Manual of Procedures
Developmental and HyperActive Ras Tumor (DHART)
Specialized Program of Research Excellence (SPORE)

Biospecimen Collection, Processing, and Shipment Manual

dhartspore.org

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1.0 ABBREVIATIONS

CPC	Circulating Progenitor Cell
CPT	Cell Preparation Tube
DHART SPORE	Developmental and HyperActive Ras Tumor Specialized Program of Research Excellence
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
MNC	Mononuclear Cell
PBMC	Peripheral Blood Mononuclear Cell
PFC	Polychromatic Flow Cytometry
PN	Plexiform Neurofibroma
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

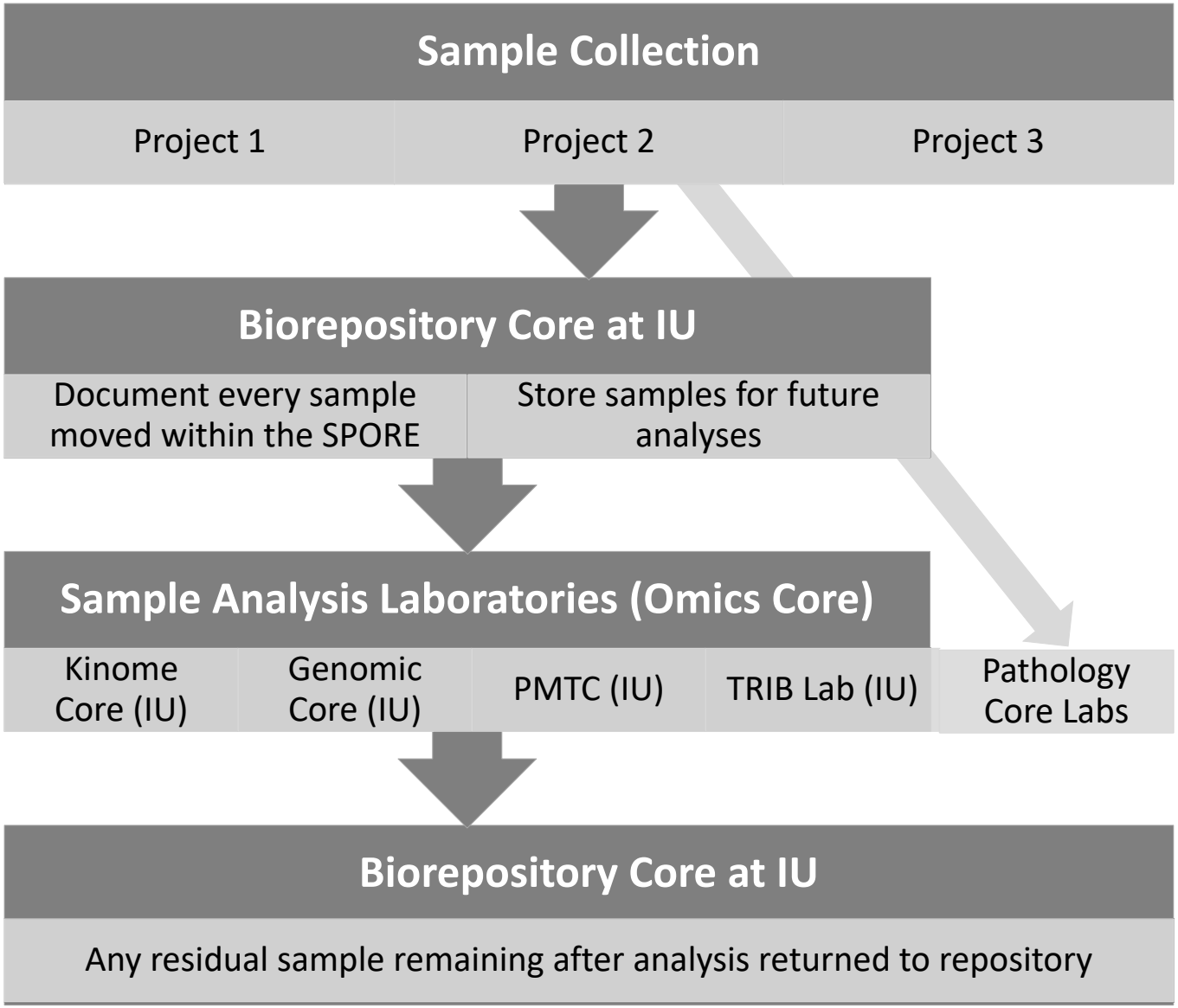
2.0 PURPOSE

The purpose of this manual is to provide DHART SPORE contributing researchers (PIs, study coordinators, and the sample collection and processing teams) with guidelines for collection and submission of biological samples to the DHART SPORE Cores, where specimens will be analyzed and catalogued for biobanking. This manual includes detailed instructions for shipping biospecimens to the Omics, CPAC, TRIB, PMTC, and Biorepository Cores at Indiana University and the Path Core at University of California, San Francisco. This manual includes the instructions for submitting the following samples to the DHART SPORE Biorepository:

