

Specimen Collection and Processing by Collection Site and Biorepository for CIMAC Studies

VERSION 8: NOVEMBER 19, 2019

1 SCOPE

The purpose of this Standard Operating Procedure (SOP) is to establish a consistent process for the sites and Biorepositories involved in CIMAC studies to collect and process tissue, stool and blood samples for immune monitoring and profiling analyses to be performed by the CIMACs. This SOP defines ***the options to be selected for each correlative study protocol*** for collection schema, handling, processing, and freezing protocols of ***tissue, bone marrow, cerebrospinal fluid, stool, plasma, and PBMCs***. Protocol drafting suggestions are indicated as [xx].

2 SUMMARY OF SAMPLE COLLECTION AND PROCESSING

Table 1: Summary of Collection and Processing Activities Intended for Tier 1 and Tier 2 Assays

Specimen Type	Timepoints*	Section 3	Section 4		Section 5
		Collection and Processing at Site	Immediate Processing at Biobank	Processing for Distribution at Biobank**	Intended Assay Use at CIMAC
De Novo Core Needle Biopsy OR Endoscopic/Punch Biopsy OR De Novo Surgical Resection (malignant, skin punch, or BM Biopsy only)	One or more	1-2 cores OR 1 segment Each protocol will select an option: • Fix and embed on-site%, • OR Fix in formalin and ship in EtOH	Embed fixed tissue Store blocks	• Unstained slides + H&E (Imaging Assays) • DNA / RNA	Fresh Frozen Samples^ WES/germline RNA-Seq TCR-Seq# FFPE Samples IF IHC MIBI# WES/germline^ RNA-Seq^ TCR-Seq^
		1-2 cores flash frozen^ OR 1 Segment flash frozen^	Store frozen	DNA / RNA	
Archival FFPE Material	Typically, one	Ship FFPE blocks OR • Specify unstained slides, • Core punches, OR • Three 10 µm Scrolls	Store blocks OR • Vacuum-seal slides + refrigerate • Refrigerate punches + scrolls	• Unstained slides + H&E (Imaging Assays) • DNA / RNA	

Specimen Type	Timepoints*	Section 3	Section 4		Section 5
		Collection and Processing at Site	Immediate Processing at Biobank	Processing for Distribution at Biobank**	Intended Assay Use at CIMAC
Sodium Heparin Green-Top Tubes	Multiple	30 mL Draw ^{&} (Ship ambient)	Isolate plasma and PBMCs, smart tubes (TCR) Freeze aliquots	Ship smart tube, plasma or PBMC aliquots, OR; DNA (TCR-Seq)	Plasma (Olink, ELISA [#]) PBMCs (CyTOF, TCR-Seq [#])
Streck Cell-Free DNA Tubes	Multiple	10 mL Draw ^{&} (Ship ambient)	Isolate plasma and Freeze aliquots	Ship plasma aliquots	cfDNA [#]
K2-EDTA Purple-Top Tubes	Baseline for germline; Multiple for TCR-Seq	<ul style="list-style-type: none"> • 2 mL Draw (solid tumor germline) • 5-10 mL Draw (end of induction therapy germline) • 2 mL Draw (TCR-Seq) (Ship ambient) 	Freeze 0.5 to 1 mL germline aliquots 2 mL aliquots (TCR-Seq)	Extract and ship DNA aliquots	Germline DNA TCR-Seq [#]
Bone Marrow Aspirates OR Cerebrospinal Fluid	Multiple	Custom volume in K2-EDTA tubes	<ul style="list-style-type: none"> • Supernatant • Cell fraction 	<ul style="list-style-type: none"> • Ship Aliquots • Unstained slides + H&E (Imaging) • DNA / RNA 	CyTOF Olink IF IHC MIBI [#] RNA-Seq
Stool Samples	Multiple	Self-collection (Ship ambient or frozen depending on kit)	<ul style="list-style-type: none"> • 2 mL DNA-stable aliquots • Frozen stool Store frozen	Ship frozen aliquots	16S rRNA Gene Amplicon or Whole Genome Shotgun Seq [#] Metabolomics Microbe Characterization [#]
Sample Data	All	<ul style="list-style-type: none"> • Collection + processing times • Core number • Clinical reports[§] 	Microbiome data Processing and storage details	<ul style="list-style-type: none"> • Clinical reports[§] • Sample QC • Thawing and shipment details 	All

* Detailed description of Timepoints will be given in the Specimen Collection table in each Protocol.

**Directions for distributing sample derivatives to CIMAC labs will be provided at a later time.

^ Flash-frozen are preferred over FFPE for genomic assays.

Not a Tier-One assay at this time: Tier 1 assays are those broadly recommended for most trials collaborating with CIMAC.

% FFPE blocks will be fixed and embedded onsite for NCTN protocols.

§ Clinical reports as defined in Section 4.1.3.

& Draw volumes may be adjusted per protocol.

2.1.1 Biomarker Plan Suggestions

- An assay can be requested for different specimen types (e.g. TCR-seq should be prioritized for tissue>EDTA tubes).
- Blood timepoints should match when tissue is acquired with additional on-treatment and follow-up timepoints (up to 5 timepoints for CIMAC analysis).
- If unstained slides are requested, please indicate # of slides (or default minimum).

3 COLLECTION SITE ACTIVITIES

3.1 Tissue Collection and Processing at Collection Site

3.1.1 Pre-Analytic Information

- [Protocol must include the comprehensive list of collection site and Biorepository pre-analytical information from Appendix I].

3.1.2 Sample Labeling Recommendations

- [Follow tissue sample labeling procedures standard for each trial group].

3.1.3 Tissue Collection

NOTE: *Cold ischemia time should be minimized as much as possible, optimally less than 20 min for formalin-fixed samples and <2 minutes for flash-frozen specimens (or as indicated by each study protocol). Ischemia time stamp should be documented for every tissue core, module or segment.*

Core Needle Biopsy Tissue: for most trials, core needle biopsies will be collected using a 16-18-gauge needle (condition permitting), at [time points of collection].

- At least 4 cores (1 cm in length) should be obtained for CIMAC analysis.
- Alternating passes: First obtain a core for FFPE processing (core 1), followed by a core for flash freezing (core 2), followed by a core for FFPE (core 3), followed by a core for flash freezing (core 4).
- The number of specimens obtained will be affected by the patient's clinical condition at the time of biopsy and determined by the specialist performing the procedure.
- Each research sample must be placed in a prelabeled cassette dedicated to each study. Up to two cassettes may be used per jar.
- Record the core number for each core needle biopsy sample on the sample label.
- [Additional cores may be obtained; number of FFPE vs. frozen samples may vary for each correlative study based on assays requested]
- [Flash frozen cores are preferred for genomics assays, however FFPE blocks are acceptable]
- [Bone marrow biopsy cores will be collected and processed under this category]

Surgical Resection Tissue: for some trials, surgical resection will be obtained at [time points of collection]. From this resected tissue, harvest a part of the tumor measuring approximately 1x1x1 cm, avoiding necrotic areas, and divide this tissue **into two almost equal segments**.

- One piece will be processed as an FFPE sample and the other as a flash frozen sample. [refer to **Section 3.1.4**]
- For some clinical trials, more than two segments may be obtained as described by the protocol.

Endoscopic/Punch Biopsy Tissue: for some trials, endoscopic or tumor punch biopsies may be obtained at [time points of collection].

