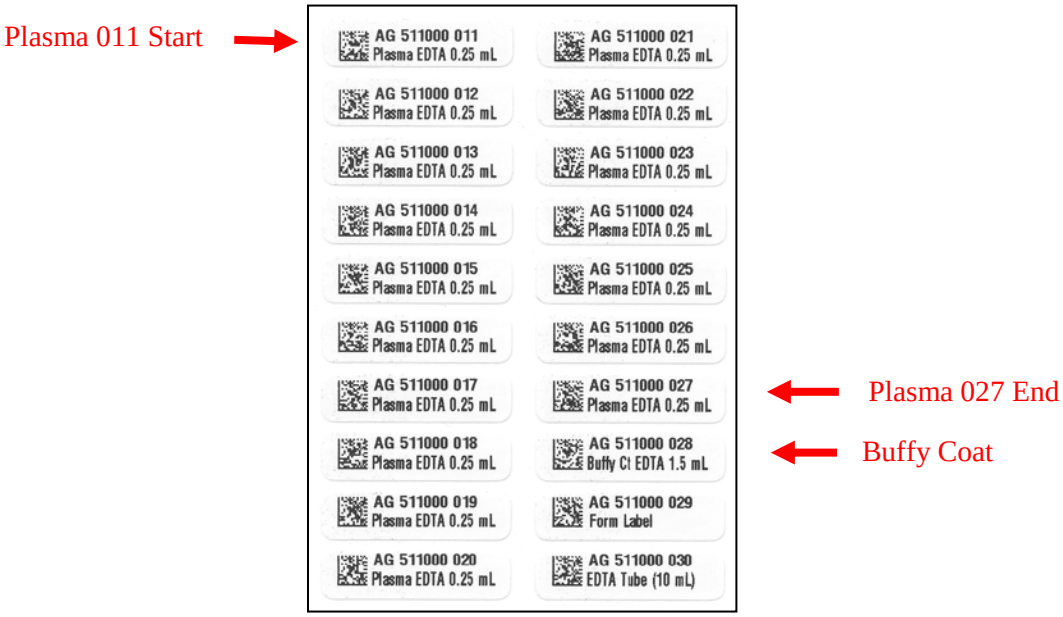
3.1.2 Collect Plasma

- To collect undiluted plasma, carefully remove the stopper from the EDTA hemogard tube by grasping the tube firmly with one hand and with the other hand, twist the stopper closure while simultaneously pushing up with the thumb of the other hand only until the stopper is loosen.
- Move thumb before lifting stopper. DO NOT use thumb to push stopper off tube.
- Carefully remove rubber stopper from tube. DO NOT REASSEMBLE THE STOPPER AND TUBE.
- Use a sterile pipette to slowly aspirate the plasma from the EDTA tube (Figure 2) down to approximately 0.5 centimeters above the buffy coat interface (see Figure 4). Be careful **NOT** to touch, mix or disturb the buffy coat (white pinkish) interface or packed red blood cells.
- Transfer the clean plasma volume to a sterile 5 or 15 mL centrifuge tube labeled with the same sample ID number on the EDTA vacutainer tube.

3.1.3 Aliquot Plasma

- Processed plasma fractions should be aliquotted and frozen within 2 hours of collection.
- Affix the cryovials with the designated plasma labels provided by the DCC that were included in the blood collection kit (see Figure 3).
- Before dispensing any plasma, verify that the sample ID number on the EDTA tube, plasma transfer tube and aliquot vials are the same.
- Create as many 250µl (0.25 mLs) aliquots as possible based on the total EDTA plasma volume (e.g. total plasma volume = 3.5 mLs – create 14 plasma vials).
- Start the label process at AG xxxxxx 011 – complete the process at the vial number based on the total volume (do not go beyond AG xxxxxx 027).