- Solid human tissue
- Plasma/Serum
- CSF
- Cell pellets or tissue for kinome analysis
- Mononuclear cells
- Animal tissue

This manual includes guidelines for snap freezing tissue, uniform collection and fractionation of human blood, aliquoting, labeling, storage prior to shipping, and shipping to the DHART SPORE Cores.

3.0 DHART SPORE Sample Flow Overview



4.0 BIOSPECIMEN/PATHOLOGY CORE

The Biospecimen/Pathology Core will be to serve as the central site to track and store samples and pathological data from the human and mouse biospecimens collected as part of the four SPORE projects. This core will perform pathological review of all samples and will make these data available to SPORE investigators and the broader research community. In addition, the Biospecimen/Pathology Core will maintain an electronic catalog which will link the specimens and tissue to the pathological reports as well as data generated in the projects for each study subject. The project investigators will use this electronic catalog to select the samples needed for analyses. The Biospecimen/Pathology Core includes the Biorepository/Indiana University Genetics Biobank (IUGB), CPAC, TRIB Lab, Omics, and PMTC at Indiana University, as well as the Path Core at University of California, San Francisco.

4.1 BIOREPOSITORY CORE (IUGB) AT INDIANA UNIVERSITY

4.1.1 Contacts

http://DHARTSPORE.org
dhartbio@iu.edu

Tatiana Foroud, PhD, Biorepository Core Leader
Phone: 317-274-2218
Email: tforoud@iu.edu

Claire Wegel, MPH, CCRP, Project Manager
Phone: 317-278-6158
Email: cwegel@iu.edu

Sample Receiving Address
DHART SPORE Biorepository
IU School of Medicine
351 W. 10th Street, TK-217
Indianapolis, IN 46202

Heather Daniel, Administrative Core Contact
Phone: 317-278-9290
Email: hdaniel@iu.edu

4.1.2 Hours of Operation

Biorepository Core business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of both ambient and frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.1.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with DHART SPORE Core staff.**

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd.
Please see: https://DHART SPORE.org/holiday_closures.html for additional information.

4.2 CPAC CORE AT INDIANA UNIVERSITY

4.2.1 Contacts

Andi Masters, M.S.
Core Director
Office: 950 W. Walnut Room E466
Phone: (317) 274-3053
argrove@iu.edu

Zeruesenay Desta, PhD
Scientific Director
Phone: (317)-274-2823
zdesta@iu.edu

Sara Quinney, PharmD, PhD
Associate Scientific Director
Phone: (317)-274-2823
squinney@iu.edu

Christine Bach, B.A.
Analyst
Office: 950 W. Walnut Room E451
Phone: (317) 274-3053
bachc@iu.edu

Xiaomei Zheng, M.S.
Analyst
Office: 950 W. Walnut Room E409
Phone: (317) 274-2768
zhengx@iupui.edu

Sample Receiving Address
Samples for the CPAC Core at Indiana University will be accessioned by the Biorepository Core for tracking purposes before being transferred to the CPAC Core at Indiana University for analysis. Please email the completed manifest to dhartbio@iu.edu prior to shipping samples.

DHART SPORE
Indiana University School of Medicine
351 W. 10th St., TK-217
Indianapolis, IN 46202

4.2.2 Hours of Operation

CPAC Core business hours are from 7 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.3 PRECLINICAL MODELING AND THERAPEUTICS CORE (PMTC) AT INDIANA UNIVERSITY

4.3.1 Contacts

Karen Pollock, PhD, PMTC Director
Phone: 317-274-8891
Email: kpollok@iu.edu

Emily Sims, Core Manager
Phone: 317-278-7232
Email: ecwillar@iupui.edu

Matthew Repass
Email: mjrepass@iupui.edu

Sample Receiving Address
Preclinical Modeling and Therapeutics Core (PMTC)
Attn: Emily Sims
980 W. Walnut St., R3-343
Indianapolis, IN 46202