- Endoscopic/punch biopsy of at least 3 mm diameter should be obtained for CIMAC analysis.
- Endoscopic/punch biopsies should be processed as FFPE blocks or Freshly Frozen. [refer to **Section 3.1.4**]

Bone Marrow Aspirates: for some trials, bone marrow aspirates may be obtained at [time points of collection] and collected using K2-EDTA vials.

- [Number of draws, needle pulls and volumes will be selected for each trial].
- [Include 5-10 mL draw K2-EDTA whole blood at end of induction therapy for germline].

Cerebrospinal Fluid (CSF): for some trials, CSF may be obtained at [time points of collection] and collected using K-2 EDTA vials.

- [Number of draws and volumes will be selected for each trial].

NOTE: Fine needle aspirations (FNAs) are not an acceptable replacement for tissue cores intended for CIMAC assays.

3.1.4 Tissue Processing

Formalin Fixation of Tissue Samples

- ***The preferred method is to fix and embed the tissue in paraffin at the collection site if all requirements can be followed.*** If FFPE samples cannot be processed on-site as described, the clinical site should formalin-fix tissues as described in option #2 below, and then transfer to in 70% Ethanol to send to the Biorepository for embedding.
- Neutral-buffered formalin ***must be used*** as fixative (no acid-based products).
- Bone marrow biopsy tissue used for imaging studies should be decalcified on-site using 10% formic acid for 4-6 hours prior to embedding.
- [Tissue will be embedded at the collection site for hybrid study protocols that indicate use of ETCTN samples for testing at the MoCha lab].
- [Ideally, each study should choose and implement ***one processing option to all samples*** if possible, otherwise allow collection sites to choose].

Fixation Options

One of the following options should be selected by each protocol:

Option #1: Embedding Tissue at Collection Site [required for NCTN]:

- Samples must be fixed in formalin for ***12-24 hours*** and embedded directly at the collection site. Embedding must be completed ***within 72 hours*** of adding 70% ethanol to tissue.
- Sites must use automated tissue processors and ***not use*** microwave tissue processors.
- Sites should follow embedding protocols where the total processing time from 70% ethanol to block embedding ***exceeds 4 hours.*** [protocol should include table from ***Appendix II***]

Option #2: Shipping Formalin-Fixed Tissue to Biorepository in Ethanol: [not permitted for NCTN]

- Samples must be fixed in formalin for a ***minimum of 12 hours but no more than 24 hours*** before being transferred to 70% ethanol. Tissue can be shipped in ethanol to the biorepository.
- Samples must be placed into an automated processor ***within 72 hours*** of adding the fixed tissue to ethanol. Record Ethanol start and end times as part of pre-analytical information.

Tissue samples fixed in formalin for 24-36 hours ***will be collected and shipped*** but will be recorded as non-compliant by CIMAC labs based on the pre-analytical data collected.

Flash Freezing of Core Needle Biopsy and Surgical Resection Samples

Surgical Resections

- Samples should be dissected soon after the specimen is released by the supervising physician and each module or segment should be placed in a separate pre-labeled cryovial.
- Prefer a minimum of 1x1x1 cm (~25 mg).

Core Needle Biopsies

- Each core sample should be placed directly into a separate pre-labeled cryovial.

Flash Freezing on Dry Ice

- Each specimen contained in its cryovial should be flash frozen using a dry ice/alcohol slurry (freezing in liquid nitrogen vapor is an acceptable alternative).
- Frozen specimens should be shipped (the day of collection) Priority Overnight on dry ice in an insulated shipper or a dual temperature-chambered kit.

3.1.5 Archival FFPE Tissue

Even when patients are able to provide a biopsy/resection specimen, a prior (archived) representative tumor tissue block may be requested. If previously-collected FFPE will be submitted, then the following criteria must be met:

- Tissue should ideally have been collected within 6 months prior to registration, however older cases will be accepted.
- A copy of the original pathology report must be provided, and the tissue collection date must be recorded so the sample age can be derived.
- Formalin-fixed paraffin-embedded tumor tissue block(s) **must be submitted or used to provide the specimens listed below**. Preferred specimen requirement is as follows:
 - Block should contain at least 30% tumor, however less tumor content is acceptable for imaging studies.
 - Material requested **exclusively for genomics** should ideally contain at least 70% tumor although less is acceptable.
 - Surface area: 25 mm² is optimal. Minimum is 5 mm².
 - Volume: 1 mm³ optimal. Minimum volume is 0.2 mm³.

If the archival block cannot be submitted, the following can be provided:

- One sectioned H&E slide, **AND**;
- [specify number] 4 µm unstained air-dried (unbaked) plus slides, **OR**;
- One (1) or more core punches (minimum of 1-2 mm diameter) from tumor block placed into a clean vial, **OR**;
- **For nucleic acid extraction only**: At least three 10µm FFPE scrolls [OR specify amount] cut from blocks and placed into a clean vial.

3.1.6 Tissue Shipment from Collection Site to Biorepository

Do not send samples the day before a national holiday or on Friday (unless Biorepository is able to process on Saturdays). **FedEx Priority Overnight is mandatory for all samples.**

- An external sample label should be fixed to the shipping container to alert the Biorepository of **Formalin-fixed** sample **time** and **date** it was placed into **Ethanol** (this helps to identify and prioritize received samples that have processing time requirements—Option #2).
- [Archival material does not need to be shipped on the day of collection].

- [The Biorepository will provide sample kits based on contents selected in Table 2 OR what has been selected for the clinical trial].

Table 2. Shipping Conditions for Tissue Samples

Tissue Sample	Collection Kit Contents	Shipping Schedule *	Shipment Conditions
Option 1 (select option 1 or 2 for a given protocol) FFPE blocks, slides, core punches, or scrolls	No kit provided if only FFPE blocks are shipped, dual chambered kit provided when FFPE and fresh frozen material are shipped together (if available).	Monday through Thursday (FedEx Priority Overnight)	Ambient, include a gel-pack or cold-pack (NOT a frozen pack) on hot days and insulation on cold days
Option 2 (select option 1 or 2 for a given protocol) Tissue fixed in formalin and shipped in 70% ethanol	Formalin-prefilled jars and cassettes, Single or Dual Chambered Kit, depending on protocol-specific details	Fixed in formalin on site for 12-24 hours and placed in ethanol for shipment to biorepository for embedding within 72 hours of Ethanol Tissue collected Monday through Thursday and shipped in ethanol (after fixation) overnight (FedEx Priority Overnight)	Ambient, include a gel-pack or cold-pack (NOT a frozen pack) on hot days and insulation on cold days
Snap-frozen specimens	Single or Dual Chambered Kit depending on protocol-specific details	Monday through Thursday (FedEx Priority Overnight)	Frozen, on dry ice
Bone Marrow Aspirates/CSF	Vacutainer or specialized tubes may be provided	Monday through Thursday (FedEx Priority Overnight)	Ambient

*For samples shipped late in the week, collection sites will work with the Biorepository to determine the most optimal sample processing conditions.

3.2 Blood Collection and Processing at Collection Site

3.2.1 Time Points of Collection

Blood will be collected at [timepoints of collection]. [Total volume may be adjusted for each trial and may be less for pediatric trials based on maximal draw limits put in place by individual protocols]

3.2.2 Sodium Heparin Green-Top Tubes (30 mL Total Draw per timepoint)

- Label Sodium Heparin Green-Top Tubes (Vacutainer®), Becton Dickinson Cat No. 367874 (or equivalent).
- Collect a total of **30 mL** of peripheral blood in Sodium Heparin Green-Top Tubes (use 5- or 10-mL tubes).
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of sodium heparin. Maintain specimens at ambient temperature (room temperature) during collection and transport.