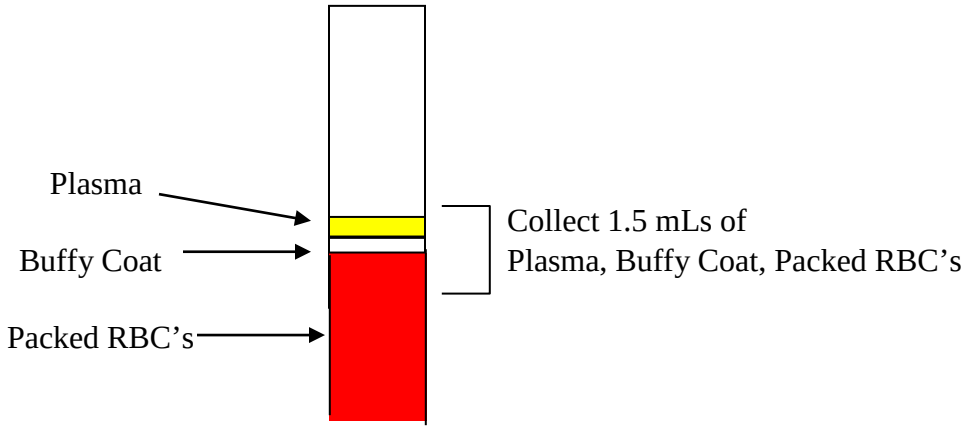
Figure 3 - EDTA and Buffy Coat Aliquot Labels



3.1.4 Collect and Aliquot Buffy Coat

- Buffy coat is sometimes difficult to see and collect. It is can be viscous and appear as a thin milky white or pinkish colored layer just above the packed red cell layer.
- Some contamination of the buffy coat with the RBC layer is to be expected. However, specimens that have an extremely high WBC count will have a thicker and more defined buffy coat layer that will be easier to collect with minimal RBC contamination.
- Affix the designated buffy coat label (AG xxxxxx 028) on a single cryovials (see Figure 3).
- Before collecting or dispensing any buffy coat, verify that the sample ID number on the EDTA tube and cryovial are the same.
- To collect buffy coat, slowly aspirate the remaining plasma and buffy coat, using a slow circular motion.
- Collect a total volume of 1.5 mLs of buffy coat per EDTA tube (see Figure 4) and dispense the buffy coat into the labeled cryovial.

Figure 4



3.1.5 Transfer Aliquots to Freezer Storage

- Immediately after aliquoting has been completed, transfer the buffy coat and plasma aliquots to a -80^{0}C mechanical freezer for short term storage.
- Store the specimen aliquots in specimen boxes provided by RTI to avoid re-transferring frozen plasma and buffy coat specimens for shipment.
- Check the lid of the specimen box to confirm that it is the right storage box according to SSIMS (refer to Chapter 6) of the GenTAC Operations Manual.
- If additional storage is needed, register a new storage box in SSIMS before storing the samples.
- Enter all processed specimens into SSIMS and notate their storage location.

4.0 Pack and Ship Biospecimens

4.1 Introduction

A critical component of the GenTAC Registry is the shipment of fresh and frozen biospecimens from the GCCs to the RTI biorepository facilities located in Research Triangle Park (RTP), North Carolina. To assist you in the successful completion of this important task, carefully review the following guidelines prior to the shipment of any specimens. In particular, please pay close attention to the regulatory requirements and packaging guidelines for shipping exempt human specimens as defined by the 52nd edition of the International Air Transport Association (IATA) Dangerous Goods Regulations.

It is important to remember that all individuals who act as the GCC shipper must be trained or have been retrained within 24 months of their initial or recurrent training session and have taken a test and been issued a certificate to verify that they successfully passed the test (IATA 1.5). The importance of individual responsibilities as a shipper can never be overemphasized because as the shipper, you are ultimately responsible for properly identifying, preparing, classifying, packing, marking, labeling, and documenting each dangerous goods shipment.

4.2 Shipping Regulations

All national air carriers are members of IATA and are required to follow the IATA Dangerous Goods Regulations to ensure the safe shipment of biological specimens and other dangerous goods by air transport. The IATA DGR is in full compliance with the International Civil Aviation Organization Technical Instructions (ICAO/TI). The US Government, through the Department of Transportation (DOT), authorizes the use of the ICAO regulations within the Code of Federal Regulations, Title 49 (49 CFR 171.11).

4.2.1 Exempt Human Specimens

Currently, most biological specimens being collected for GenTAC are classified and shipped as patient specimens (IATA 3.6.2.2.3.6) with the outer packaging marked as “exempt human specimens”. The IATA requirement noted above further mandates that liquid specimens (frozen or fresh) must be packed under the following guidelines; (1) leak-proof primary receptacle(s), (2) leak-proof secondary packaging, and (3) an outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm.

For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material and when multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.

4.2.2 Biological Substances, Category B

All specimen shipments (e.g. ambient or frozen) that are expected to be identified as infectious must be classified and packed as Biological Substances, Category B (UN3373) according to IATA requirement 3.6.2.2.2.2 and IATA Packing Instruction (PI) 650. Shipments of this type must use secondary packagings and outer containers that are pre-tested as a complete set by the manufacturer to meet IATA packing requirements. If you are planning to make a Category B shipment, please contact the RTI Specimen Manager at least several days in advance so that the required packaging supplies and instructions can be sent to your site.