4.3.2 Hours of Operation

PMTC business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. For packing and shipment details of ambient samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.4 PATH CORE AT UNIVERSITY OF CALIFORNIA SAN FRANCISCO

4.4.1 Contacts

Andrew Horvai, MD, PhD, Path Core Director
Phone: 415-885-7313
Email: Andrew.Horvai@ucsf.edu

Melike Pekmezci, MD, Pathology Core Director
Phone: 415 502 4250
Email: melike.pekmezci@ucsf.edu

Sample Receiving Address
If shipping physical samples for the Path Core at University of California San Francisco (UCSF), they should be sent directly to the Path Core at the following address below. Please notify dhartbio@iu.edu of the shipment for tracking purposes.

UCSF Medical Center Mission Bay
Attn: Dr. Andrew Horvai
1825 4th St., Room M2354
San Francisco, CA 94158

Digitial slides
Digital slides in Aperio .svs format may be uploaded to the DHART SPORE Synapse cloud server, All SPORE Data folder:
https://www.synapse.org/#!Synapse:syn10659741

4.4.2 Hours of Operation

Pathology Core business hours are from 8 AM to 5 PM Pacific Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. For packing and shipment details of ambient samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.5 CHILDREN'S CLINICAL RESEARCH CENTER (CCRC), TRANSLATIONAL RESEARCH AND INTEGRATED BIOLOGY (TRIB) LAB OF JAMIE RENBARGER, MD, MS AT INDIANA UNIVERSITY

ANALYSIS TO BE PERFORMED: CYTOKINE ANALYSIS

4.5.1 Contacts

Khadijeh (Sholeh) Bijangi-Vishehsaraei, PhD, MS
Associate Research Professor and Scientific Coordinator of CCRC,
Renbarger Laboratory
Lab Phone: (317) 278-3050
Email: khbijang@iupui.edu

Brian Ashmore, TRIB lab Technician
Phone: 317-278-3050
Email: bashmore@iupui.edu

Sample Receiving Address
Samples for the TRIB Laboratory at Indiana University will be accessioned by the Biorepository Core for tracking purposes before being transferred to the TRIB Laboratory at Indiana University for analysis. Please email the completed manifest to dhartbio@iu.edu prior to shipping samples.

DHART SPORE
Indiana University School of Medicine
351 W. 10th St., TK-217
Indianapolis, IN 46202

4.5.2 Hours of Operation

Lab business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.
Frozen samples must be shipped Monday through Thursday only.
Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

5.0 OMICS CORE AT INDIANA UNIVERSITY

The Center for Medical Genomics (CMG) and the Kinome Core (Angus LAB) at Indiana University, referred to collectively as the Omics Core for DHART SPORE purposes, will collaborate with tissue processing and nucleic acid extraction cores/facilities to accept extracted and purified DNA and RNA samples, to QC those DNA and RNA samples, and to prepare NGS libraries for sequencing. Specimens to be analyzed by the Omics Core will be shipped to the Biorepository Core. Samples for kinome profiling must be flash-frozen and may be tissue or frozen cell pellets, ready for lysis and protein extraction. The Biorepository Core is responsible for receipt of the samples, assigning specific tracking information, relabeling the samples with that information and inputting the samples into an electronic database and Lab Information Management System (LIMS). Sample tracking information will be made available to Sage (Synapse). Relabeled and trackable samples are then transferred to the Omics Core facility where they are processed according to the directives of the specific project. CMG extracts nucleic acids, QCs those RNAs or DNAs, and makes recommendations on the downstream library preparation process that will maximize the potential success of the library preparation and ultimate sequencing. For large sample numbers, CMG can use the Beckman Coulter Biomek FXP automation system to process library preparation. Sequencing may be performed using Illumina MiSeq Dx, NextSeq500, HiSeq4000, or NovaSeq6000 in CMG. CMG is also capable of performing bioinformatics for data mapping QC, alignment, variant call, gene expression profile, as well as downstream analysis, if needed. Kinome profiling will include protein extraction from cell pellets or tissue, affinity purification of the functional kinome over multiplexed kinase inhibitor beads (MIBs), LC/MS/MS analysis, data analysis and data sharing with the project members and upload to Sage of raw and processed data. All of these facets and experience will be employed to track and process DHART SPORE samples and, to whatever degree is requested, to analyze subsequent data.