3.2.3 Streck Cell-Free DNA Tubes (10 mL)

- Label one 10 mL Streck cfDNA BCT (Streck catalog # 218961, 218962, or 218992).
- Collect **10 mL** of blood into the pre-labeled tube and invert to mix. **Note: Blood must be thoroughly mixed to ensure preservation of specimen.**
- After collection, blood in cfDNA Streck BCT should **never be refrigerated**, as this will compromise the specimen. Blood collected in cfDNA Streck Tubes is stable at room temperature.

3.2.4 K2 EDTA Purple-Top Vacutainer Tubes (2, 4 or 10 mL)

- Collect [select] mL of peripheral blood into a labeled K2 EDTA Purple-Top Tube; each tube must be filled completely to ensure the correct blood/anticoagulant ratio.
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of EDTA. Maintain specimens at ambient temperature (room temperature) during collection and transport.
- [One 2 mL tube should be collected at baseline for germline analysis if WES is requested]
- [2mL of blood should be collected for TCR-Seq at each desired timepoint].
- [2-5 mL of whole blood should be collected as germline at end of induction therapy around 1 month for hematological malignancies].

3.2.5 Whole Blood Shipment from Collection Site to Biorepository

Do not send samples the day before a national holiday or on Friday (unless Biorepository is able to process on Saturdays). **FedEx Priority Overnight is mandatory for all samples.**

- Blood should be shipped ambient FedEx Priority Overnight to the biorepository where it is processed the day of receipt **within 24 hours of collection (not to exceed 48 hours)**.
- An external sample label should be fixed to the shipping container to alert the Biorepository of **blood** sample collection **time** and **date** (this helps to identify and prioritize received samples that have processing time requirements).
- [The Biorepository will provide sample kits based on contents selected in Table 3 OR what has been selected for the clinical trial].

Table 3. Shipping Conditions for Blood Samples

Blood Sample	Collection Kit Contents	Shipping Schedule	Shipment Conditions
Blood in Sodium Heparin Green-Top Tubes	Ambient shipper	Day of Collection (Samples collected and shipped together Monday through Thursday*; FedEx Priority Overnight)	Ambient
Blood in Streck Cell-Free DNA Tubes	Streck tubes provided with ambient shipper		
Blood in K2 EDTA Purple-Top Tubes	Ambient shipper		

* Blood samples may be shipped Friday to Biorepositories (ETCTN, COG, NRG BB-Columbus, and SWOG) which are open and able to process samples on Saturdays.

3.3 Stool Collection and Processing at Collection Site

3.3.1 Stool Samples

Partial stool samples will be collected at [time points] using provided self-collection kits and written instructions. Clinical site staff will explain to patients how to use the kits at the clinic or in the privacy of their home.

- Stool collected at the baseline timepoint will employ a Cold Chain collection using kits containing the following: a ThermoSafe Multi-Purpose Foam Container packaged with 1) Two ThermoSafe U-tek Phase Change Materials 2) Ziploc bag “A” containing stool collection and packing instructions and tape to close the ThermoSafe box 3) Ziploc bag “B” containing two EZ sampler paper hats, a pair of disposable gloves, a biohazard transport bag and a barcoded Covidien Precision Stool Collector.
- The stool sample must be packaged in the ThermoSafe Multi-Purpose Foam Container sandwiched between two frozen ice-packs.
- Baseline and subsequent timepoints will use OMNIgene GUT kits (OMR-200.100—shipped ambient) which include a DNA stabilizing solution.
- Collection kits will include directions and a Sample Collection Form to be completed by the patient to record selected preanalytical details including the Bristol Stool Scale to classify their sample.
- Collection site staff will record data from the Sample Collection Form into an electronic CRF.

3.3.2 Stool Sample Shipment from Collection Site to Biorepository or CIMAC lab

- Study participants will collect and return stool samples to the clinical site which will ship each specimen to the Biorepository or CIMAC lab where they will be homogenized, aliquoted and stored frozen.
- It is recommended that patients keep the Cold Chain samples cold after collection and return collection kits with frozen ice-packs within 24 hours. The clinical site should store the samples immediately upon receipt at -80°C and ship samples on dry-ice to the CIMAC lab for further processing. The OMNIgene GUT collected samples can remain at ambient temperatures for a maximum of 60 days.

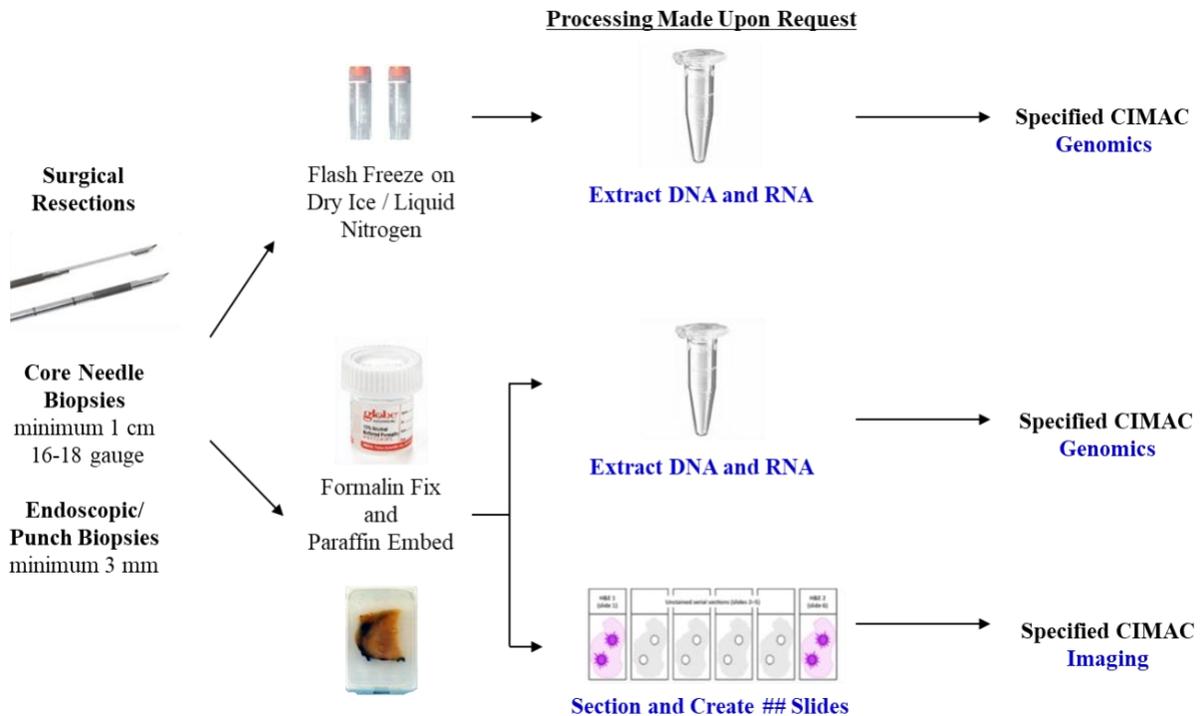
Table 4. Shipping Conditions for Stool Samples

Sample	Collection Kit Contents	Shipping Schedule	Shipment Conditions
Stool Samples	Collection container, bags, collection aids, DNA stabilizing solution, Sample Collection Form, and instructions	Samples should be shipped Monday through Thursday only (FedEx Priority Overnight)	Frozen for Cold Chain Ambient for OMNIgene GUT

4 BIOREPOSITORY ACTIVITIES

4.1 Tissue Processing by Biorepository

Overall Tissue Processing Schema for Tier 1 Assays



4.1.1 Pre-Analytic Information

- [Protocol must include the comprehensive list of collection site and Biorepository pre-analytical information from Appendix I].