4.3 Shipping Schedules

GenTAC biospecimen shipping schedules will vary due to the type of specimen collected or specimen storage limitations. However, based on study specific requirements the GenTAC Registry requires that all fresh blood specimens be shipped on the day of collection and arrive at the RTI Biorepository the next day. All specimen shipments will use Federal Express priority overnight service to expedite delivery at the final destination. However, GCCs **should not** attempt to ship bio-specimens on dates surrounding national holidays that are established for RTI staff as holidays (see below). The displayed table will be updated annually and forwarded to each participating GCC 30 days prior to the start of the new calendar year.

Note: It is understood by the DCC that fresh blood specimens collected and shipped on a Friday will not be delivered until the following Monday. The scheduling of shipments for any day other than Monday through Thursday must receive pre-approval from the RTI Biospecimen Manager.

Blackout Shipping Dates

Calendar Year 2013	
Friday Before Memorial Day	Friday, May 24
Wednesday Before Independence Day	Wednesday, July 4
Friday Before Labor Day	Friday August 30
Day Before Thanksgiving Day	Wednesday, November 27
Thanksgiving Day	Thursday, November 28
Friday Before Christmas Eve	Friday, December 29
Christmas Eve	Tuesday, December 24
Day Before New Year's Eve	Monday December 30
New Year's Eve	Tuesday, December 31

4.4 Pre-shipment Notification

Prior to shipping any type of specimens, fresh or frozen, the RTI Biorepository requires electronic notification to confirm the type of shipment, amount/volume to be expected, carrier name, tracking number and point of origination. Electronic notification for both fresh and frozen shipments will be made through SIMMS (see Chapter 6.0) unless otherwise directed by the Biospecimen Manager.

4.5 Shipping Supplies

The DCC will provide each GCC with the necessary shipping containers and supplies to properly package, label, and transport their collected blood samples (fresh or frozen) as "exempt human specimens". The supplies will include preprinted Federal Express billable stamps, Federal Express clinical paks and the necessary packing materials to meet all IATA packaging specifications for "exempt human specimens". GCCs will need to contact the RTI Biospecimen Manager at least 2 weeks in advance to receive additional shipping supplies. Requests for Biological Substances, Category B shipments must be made in advance to the DCC Biospecimen Manager.

4.5.1 Fresh Blood Packing Supplies

Refer to section 2.4.1.

4.5.2 Pack Fresh Blood Specimens

Step 1. Slide the ACD whole blood tube(s) into the empty slots of the Aqui-Pak absorbent pouch to protect the tube during transit and to meet the absorbent requirement.

Step 2. Roll up the pouch and place the protected ACD tube inside the plastic self-closing bag with the biohazard symbol. Use one bag per subject in the event a tube leaks.

Step 3. Seal the bag and place the bagged tubes in the white fiberboard box. Insert a hardcopy of the completed specimen collection form. Close and secure the box using the locking side-tabs.

Step 4. Place the fiberboard in a FedEx Clinical Pak and seal the pak. Affix an "Exempt Human Specimen" label and the FedEX billable stamp to the outside of the clinical pak.

4.6 Frozen Blood Specimens

RTI will provide each GCC with the necessary shipping supplies to properly package, label, and transport frozen blood samples as "exempt human specimens". The supplies will include preprinted Federal Express airbills, prelabeled insulated shippers, and the necessary associated packing supplies to meet all IATA packaging specifications for "exempt human specimens".

4.6.1 Frozen Specimens Packing Supplies

The following supplies (see 2.4.2) will be made available for packaging frozen biospecimens packed on dry ice that are classified as "Exempt human specimens" according to IATA 3.6.2.2.3.6.

- Well constructed one piece expanded polystyrene shipper and tight fitting lid designed to maximize temperature control and withstand the hazards of transport. The outer walls of the insulated shipper will be at least 1.5 inches thick.
- The insulated shipper will be inserted into a rigid 200-lb. test corrugated carton that can be sealed to prevent any loss of contents that might be caused under normal conditions of transport.
- Self-sealing leakproof secondary bags.