5.1 KINOME CORE (ANGUS LAB) AT INDIANA UNIVERSITY

Snap-frozen cell pellets or tissue samples submitted for kinome profiling should be sent to the Biorepository Core on dry ice (refer to Section 4.0 above for shipping address and business hours). This process will ensure that residual tissue following kinome profiling (or sequencing, or both) may be easily tracked and stored. In cases of limited tissue amount, the investigator should indicate prioritization of analyses. Please feel free to contact the Omics Core prior to submission with any questions.

5.1.1 Contacts

Steven Angus, PhD, Kinome Core Director
Phone: 919.619.7312 cell, office number: 317-274-8911
Email: sangus@iu.edu

Please contact Steve with any clarifications, specific requests, or questions prior to sample submission.

Kinome Sample Receiving Address (Frozen samples on dry ice)
Frozen samples for the Kinome Core at Indiana University will be accessioned by the Biorepository Core for tracking purposes before being transferred to the Kinome Core at Indiana University for analysis. Please email the completed manifest to dhartbio@iu.edu and copy to Steve Angus (sangus@iu.edu) prior to shipping samples. Samples should be shipped on ample dry ice to the Biorepository Core and according to the shipping instructions in section 4.1. Kinome samples will always be shipped on dry ice and therefore shipped must be shipped *Monday through Wednesday only*. For packing and shipment details, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

Sample Receiving Address
DHART SPORE
Indiana University School of Medicine
351 W. 10th St., TK-217
Indianapolis, IN 46202

5.1.2 Hours of Operation

Kinome Core Business hours: from 8am to 5pm Eastern. Monday through Friday.

5.2 GENOMIC CORE AT INDIANA UNIVERSITY

5.2.1 Contacts

Yunlong Liu, Ph.D, CMG Director
Phone: 317-278-9222
Email: yunliu@iu.edu

Xiaoling Xuei, Ph.D, CMG Technical Director
Phone: 317-278-5201
Email: xxuei@iu.edu

Maks Luthra, MPH, CMG Program Manager
Phone: 317-278-9186
Email: maluthra@iu.edu

Center for Medical Genomics
Phone: 317-278-9744

Sample Receiving Address
Frozen samples for the Genomic Core at Indiana University will be accessioned by the Biorepository Core for tracking purposes before being transferred to the Genomic Core at Indiana University for analysis. Please email the completed manifest to dhartbio@iu.edu prior to shipping samples.

Sample Receiving Address
DHART SPORE
Indiana University School of Medicine
351 W. 10th St., TK-217
Indianapolis, IN 46202

5.2.2 Hours of Operation

GMG Business hours: from 7:30am to 5pm. Monday through Friday.

Frozen samples must be shipped Monday through Wednesday only. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

6.0 SPECIMEN COLLECTION AND PROCESSING PROCEDURES

Consistency in sample collection and processing is essential for biomarker studies. All samples are drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

6.1 Blood and Tissue Samples for the CPAC Core at IU

- CPAC Protocol for Solid Tissue Collection and Storage **(Appendix A)**
- CPAC Protocol for Plasma Collection and Storage **(Appendix B)**

6.2 Blood for the PMTC at IU

- Blood Collection for Polychromatic Flow Cytometry & Identification of Circulating Progenitor Cells **(Appendix C)**

6.3 Unstained Slides for the Path Core at UCSF

- **Appendix D**

6.4 Blood for the TRIB Laboratory at IU

- **Appendix I**

6.5 Samples for the Kinome Core at IU

- Submitting Frozen Tissue or Cell Pellets for MIB (Kinome) Analysis **(Appendix E)**
- Instructions for Preparing and Submitting Samples for the DHART-SPORE Project to the Omics Core at IU **(Appendix G)**

6.6 Samples for the Genomic Core at IU

- Instructions for DNA and RNA sample shipment to the Genomic Core at IU **(Appendix F)**
- Instructions for Preparing and Submitting Samples for the DHART-SPORE Project to the Omics Core at IU **(Appendix G)**
- Omics Core Preferred Volumes **(Appendix H)**

7.0 PACKAGING AND SHIPPING INSTRUCTIONS

There are strict Federal guidelines for the shipment of human specimens. If you are unsure of the requirements, please visit DHARTSPORE.org to watch the shipment training videos. If you do not have the necessary supplies (such as UN3373 labeling), please contact dhartbio@iu.edu.