4.1.2 Sample Labeling Recommendations

- [Each Biorepository may use their own labeling sample schema until a time when the CIMAC network provides instructions for generating CIMAC Network IDs for patients and their sample derivatives].

4.1.3 Collection of Clinical Reports

Collect all relevant pathology reports, surgical reports, and molecular reports **for each sample time point** and provide redacted copies to CIMAC investigators along with each associated sample/derivative. If report is not available, please use the pathology verification form included in appendix [VII].

- [ETCTN: path reports (or pathology verification forms), surgical/procedural reports, molecular reports].
- [NCTN: all standard-of-care pathology reports, pathology verification forms and procedural reports].
- [NRG: path reports may need to be obtained from a Data Center for some sites (Pittsburgh)].
- [Archival samples: collect original diagnostic pathology reports].

ONLY INSTRUCTIONS FOR SPECIMEN COLLECTION, IMMEDIATE PROCESSING AND STORAGE ARE DESCRIBED. SUBSEQUENT PROCESSING AND DISTRIBUTION DETAILS WILL BE PROVIDED AT A LATER TIME

4.1.4 Quality Control Activities (QC) by the Biorepository

Before distributing samples to CIMACs, the Biorepository will perform the following:

- Histology preparation such as H&E staining and mounting unstained whole sections for immunohistochemistry and immunofluorescence.
- **Histology concordance confirmation and percent viable tumor evaluation** of tissues. [Include **Appendix VI**]
- Quality assessment of extracted DNA and RNA to ensure sufficient amount and quality of material is shipped to CIMAC labs for testing (use existing Biorepository practices.).
- **Note the condition of blood samples** for processing [refer to **Appendix IV and V: Plasma Isolation** sections].

4.1.5 Formalin-Fixed Tissue Samples Arriving in Ethanol

Upon receiving a formalin-fixed sample shipped in ethanol, the Biorepository will process and embed each sample in paraffin to create separate formalin-fixed paraffin-embedded (FFPE) block(s):

- [Include **Appendix II**, "Processing and Paraffin Embedding of Tissue" for details].
- For tissue arriving in 70% ethanol: Processing should occur within **72 hours** of the specimen having been placed in ethanol.

4.1.6 Frozen Tissue

Frozen tissue specimens received from the collection site should be stored in liquid nitrogen vapor phase **until a request** for sample processing is made by CIMAC:

- **DNA/RNA will be co-extracted by the Biorepository for genomics assays.** [refer to **Appendix III** for SOPs]

4.1.7 FFPE Tissue

FFPE blocks received from the collection site or blocks embedded by the Biorepository should be stored at room temperature **until a request** for sample processing is made by CIMAC:

- **DNA/RNA will be co-extracted by the Biorepository for genomics assays** [refer to **Appendix III** for SOPs]
- **H&E slides:** create at least one H&E slide per block.
- **Unstained air-dried plus slides for IHC/IF imaging only:** cut at least 5 [OR number requested by study] tissue sections of [4 or 5] microns per case, using a microtome, and mount on "plus" (charged) glass slides.
- **Unstained air-dried plus slides for Mt. Sinai IHC/IF imaging only:** cut at least 5 [or number requested by study] tissue sections of [4 or 5] microns per case, using a microtome, and mount on "plus" (charged) Leica Superfrost Plus slides centered at 12 mm (left-label), 3 mm (top-bottom), and 7 mm (right).
- **Unstained air-dried gold-coated slides for MIBI imaging only:** cut at least 2 [OR number requested by study] tissue sections of [4 or 5] microns and mount on IONpath slides (Cat #567001).

- Vacuum seal unstained slides for long-term storage if sections need to be cut immediately upon specimen arrival at the Biorepository, otherwise cut sections in batch upon request and distribute to CIMAC labs.
- **FFPE scrolls:** Cut fresh scrolls for nucleic acid extraction to be performed by Biorepository.

4.1.8 Archival Tissue

Upon receiving FFPE blocks, slides, scrolls, or core punches, from the collection site, the Biorepository should perform the following until a request for shipment is made:

- Store each FFPE block at room temperature.
- Vacuum seal unstained slides and store refrigerated.
- FFPE core punches and scrolls should be stored refrigerated.
- **DNA/RNA will be co-extracted by the Biorepository for genomics assays** [refer to **Appendix III** for SOPs]

4.1.9 Bone Marrow Aspirates

Upon receiving bone marrow aspirates from the collection site, the Biorepository should:

- Use a Ficoll separation protocol to isolate supernatant and mononuclear cell fractions.
- Process bone marrow aspirate according to Biorepository practices and store an appropriate number of frozen aliquots.

4.1.10 Cerebrospinal Fluid Samples

Upon receiving CSF samples from the collection site, the Biorepository should:

- Use a centrifugation protocol to isolate supernatant and cell pellet fractions.
- Process CSF samples according to Biorepository practices and store an appropriate number of frozen aliquots.

When a request for bone marrow or CSF material is made, the Biorepository will provide:

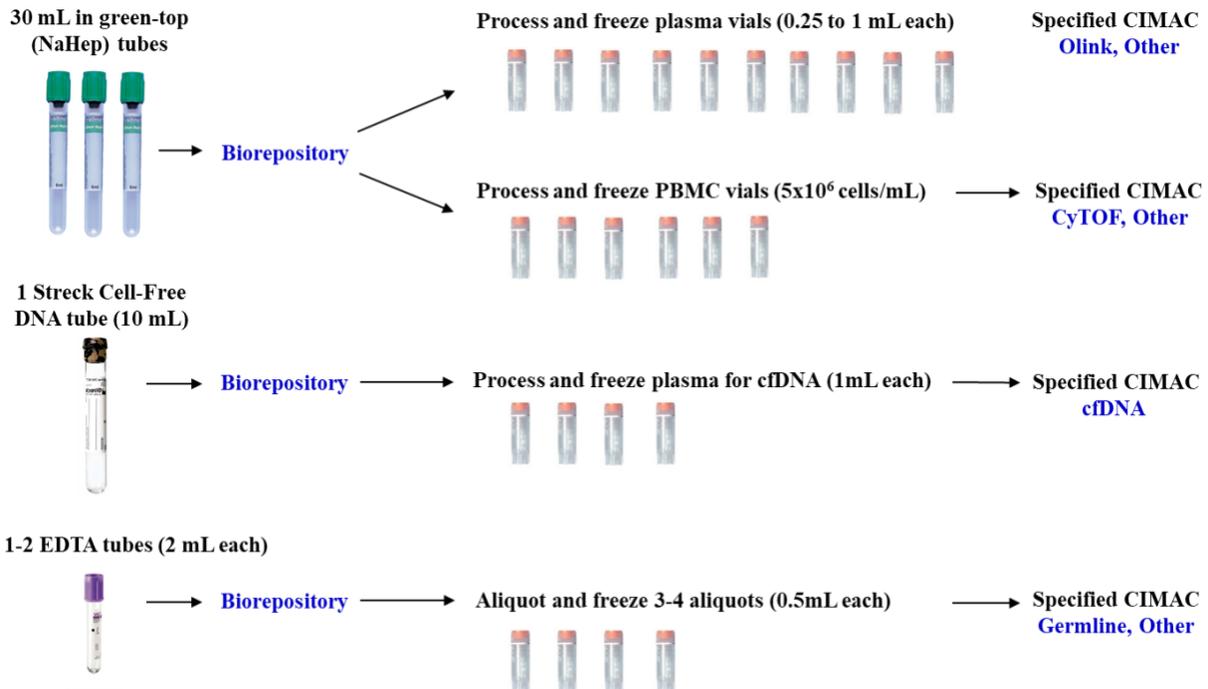
- Frozen aliquots of bone marrow cell fractions.
- **Unstained air-dried plus slides for IHC/IF imaging only:** cut at least 5 [or number requested by study] tissue sections of [4 or 5] microns per case, using a microtome, and mount on "plus" (charged) glass slides.
- **Unstained air-dried plus slides for Mt. Sinai IHC/IF imaging only:** cut at least 5 [or number requested by study] tissue sections of [4 or 5] microns per case, using a microtome, and mount on "plus" (charged) Leica Superfrost Plus slides centered at 12 mm (left-label), 3 mm (top-bottom), and 7 mm (right).
- **Unstained air-dried gold-coated slides for MIBI imaging only:** cut at least 2 [or number requested by study] tissue sections of [4 or 5] microns and mount on IONpath slides (Cat #567001).
- **DNA/RNA will be co-extracted by the Biorepository for genomics assays** [refer to **Appendix III** for SOPs]