- Absorbent strips and Additional specimen storage boxes with 81 space dividers.
- Assorted hazard, packing and address labels that are affixed to the corrugated outer carton container.
- Federal Express hardcopy airbill (partially completed)

4.6.2 Prepare Specimens for Shipment

Begin packaging preparations at least 3 days prior to the scheduled shipping date. The shippers will come pre-labeled from RTI that will show the following information (see Figure 4.2);

- Full name, address and telephone number of the shipper.
- Full name, address and telephone number of the recipient.
- Class 9.0 Hazard Label for Dry Ice, and directly adjacent the corresponding UN number (UN 1845) and the net weight of the dry ice in kilograms.
- Exempt Human Specimen label.
- Package orientation arrows on opposite ends of the box.

Prepare an electronic file that details the bio-specimens being shipped.

- The file can be created using SIMMS and can generate a hardcopy "Itemized List of Contents" that will be packed with the shipment.
- The list of contents will include the following; Sample ID, Vial/Sequence ID, Material Type, Sample Modifiers, Box location.
- During the packing procedure work quickly to minimize frozen specimens exposure to room temp.
- Prior to packing - verify that each box is identified and that a hardcopy list has been created for each box.
- Secure the contents of each box by wrapping two rubber bands crosswise around the lid and base.
- Pack each secured box in a leakproof secondary packaging with an absorbent strip and seal the packaging.
- Return the packaging to the freezer and pack any additional specimens using the same methods.

4.6.3 Pack Frozen Specimens

On the day of shipment fill the bottom of the insulated shipper with a two to three inch layer (75 cm) of dry ice pellets (see Figure 1). Carefully stack the first layer of vial boxes on top of the 3 inches of dry ice. Add more dry ice and repeat the process the process for the number of boxes you plan to ship (see Figure 2). Cover the stacked vial boxes with a sufficient volume of dry ice pellets to fill the entire inner space of the shipper to a height that is 1 inch below the top of the shipper.

As you add dry ice, occasionally shake the shipper to fill any air pockets. **Do not** partially fill the shipper with other materials such as styro-foam pellets, air bags, "chemical ice packs" or wet ice. Insert the flexible sub-lid of the shipper. Then place a complete and correct hardcopy (itemized list of contents) of all specimen boxes on the top of the sub-lid. Then close and seal the shipper's fiberboard box flaps with packing tape.

Attach the completed hardcopy FedEx airbill to the top of the box using the Fed Ex self-adhering document bag – ensure that the text "Exempt Human Specimens" is printed on the airbill.

4.6.4 Shipment Notification

All frozen shipment notifications will be performed through SSIMS. The information will include;

- Date of shipment
- Name of carrier
- Airway bill number
- Electronic file attachment (e.g. specimens type(s), number of vials)

Figure 1.

Side View of Insulated Shipper

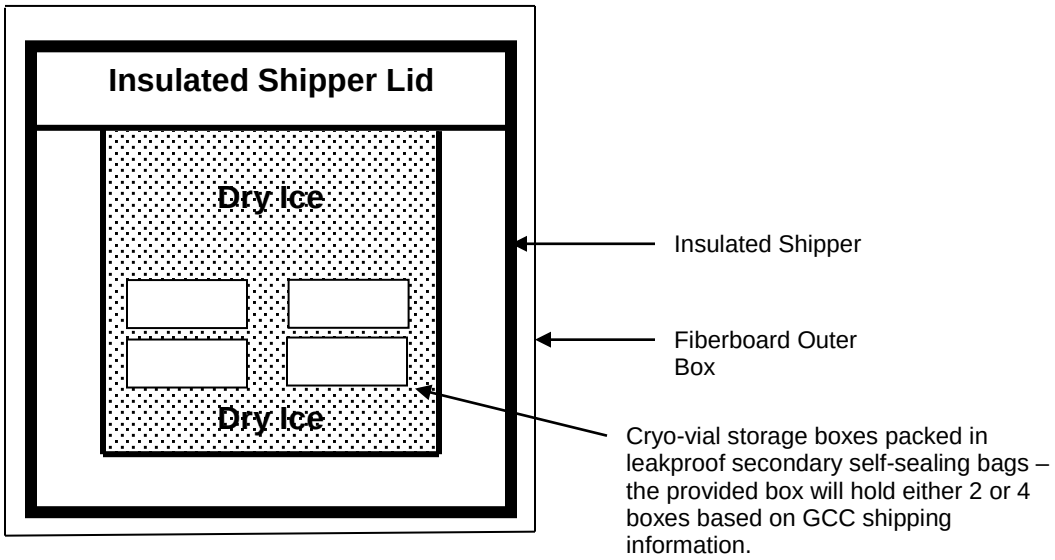


Figure 2.

Example of Outer Box Markings and Labels for
Exempt Human Specimen Dry Ice Shipment