7.1 Ambient Shipping Instructions

- **IMPORTANT!** Ambient samples ***must*** be shipped ***Monday through Thursday*** using priority overnight service.
- Please do NOT draw blood for ambient shipments on Fridays.
- Notify the recipient Core and the Biorepository Core of all shipments.

Ambient whole blood tube and tissue shipments are Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest edition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B" UN3373

7.2 Frozen Shipping Instructions

- **IMPORTANT!** Frozen samples ***must*** be shipped ***Monday through Wednesday*** using priority overnight service.
- Please be aware of holidays and inclement weather, and plan your shipments accordingly.
- Notify the recipient Core and the Biorepository Core of all shipments.

Specimens being shipped to the DHART SPORE Biorepository are Category B UN3373 specimens and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest eEdition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material must be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

Appendix A: CPAC Protocol for Solid Tissue Collection and Storage

1. Extract tissue from animal.
2. If highly perfused, flush the tissue with saline.
3. Flash freeze in liquid nitrogen.
4. Label a polypropylene tube (screw cap cryogenic vial) with date of tissue collection and animal ID.
5. Place frozen tissue sample in the screw cap cryogenic vial and store in a -80 freezer.
6. Complete the CPAC Submission e-Forms:

   Note: there are two forms that needs to be completed

   Form #1 : https://dhartspore.org/manual_and_manifests.html

   Form #2: https://www.cancer.iu.edu/research-trials/facilities/cpac/documents/CPAC%20Project%20Authorization%20Form%2008101 8.pdf

7. Notify the CPAC Core (argrove@iu.edu) and Biorepository Core (dhartbio@iu.edu) of incoming specimen shipment.
8. Ship frozen samples on dry ice to the Biorepository Core at Indiana University with the accompanying sample manifest for cataloging purposes and distribution to the CPAC Core.

Notes: *Store tissue separately*.

Appendix B: CPAC Protocol for Plasma Collection and Storage

1. Collect blood sample in EDTA treated tubes.

2. Mix blood with anticoagulant in tubes by inverting 6-8 times.

3. Allow to set at room temperature for 15 minutes.

4. Centrifuge sample at 3500 rpm for 10 min.

5. Label a polypropylene tube (screw cap cryogenic vial) with date of blood collection and subject ID.
6. Draw off the plasma with a sterile pipette and transfer the plasma into the cryogenic vial.

7. Store plasma in -80 freezer within 2 hours of blood collection.

9. Complete the CPAC Submission e-Forms:

   Note: there are two forms that needs to be completed

   Form #1 : https://dhartspore.org/manual_and_manifests.html

   Form #2: https://www.cancer.iu.edu/research-trials/facilities/cpac/documents/CPAC%20Project%20Authorization%20Form%20081018.pdf

10. Notify the CPAC Core (argrove@iu.edu) and Biorepository Core (dhartbio@iu.edu) of incoming specimen shipment.

10. Ship frozen samples on dry ice to the Biorepository Core at Indiana University with the accompanying sample manifest for cataloging and distribution to the CPAC Core.

Appendix C: Blood Collection for Polychromatic Flow Cytometry (PFC) & Identification of Circulating Progenitor Cells (CPCs)

1. Two 8 ml sodium heparin CPT tubes and two 6 ml EDTA tubes will be required for this collection. Label each tube with **DATE**, **TIME OF COLLECTION**, and **PATIENT ID NUMBER**.

2. Collect a total of 16 mL of peripheral blood into two 8 ml sodium heparin CPT tubes. These tubes have a red/green closure.

3. Gently invert eight times to mix blood with anticoagulant.

4. **CRITICAL: The sample must be processed within 2 hours of blood collection.**

5. Centrifuge at room temperature for 30 minutes at 1500-1800 xg RCF. Please note, due to the height of these tubes, a swing-bucket rotor centrifuge should be used.

6. The centrifugation will separate the plasma and the mononuclear cell (MNC) fraction above the gel barrier. The red blood cells (RBCs) pass through the gel barrier to the bottom of the tube. To re-suspend the MNCs, **gently** invert the CPT tubes **one** time.

7. Uncap the two EDTA tubes.

8. Transfer the plasma/MNC cell suspension from each CPT tube into separate 6 ml EDTA tubes using a disposable transfer pipette. Do not combine the plasma from the two CPT tubes. Use care to prevent piercing the gel barrier and disturbing the RBC layer. Recap the EDTA tubes.