4.1.11 Stool Samples

Upon receiving a self-collection kit with a stool sample, the Biorepository should:

- Record the following information:
 - Date of sample receipt
 - Record if Cold Chain collected specimens arrived cold/frozen, not ambient.
 - Record the weight of the OMNIgene GUT tube before processing.
- Store the Cold Chain collected samples at -80°C until request for shipment on dry-ice to CIMAC laboratory for further processing.
- Process the ambient OMNIgene GUT collected specimens as follows:
 - Vortex the sample vigorously for 60 seconds (medium setting). This will typically break up the matrix making it more liquid and visibly homogenous.
 - With the purple cap still screwed on, unscrew the yellow portion of the tube and set aside on a clean surface.
 - Using a wide bore pipette tip, pipette out the sample and divide homogenized fecal sample evenly between two 2 mL cryovials with o-rings. If the sample is too viscous to pipette, put the purple cap and yellow portion back on the tube tightly, incubate the sample at 50°C for 30 minutes, then immediately redo steps 3 and 4. If the incubation at 50°C above does not facilitate the transfer of your viscous samples, please obtain the Liquefaction Reagent OM-LQR (400/1600).
 - Freeze aliquots at -80°C until request for shipment on dry-ice.

4.2 Blood Processing by Biorepository



4.2.1 Important Notes

- [Number and volume of aliquots may vary based on total blood volume, PBMCs collected, and assay needs, custom-aliquoting may be requested for each protocol].
- [If possible, record Time/Date blood processing was initiated].
- [Each Biorepository may use their own labeling sample schema until a time when the CIMAC network provides instructions for generating CIMAC Network IDs for patients and their sample derivatives].

4.2.2 Sodium Heparin Green-Top Tubes

Upon receiving the Sodium Heparin Green-Top Tubes from the collection site, the Biorepository will **pool all samples** from a 30 mL draw at one timepoint and prepare Plasma and PBMCs following a Ficoll-Paque protocol. [Described in **Appendix IV**]

- **For proteomic stabilization of whole blood only:** aliquot 1 mL of whole blood directly into Smart Tube (MTS1P 100/CS) or equivalent vessel and store frozen using PROT1 stabilization buffer (Smart Tube Inc). [Select this option only when requested by CIMAC]
- Create ~12 plasma vials in 1 mL aliquots (or as many as can be obtained) and store at -80°C.
- Create ~6 PBMC vials (or as many as can be obtained) in **Recovery™ Cell Culture Freezing Medium** (Invitrogen Cat# 12648-010) at 5 x 10⁶ cells/mL depending on blood volume and study need. Typical recovery can expect 1 x 10⁷ cells from each 10 mL tube.
- Slow-freeze PBMC aliquots at -80°C in a freezing container <24 hours (or over the weekend) followed by long-term cryopreservation in a liquid nitrogen vapor phase freezer.
- If requested, the Biorepository will extract DNA/RNA.

4.2.3 Streck cfDNA Tubes

Upon receiving the Streck cfDNA Tube from the collection site, the Biorepository should prepare Plasma. [Described in **Appendix V**]

- For each 10 mL Streck tube, create at least 4 plasma vials of 1 mL aliquots (or as many as can be obtained) and store at -80°C.

4.2.4 K2 EDTA Purple-Top Tubes

Upon receiving K2 EDTA Purple-Top Tubes from the collection site, the Biorepository should **pool all samples from a blood draw** and: [Select only those that apply to biomarker plan]

- For each 2 mL of EDTA Purple-Top Tubes **intended for TCR-Seq**, create ~2 mL aliquot and store at -80°C as follows:
- For each 2 mL EDTA Purple-Top Tube **intended for solid tumor germline analysis**, create 3-4 whole blood vials of 0.5 mL aliquots and store at -80°C, as follows:
- For each 2-5 mL EDTA Purple-Top Tube **intended for hematologic malignancy germline analysis**, create an appropriate number of whole blood vials in 1.0 mL aliquots and store at -80°C, as follows:
 - Invert the tube gently about 5 times; excess inversion can cause changes in the integrity of the sample.
 - Aliquot 500 µL of whole blood cell pellet using a sterile pipet into each of three or four prelabeled 1.8 or 2 mL cryovials (discard as waste if less than 0.5 mL remains).
 - Store blood samples in a -80°C freezer.
- When requested, the Biorepository will extract genomic DNA for germline analysis.

4.3 Shipment of Samples and Derivatives from Biorepository to CIMAC

Instructions on how to thaw, aliquot and distribute sample derivatives to the downstream CIMAC laboratories will be added at a later time.

- Ship samples as batches on dry ice (or equivalent container depending on practices) upon discretion based on shipping and receiving locations taking weather and other pending conditions into consideration.

Table 5. Shipping Conditions for Biorepository Samples

Sample	Shipping Schedule	Shipment conditions
All slides (imaging)	Upon discretion except before Federal Holidays, Monday through Wednesday (FedEx Priority Overnight)	Ambient, Storage box that prevents slide contact.
Frozen Tissue		Frozen, Cryoport/equivalent or dry ice
Stool Aliquots		
Plasma Aliquots		
PBMC Aliquots		
Whole Blood Aliquots		
DNA/RNA (from tissue, stool, blood, CSF or bone marrow aliquots)		

Table 6. Contact Information for Shipping Samples from Biorepository to CIMAC Lab.

CIMAC Site Name	Study PI	Contact(s) (Attn to:)	Address
CIMAC 1-- MD Anderson Cancer Center	Ignacio Wistuba iwistuba@mdanderson.org	Elena Bogatenkova ebogatenkova@mdanderson.org Beatriz Sanchez-Espiridion bsanchez2@mdanderson.org 713-745-7047	Institutional Tissue Bank (ITB) 1515 Holcombe Blvd, Rm G1.3586 Houston, TX 77030
	Chantale Bernatchez cbernatchez@mdanderson.org		
	Gheath Al-Atrash galatras@mdanderson.org		
CIMAC 2-- Icahn School of Medicine at Mount Sinai	Sacha Gnjjatic Sacha.gnjatic@mssm.edu	Diane Del Valle diane.delvalle@mssm.edu 212-824-9624 Jose Lacunza jose.lacunza@mssm.edu 212-824-9344	Hess Center for Science and Medicine 5th floor, rooms 310/313 Human Immune Monitoring Center (HIMC) Icahn School of Medicine at Mount Sinai 1470 Madison Avenue New York, NY 10029
	Adeeb Rahman Adeeb.rahman@mssm.edu		
	Seunghee Kim-Schulze Seunghee.kim-schulze@mssm.edu		
	Gerold Bongers Gerold.bongers@mssm.edu		
CIMAC 3-- Dana-Farber Cancer Institute	Catherine Wu cwu@partners.org	Mariano Severgnini Mariano_Severgnini@dfci.harvard.edu Srin Ranasinghe Srinika_Ranasinghe@dfci.harvard.edu	Dana-Farber Cancer Institute 450 Brookline Ave, Mayer Building Room 305 Boston, MA 02215 Tel: 617-632-2421
	Stephen Hodi Stephen_Hodi@dfci.harvard.edu		
CIMAC 4-- Stanford University	Holden Maecker maecker@stanford.edu	Bita Sahaf bsahaf@stanford.edu Mina Pichavant minapich@stanford.edu	Attn to : Caroline Duault Fairchild Science Building, room D033 (location 07-520, basement) 299 Campus Drive Stanford, CA, 94305- 5124 Phone: (650) 725-0229
	Sean Bendall bendall@stanford.edu		
CIDC--Dana- Farber Cancer Institute	Xiaole Shirley Liu xsliu@jimmy.harvard.edu	Joyce Hong jhong@jimmy.harvard.edu	Liu Lab Center for Life Science Building 3 Blackfan Circle, 11th Floor Boston, MA 02115
	Ethan Cerami cerami@jimmy.harvard.edu		

5 CIMAC ASSAYS

Category	CIMAC Assays	Specimen Types
Genomics Tier 1	WES	Flash-Frozen Tissue (preferred) FFPE tumor tissue Bone Marrow Aspirate CSF EDTA Whole Blood EDTA Whole Blood (germline)
	RNA-Seq	
	Nanostring	
Genomics Tier 2	TCR-Seq	Flash-frozen / FFPE PBMCs/ EDTA whole blood
	Single-Cell Sequencing	Flash-Frozen Tissue Bone Marrow / CSF
	ATAC-Seq	Heparin PBMCs
Imaging Tier 1	IHC Singleplex	FFPE Bone Marrow
	IF Singleplex (PD-L1 FDA approved assay)	
	IHC/IF Multiplex	
Imaging Tier 2	MIBI	
Proteomics/Seromics Tier 1	CyTOF OR CyTOF Smart-Tube	Heparin PBMCs; OR Heparin whole blood in Smart Tubes
	Olink	Heparin Plasma
Proteomics/Seromics Tier 2	ELISA/Grand Serology	
Microbiome Tier 2	16S rRNA Amplicon Seq or Whole Genome Shotgun Seq	Cryopreserved stool (Cold Chain Collection) And DNA Stabilized Stool Aliquots (OMNIgene GUT)
	Metabolomics	
	Microbe Characterization	
Liquid Biopsy Tier 2	ctDNA	Streck Tube Plasma

APPENDICES

Appendix I. Pre-Analytic Information

Pre-Analytic Information

Collection site must record all preanalytical information and enter the following into a specimen tracking system (STS) used by each trial network or record and provide with shipping manifest:

1. Time/date blood or tissue sample collection was made as ***Time/Date Specimen Collected***.
2. Ischemia start time (time when sample was devascularized OR estimated time of surgery)—***Tissue Collection Time/Date***.
3. Ischemic end time ***for each tissue core and surgical segment*** (time when sample was moved to preservative such as formalin or dry ice)—***Tissue Processing (Formalin Start) Time/Date***.
4. Completion of formalin fixation should be recorded as ***Formalin End Time/Date*** in the STS (or under “comments” if field is not available).
5. Start of 70% Ethanol dehydration should be recorded as ***Ethanol Start Time/Date*** in STS (or under “comments” if field is not available)
6. Time when fixed tissue, held in Ethanol, was placed into an automated processor should be recorded as ***Ethanol End Time/Date*** in the STS (or under “comments” if field is not available).
7. Core # for each core needle biopsy obtained. Each core should be recorded in the STS as a separate specimen with a unique Specimen ID that captures the chronological order in which the biopsy cores were obtained.
8. Segment # for each surgical resection. These can be hand-labeled on the sample and ***captured electronically as separate specimens in the STS***.

Biorepository will collect the following information for received specimens:

1. Date of sample receipt.
2. Time/date formalin-fixed tissue in Ethanol is moved into an automated processor—***recorded as Ethanol End Time***.
3. Record if frozen tissue sample arrived with insufficient amount of dry ice.
4. Collected pre-analytic information will be entered into the shipping manifest (NCI specimen tracking system).

Appendix II. Processing and Paraffin Embedding of Tissue at Collection Sites and Biorepository

Core Needle Biopsy (including bone marrow biopsy), Small Biopsy, and Surgical Resection Samples

- Tissue **must be fixed** in neutral-buffered formalin (no acid-based products).
- **For collection sites shipping samples in Ethanol**, formalin fixed tissue will be transferred to 70% ethanol at room temperature for **up to 72** hours before processing (Steps 3 to 13, Table 1) is completed at the Biorepository.
- The tissue will be processed on an **automated tissue processor** following Steps 3 to 12 **as suggested** in Table 1 so long **as total time from ethanol to embedding (in gray) exceeds 4 hours**.
- Do **not** use a microwave processor.
- The tissue will be embedded in paraffin (Step 13, Table 1).

Table 1. Main stages of tissue processing. Steps 3-12 performed in an automated tissue-processor (no microwave processors).

Step/Process	Solution	Time
1. Fixation	10% buffered formalin	12-24 hours
2. Dehydration	70% Ethanol	30 minutes or up to 72 hours
3. Dehydration	95% Ethanol	30 minutes
4. Dehydration	95% Ethanol	30 minutes
5. Dehydration	100% Ethanol	30 minutes
6. Dehydration	100% Ethanol	30 minutes
7. Dehydration	100% Ethanol	30 minutes
8. Clearing	Xylene	30 minutes
9. Clearing	Xylene	30 minutes
10. Infiltration	Paraffin Wax	30 minutes
11. Infiltration	Paraffin Wax	30 minutes
12. Infiltration	Paraffin Wax	30 minutes
13. Blocking Out	Paraffin Wax	n/a

Appendix III. DNA/RNA Extraction from Tissue and Blood Samples

NOTE: For genomics assays, Biorepositories will perform DNA and RNA co-isolation using the following kits, for other samples and assay types please refer to table listings.

Tumor Tissue

- For Frozen Tissue: AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).
- For FFPE: AllPrep DNA/RNA FFPE Kit (QIAGEN) plus High Pure (Roche).

DNA/RNA from Bone Marrow Aspirate Mononuclear Cells

- AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).

DNA/RNA from CSF Cell Fraction

- AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).

DNA/RNA from Bone Marrow Biopsy Frozen Cores

- AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).

DNA/RNA from Heparin PBMCs for CyTOF or TCR-Seq (Section 4.2.2)

- AllPrep DNA/RNA Kit (QIAGEN) plus MirVana Kit (Applied Biosystems).