9. Complete the PMTC DHART SPORE Sample Submission e-Form (https://dhartspore.org/docs/angiomanifest.xlsx) and email a copy to Emily Sims (ecwillar@iupui.edu), Matthew Repass (mjrepass@iupui.edu) and dhartbio@iu.edu.

10. Ship the EDTA tubes according to the Ambient Shipping Instructions (Section 10.1 of this manual). Include a hard copy of the submission form in the shipment.

11. **CRITICAL: The MNCs are stable for 24 hours post collection after centrifugation and MUST be received within those 24 hours.**

Appendix D: Creating slides for the Path Core

1. From a representative paraffin block, cut 5-10 unstained sections (depending on amount of available tissue remaining in block, 10 is preferred), 4 micrometers thick, on charged (+) glass slides.

2. Label the slides with the date and patient ID.

3. Notify the pathology core (Andrew.horvai@ucsf.edu, melike.pekmezci@ucsf.eduscott.kogan@uscf.edu) Biorepository Core (dhartbio@iu.edu) of the inbound specimen, and also print out the email and enclose with the slides. Please include the following information in the email:
    a. Tumor site
    b. Gross findings
    c. Other comments relevant to the path analysis

4. Package the slides in a plastic slide carrier.

5. Ship Monday to Thursday, overnight express, ambient temperature, in a padded envelope or box with foam to:

    UCSF Medical Center Mission Bay
    Attn: Dr. Andrew Horvai
    1825 4th St., Room M2354
    San Francisco, CA 94158

6. For Apreio scanned .svs files, upload to the DHART SPORE Synapse cloud server All SPORE Data folder: https://www.synapse.org/#!Synapse:syn10659741

Appendix E: Submitting Frozen Tissue or Cell Pellets for MIB (Kinome) Analysis

For tissue samples:

1. Snap-freeze tissue from animal models or clinical samples in liquid nitrogen as soon as possible (ideally within 5 minutes as phosphatases act very quickly). See Appendix F for further detail on snap-freezing fresh tissue in liquid nitrogen.
2. Submit individual samples for analysis in separate, labeled cryovials, which may be stored in liquid nitrogen or -80 prior to shipping on dry ice to the Biorepository Core (IU) with an accompanying sample manifest for cataloging and re-distribution to the Kinome Core.

For adherent cell lines:

1. Wash adherent cell lines once with a plate volume of PBS.
2. Dislodge cells in small volume of PBS (e.g. 1 ml of PBS per 15-cm dish) using cell lifter.
3. Collect cells in a single 15ml conical tube for each replicate and condition.
4. Pellet cells in a tabletop centrifuge (usually 1500 rpm, 5 min).
5. Aspirate PBS supernatant and flash-freeze cell pellet in liquid nitrogen or dry-ice methanol bath.
6. Flash-frozen cell pellets may be stored at -80 prior to shipping.
7. Ship on dry ice to the Biorepository Core (IU) with an accompanying sample manifest for cataloging and re-distribution to the Kinome Core.

For suspension cell lines:

1. Pellet suspension cells, resuspend cells in PBS.
2. Pellet cells again.
3. Aspirate PBS supernatant and flash-freeze cell pellet in liquid nitrogen or dry-ice methanol bath.
4. Flash-frozen cell pellets may be stored at -80 prior to shipping.
5. Ship on dry ice to the Biorepository Core (IU) with an accompanying sample manifest for cataloging and re-distribution to the Kinome Core.

The goal is to have at 2 – 5 mg of total protein after extraction for each sample to be analyzed. If necessary, lower protein input amounts may be processed but fewer kinases will be identified and quantified. Clinical specimens submitted for kinome profiling will be handled with extreme care to maximize yield.

From tissue, we have found that we can expect to extract approximately 1 mg protein per 10-20mg tissue (wet weight). As a rough guide, PDX samples and other soft tissue samples approximately the size of pencil eraser are generally sufficient to yield 2 mg total protein for analyis. Provided by Dr. Angus's Laboratory, IU 2019.

Please contact Steve Angus (sangus@iu.edu) with any questions or considerations about submitting your samples for MIB/MS kinome profiling or inhibitor competition profiling.

For inhibitor binding competition, we recommend providing enough cells (as a frozen pellet) or tissue to yield at least 16mg total protein. This permits two biological replicates of vehicle and three different concentrations of drug, using 2mg protein for each MIB/MS sample.