DNA from EDTA Whole Blood for TCR-Seq (Section 4.2.4)

- QIAGEN QIAamp DNA Blood Mini/Midi Kit
- QIAGEN Symphony (performed at Adaptive for a fee)

Germline from EDTA Whole Blood (Section 4.2.4)

- QIAGEN QIAamp DNA Blood Mini/Midi Kit

Specimen Type	Processing Step	Processing Protocol	Processing Time Requirement	Assay	Analyte Required	Extraction Kit	
K2-EDTA Blood	2 mL of whole blood	Centrifugation	Within 48 hours of collection	WES; germline for solid tumors	Genomic DNA	QIAGEN QIAamp DNA Blood Mini/Midi*	
	5-10 mL whole blood; End-of-Induction therapy (1 mo)			WES; germline for heme malignancies	Genomic DNA		
	2 mL of whole Blood			TCR-Seq (Adaptive kit)	Biobank DNA extraction Adaptive DNA extraction		QIAGEN Symphony
				Peds-MATCH germline	Genomic DNA		QIAGEN QIAamp DNA Blood
Sodium Heparin Blood	1 mL Plasma	Ficoll Prep*	Within 48 hours of collection	Olink/ELISA Grand Serology	NA	NA	
	5x10 ⁶ PBMCs/vial			CyTOF	DNA/RNA Co-extraction	AllPrep + MirVana*	
				TCR-Seq			
Streck Blood	Plasma	Centrifugation	Flexible	TBD	cfDNA/ctDNA	TBD	
Tissue (Resection, CNB, Punch/Endoscopic)	FFPE	Automated Processor	Fixation 12-24 hours	mIF, IHC, MIBI (or Genomics)	Slides or DNA/RNA	AllPrep + Roche High Pure	
	Flash Frozen	LN or Dry Ice	Immediate	WES/RNA-Seq/TCR-Seq	DNA/RNA coextraction	AllPrep + MirVana*	
Bone Marrow Biopsy	Decalcified: FFPE	Standard Embedding	Fixation 12-24 hours	Imaging	NA	NA	
	Flash Frozen	LN or Dry Ice	Immediate	WES/RNA-Seq/TCR-Seq	DNA/RNA co-extraction	AllPrep + MirVana*	
Bone Marrow Aspirate (K2-EDTA Tube)	Plasma	Ficoll Prep*	Within 48 hours of collection	Olink/ELISA Grand Serology	NA	NA	
	Mononuclear			CyTOF/WES/RNA-Seq/TCR-Seq	DNA/RNA co-extraction	AllPrep + MirVana*	
CSF (K2-EDTA Tubes)	Supernatant	Centrifugation	Within 48 hours of collection	Olink/CyTOF ⁵	NA	NA	
	Cell Pellet			TCR-Seq/RNA-Seq ⁵	DNA/RNA co-extraction	AllPrep + MirVana*	
Stool Samples	DNA stabilization	2 mL aliquots (frozen)	Return to site (24 hrs) and ship to bank within 72 hrs	16S rRNA seq/ metabolomics / microbe characterization	DNA extraction at CIMAC	Custom extraction	
	Frozen at -80°C	Frozen					

* shared protocols

Appendix IV. Processing of Green-Top Tubes: Isolation of Plasma and PBMC

NOTE: The following protocols are given as examples; equivalent SOPs may be followed according to Biorepository practices.

Equipment

- Benchtop centrifuge (Allegra X-15R, Beckman Coulter) or equivalent
- Tali Image Based Cytometer (Invitrogen) or equivalent
- Pipette Gun (Drummond) or equivalent
- p200, p1000 micropipettes (Rainin) or equivalent

Materials

- Sodium Heparin Green-Top Tube (Fisher, # 367874)
- 1.8 mL Cryotube vials (Fisher, #375418)
- Micropipette tips
 - Sterile, filtered, p200 micropipette tips
 - Sterile, filtered, p1000 micropipette tips
- 50 mL conical tube (Fisher, #352070)
- Tali Cellular Analysis Slide (Invitrogen, #110794) or equivalent
- CoolCell (Fisher, #NC9883130 or Biocision Inc., BCS-405) and CoolBox or equivalent
- 2 mL, 5 mL, 10 mL, 25 mL, and 50 mL sterile serological pipettes (Fisher, #356507, #356543, #356551, #356525, #356550, respectively)

Reagents

- Ficoll-[®]-Paque (GE Healthcare, 17144003)
- SepMate™ (StemCell Technologies, 85450)
- PBS (without Ca²⁺, Mg²⁺) (Invitrogen, 10010-049)
- Recovery™ Cell Culture Freezing Medium (Invitrogen Cat# 12648-010)

PLASMA Isolation

1. Whole blood samples will be received by the laboratory collected in Sodium Heparin Vacutainer® Green-Top Tubes.
2. **Note the condition of the samples upon receipt.** Observations may include labeling errors, obvious clotting, degree of hemolysis, low blood volume, leakage or breakage, etc.
3. Sodium Heparin tubes will not be processed if any of the following conditions exist:
 - a. Samples which cannot be identified.
 - b. Clotted or excessively hemolyzed (dark red/mahogany-colored plasma) samples.
 - c. .
4. In a 50 mL conical tube, **pool all blood samples from each case** (30 mL total volume) and measure the volume of heparinized whole blood and record it (in mL).
5. Pre-chill at 2-8°C or on ice cryo-vials that have been pre-labeled with pertinent patient and sample information.
6. Load the blood samples in the centrifuge such that the load is properly balanced. Tubes of the same type and size should be compared and balanced according to fill volumes. If an odd number of tubes will be centrifuged, “balance tubes” containing water must be used.

7. Centrifuge the samples at 250 xg for 6 minutes at 18-20°C with acceleration set at 9 and the brake turned off.
8. Following centrifugation, carefully transfer all upper (plasma) phase to a fresh conical tube making sure you do not disturb the lower phase. Mix the plasma-containing tube with a pipette. **Save the lower part which will be used for the PBMC isolation.**
9. Centrifuge the plasma samples at 400 xg for 10 minutes at 18-20°C with acceleration set at 9 and the brake turned off, to remove platelets.
10. Label sample cryovials with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
11. Transfer all plasma to a fresh conical tube. Aliquot the plasma (~**12 x 1 mL**) into pre-labeled cryovials. The exact aliquot volume may be adjusted based on requirements of the specific study.
12. Cryovials containing plasma will be stored at -80°C until used, transferred, or shipped.
13. Record the storage location on the corresponding worksheet/database.

PBMC Isolation Using Ficoll-Paque

1. Dilute blood 1:1 with PBS (without Ca²⁺, Mg²⁺). (Blood amount should not exceed 25 mL per tube.)
2. Take 2 new 50 mL conical tubes and add 12 mL Ficoll-Paque (Cat# 17144003; GE Healthcare) per tube.
3. Slowly and gently layer the diluted blood on top of the Ficoll-Paque of the tube with a maximum volume of 35 mL. Minimize blood entering into the Ficoll layer and avoid air bubbles
4. Centrifuge the tube at 500 xg for 20 min at room temperature with slow acceleration (#7) and deceleration (#7) (Sorvall Legend XTR centrifuge).
5. A white ring of PBMC will be observed between the upper layer (diluted plasma) and middle layer (Ficoll-Paque). The lower layer is composed of pelleted red blood cells. Discard the upper layer (diluted plasma) carefully using a pipette. Remove the PBMC layer from the tube and transfer into a 50 mL conical tube. Do not transfer the red blood cell pellet.
6. Completely fill conical tube containing isolated PBMC with PBS, mixing well by inverting capped tube 2-3 times.
7. Centrifuge the PBMCs at 250 xg for 10 minutes.
8. Aspirate the supernatant and resuspend the cells in 48 mL of PBS.
9. Count the cells using the Tali Counter (or lab's preferred cell counting method) and record viable cell count and total count.
10. Label sample cryovials to be cryopreserved with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
11. Prechill labeled cryovials for at least 10 minutes on wet ice.
12. Centrifuge the conical vial at 250 xg for 10 minutes. Based off the viable cell count, calculate the number of vials and volume of freezing medium that will be needed
 - a. PBMCs should be aliquoted into each cryovial at **~5x10⁶ (5 million) cells per mL**. The total mL amount of freezing media needed is equal to the total number of aliquots needed.
13. Aspirate the supernatant and discard.
14. Resuspend the cells in Recovery Cell Freezing Medium.
15. Quickly aliquot **1 mL of cell suspension in each prelabeled cryovial** with CIMAC label and biorepository label. on ice (delay in this step reduces viability).
16. Place the cryovials into a CoolCell (or equivalent container) and into a -80°C freezer for 2 hours or overnight (alternatively a Mr. Frosty or controlled rate freezer can be used).
17. Following this, immediately put the PBMCs cryovials into liquid nitrogen for long term storage.

Appendix V. Processing of Streck Cell-Free DNA Tube: Isolation of Plasma

NOTE: *The following protocols are given as examples; equivalent SOPs may be followed according to Biorepository practices.*

Plasma Isolation

1. Blood samples will be received by the laboratory collected in Streck Cell-Free DNA Tubes.
2. **Note the condition of the samples upon receipt.** Observations may include labeling errors, obvious clotting, degree of hemolysis, low blood volume, leakage or breakage, etc.
3. Streck tubes will not be processed if any of the following conditions exist:
 - a. Samples which cannot be identified.
 - b. Streck tube samples that have been refrigerated.
 - c. Clotted or excessively hemolyzed (dark red/mahogany-colored plasma) samples.
4. Load specimen tubes in the centrifuge. Ensure the centrifuge is balanced. Use an appropriate counterbalance if needed (i.e., Streck tube filled with water--Streck catalog # 218961, 218962, or 2189921).
5. Centrifuge the samples at 250 xg for 6 minutes at 18-20°C with acceleration set at 9 and the brake position off.
6. After centrifugation transfer the top layer (plasma) into a 15mL clean conical tube.
7. Centrifuge the plasma samples collected in step 3 at 400 xg for 10 minutes at 18-20°C with acceleration set at 9 and the brake turned off.
8. Label sample cryovials to be cryopreserved with two thermostable labels: a CIMAC network label and a label typically used by the Biorepository.
9. After second centrifugation aliquot **1 ml of plasma** cell-free DNA (cfDNA) into each pre-labeled cryovial. Don't pipet the pellet built on the bottom of 15 ml tube, instead discard it all together after plasma collection. The exact aliquot volume may be adjusted based on requirements of the specific study.
10. Store all plasma cfDNA samples at -80°C.

Appendix VI. Quality Control of Tissue Specimens

Histology/Cytology examination: H&E-stained sections from CNBs, surgical resections and archival tissues will be used to confirm the presence of tumor cells, as well as their relative abundance (tumor cellularity), and the composition of the tumor associated stroma and lymphocytic infiltrates.

The following pathological analysis may be performed by the Biorepository if requested:

1. Use the pathology/clinical report provided with each sample timepoint to confirm the tumor diagnosis concordance and record classification using established Biorepository practices.
2. Score the percentage of viable tumor cells comprising the tumor bed area **to select the most suitable material for nucleic acid extraction using established practices at the Biorepository.** Record this information on the provided shipping manifest.

The following pathological analysis will be performed by CIMACs and reported to CIDC as part of assay data:

1. Score the percentage of tumor (including tumor bed) tissue area of the slide (e.g. vs non-malignant or normal tissue); and
2. Score the percentage of viable tumor cells comprising the tumor bed area; and
3. Score the evaluation of stromal elements (this indicates the % area of tumor bed occupied by non-tumor cells, including inflammatory cells [lymphocytes, histiocytes, etc], endothelial cells, fibroblasts, etc); and
4. Score the percentage area of necrosis; and
5. Score the percentage area of fibrosis.
6. If microdissection is performed, percentages for items 2 through 5 should add up to 100%

Table 8. Quality Control Metadata for Tissue Samples

Diagnosis	Tumor Tissue (% total area)	Viable Tumor (% area)	Viable Stroma (% area)	Necrosis (% area)	Fibrosis (% area)

Highlighted columns each reflect percent area of viable and damaged tumor bed that should add up to 100%.

[Include in Protocol Appendix]

A copy of the diagnostic pathology report must be shipped with all tissue specimens sent to the ETCTN Biorepository.

If the *corresponding* pathology report is not available for the biopsy, then a copy of the radiology report or operative report from the biopsy procedure and the diagnostic pathology report must be sent to the ETCTN Biorepository. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the ETCTN Biorepository.

Note: If this information is not provided with the biopsy specimen, then it will not be accepted by the ETCTN Biorepository.

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): **Primary** **Metastatic**

Time point (circle one): **Timepoint1** **TimePoint2** **Timepoint3**

Site Tissue Taken From: _____

Diagnosis: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

Clinician Signature

Date

Clinician Printed Name

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

Appendix VIII. Checklist of Minimal CIMAC Biomarker and Specimen Collection Details Required for Trial Activation

Determine if dose escalation phase requires collection of specimens for CIMAC testing:

- Can trial be activated if samples from initial phase are not assayed by CIMAC.
- Specify which specimens are collected for each timepoint:
- Some cores may need to be earmarked for integral assays.
- Indicate desired number of tissue cores in formalin.
- Indicate desired number of tissue cores snap-frozen.
- Indicate blood, aliquot size volume and number for each tube type.
- Indicate if archival blocks, slides, punches, or scrolls are requested AND confirm quantity, minimal tumor % (genomics only), section thickness, and volume for each assay.
- Confirm that clear distinction is made between archival tissue, fresh-frozen tissue, and fresh tumor biopsy core (FFPE) for each timepoint.
- Confirm agreement between Biomarker Table and Specimen Summary Table.
- Include Pathology Verification Form in protocol appendix if appropriate.

Specify sample processing details at collection site:

- Indicate if special ischemic times are required for tissue excision and fixation.
- Specify what preanalytical data is to be collected.
- Select how tissue is processed on-site (FFPE embedding on-site OR ship cores in ethanol).
If on-site embedding is selected include a table of steps for tissue auto-processor.
- Indicate processing time requirements for tissue (12-24 hours in formalin, <72 hours in Ethanol) and blood (no more than 48 hours for blood).
- Include “external sample label” language in shipping section to alert biorepository to prioritize processing *for tissue and blood samples*.
“An external sample label should be fixed to the shipping container to alert the Biorepository of Formalin-fixed sample time and date it was placed into Ethanol (this helps to identify and prioritize received samples that have processing time requirements)”
- Confirm specimens are shipped to their intended biorepository.

Specify sample processing details at Biorepository:

- Specify in the Biorepository Section that “additional sample processing and sample request details will be provided at a later time”.
- Number and size of blood aliquots, number and priority of tissue cores (FFPE and flash-frozen), and number of slides should be derived from the Intake Form.
- Processing of specialized material (stool, bone marrow aspirates etc.) should be requested on trial-by-trial basis.