Appendix F: Instructions for DNA and RNA sample shipment to the Genomic Core at IU

1. Purified DNA and RNA sample should be aliquoted to a 1.5ml microfuge tube. The proper microfuge tube should be freeze-tolerate, non-sticky (LoBind tube) and tight capped. Eppendorf LoBind Tubes are highly recommended (Eppendorf, Cat# 022431081).
2. Label all tubes properly with simple unique sample ID. Place tubes in a freeze-tolerate box or container and precool.
3. Put sample container in a large styrofoam box filled with excess amount of dry ice.
4. Seal and label shipping box according to FedEX requirement.
5. Complete the Genomic DHART SPORE Sample Submission e-Form
6. Notify the Genomic Core (xxuei@iu.edu) and the Biorepository Core (dhartbio@iu.edu) of the incoming sample shipment. Send a copy of the sample QC submission form with the email.
7. Ship samples on dry ice in dry shipper for next day delivery to the Biorepository Core (IU) for cataloging and re-distribution to the Genomic Core. Include a hard copy of the sample QC submission form in the shippment.

Appendix G: Instructions for Preparing and Submitting Samples for the DHART-SPORE Project to the Omics Core at IU

Section I: Omics Core - Sample Submission and Intake

In order to intake and process DHART SPORE samples in a timely and efficient matter, the Omics core at IU requests that samples be prepared and submitted in accordance with the following guidelines:

1. All samples of any type should be prepared, packaged and shipped as indicated in Appendices E and F
2. Samples should be first sent to the DHART SPORE Biorepository Core and then distrusted to the Center for Medical Genomics (CMG) or to the Angus lab for kinome assay and/or nucleic acid preparation from tissue once the samples have been logged.
3. Complete DHART SPORE sample manifest form, indicating preferred assays and clearly label tubes/vials.
4. The CMG accepts purified DNA, gDNA, and total RNA for library preparation as well as DNA and cDNA library for sequencing. It can be in any concentration. For submission of SF tissue for next-generation sequencing and/or kinome profiling see below.
5. If specific quantities of already extracted/purified DNA or RNA are supplied to the CMG, sample will have to be FIRST processed for quality control (QC) by Qubit, Agilent Bioanalyzer, and/or TapeStation. Please include the following information (see Sample QC submission form):
    a. PI name department, email
    b. Lab contact name, email, phone number
    c. organism
    d. extraction/purification method,
    e. sample dissolved in H2O, or specific buffer; e.g. TE, TAE, etc.
    f. sample type; DNA, gDNA, RNA, etc.
    g. requested QC type; e.g., total RNA, small RNA, PCR-DNA, gDNA, cDNA library

    Other required information, such as sample ID, NanoDrop concentration and volume, should be filled based on the Sample QC Submission Form (Attachment A).

    Please provide at least 5ul amount in volume for sample QC. Strongly recommend submission of entire original sample volume to avoid aliquoting evaporation and for convenience of downstream library preparation and sequencing. Remaining sample will be returned upon request.
6. Please supply biopsies and SF tissue in clearly labeled external screw top 2 mL vials.
7. No fewer than 12 samples will be processed via an automated work flow for sequencing. If less than 12 samples are received for such a process, the Omics core will either: a) store the sample set until additional samples arrive for the specific work flow requested; or, b) if no additional samples are forthcoming for the specific work flow, the samples

may be manually processed. For kinome profiling, please submit all samples for a given experiment in the same shipment.

8. Please make sure that each sample/aliquot has a unique identifier that you will be able to track to the original case/patient/time point, etc. and the sample tube label is the same as the name "label on tube" (See Sample QC Submission Form: Row 34 and Column B). If a sample is improperly labeled or inconsistent with the form, the CMG will re-assign a new identifier to each sample in order that the sample may be accurately tracked through all Omics core work flows, as well as through LCCC Bioinformatics.
9. Sample transfer process and documentation:

   a) Before samples are transferred to the Center for Medical Genomics (CMG) or to the Angus Lab (Kinome profiling) for entry into the processing work flow, please complete the appropriate DHART SPORE Sample Submission e-Form (https://dhartspore.org/manual_and_manifests.html). The submission form will be provided to all DHART SPORE participants. Please check your version as recent updates have been made.

   b) Amount of samples needed by process (all quantitation performed by Agilent Bioanalyzer or TapeStation):
      - DNA for Agilent Sureselect$_{xt}$ Human All Exon V7 Library Prep (Standard Input): 500 ng-1 ug DNA
        - A DNA integrity number (DIN) of 6 or higher is required for WES library prep.
      - DNA for Agilent Sureselect$_{xtHS}$ Human All Exon V7 Library Prep (Low Input): 250 ng-400 ng DNA
        - High or low quality gDNA, FFPE DNA, or cfDNA for low input WES library prep.
      - gDNA for Whole Genome Sequencing (WGS) with Illumina Nextera FLEX Library Prep: 200 ng-400 ng gDNA
        - A DNA integrity number (DIN) of 6 or higher is required for WGS library prep.
      - gDNA for Illumina TruSeq Methyl EPIC Capture (human, 100Mb) Library Prep: >800 ng gDNA
        - A DNA integrity number (DIN) of 6 or higher is required for targeted methylation library prep.
      - RNA for KAPA Stranded mRNA/total RNA HyperPrep Library Prep: 200 ng-400 ng RNA
        - A RNA integrity number (RIN) of 7 or higher is required for mRNA library prep, and a RIN < 7 will be designated to total RNA library prep.
        - Additional DNase treatment is required for total RNA library prep.
        - Depletion of Globin mRNA step will be added for RNA of whole blood origin.
      - - RNA for Takara/Clontech SMART-seq mRNA HT and Illumina Nextera XT Library Prep (Ultralow Input): 1 ng-50 ng RNA
        - A RIN of 7 or higher is required for the ultralow input mRNA library prep.

- Additional DNase treatment is required for total RNA library prep.
- Depletion of Globin mRNA step will be added for RNA of whole blood origin.

○ RNA for Takara/Clontech SMARTer total RNA Pico V2 Library Prep (Ultralow Input): 1 ng-50 ng RNA

- If RIN < 7, the ultralow input total RNA library prep is recommended.
- Depletion of Globin mRNA step will be added for RNA of whole blood origin.

○ RNA for Qiagen QIAseq miRNA Library Prep: >200 ng total RNA

- >20% miRNA in total RNA is required for miRNA library prep.

The CMG also offers several additional specialty library prep protocols, such as ChIP seq and targeted RNA seq. It's encouraged to directly discuss with Dr. Xiaoling Xuei (xxuei@iu.edu) on the amount of DNA/RNA required and Dr. Angus (sangus@iu.edu) if tissue is being submitted.

Section II: INSTRUCTIONS – Completing the Center for Medical Genomics (CMG) Sample Submission and Sequencing Request Form

The CMG Sequencing Request Forms for sample submission/QC and sequencing are available on iLab. iLab integrates submission request, communication and billing into one platform, and requires one-time registration. The process can be started by following the core webpage on iLab and registering with your IU/institutional credentials.

For DNA and RNA samples submitted to SPORE Biorepository Core or if the SF tissue samples require DNA/RNA extraction by the Omics core (Angus lab) at IU, the iLab request forms will be processed by Steve Angus, instead of individual investigators.

Investigators are asked to prioritize sequencing and kinome profiling in the sample manifest. Consultation with the Omics Core is strongly encouraged if there are any specific concerns or considerations.

1. **SAMPLE SUBMISSION/QC request form:**
   a. Please complete all fields, and at a very minimum, the required fields.
   b. The form asks for PI information, billing account and contact information, all of which are required fields to proceed with the request The contact person, if different from the PI, should be the person most knowledgeable about the specific sample submissions included on this form and who can make decisions relative to any changes in processing that may be suggested by extraction and/or QC issues. This is the person the Genomic core will contact for information on the samples.
   c. Please carefully provide the sample information, such as number of samples, type of QC requested, RNA/DNA extraction kit used and type of organism and tissue the sample is derived from.
   d. Please download the sample submission template (excel spreadsheet) from the request form, provide the requested information, carefully noting the sample names (as labeled on the tube) and uploading it back to the submission.

2. SEQUENCING request form:

a. Please select the most appropriate category for your request from the four options listed:

i. sequencing request for samples: request for library preparation and sequencing;

ii. sequencing request for libraries: request for sequencing only;

iii. sequencing request for single cell samples: request for single cell library preparation and sequencing);

iv. sequencing request for single cell libraries: request for single cell sequencing only.

b. Please complete all fields, and at a very minimum, the required fields.

c. The form asks for PI information, billing account and contact information, all of which are required fields to proceed with the request The contact person, if different from the PI, should be the person most knowledgeable about the specific sample submissions included on this form and who can make decisions relative to any changes in processing. This is the person the Genomic core will contact for information on the samples.

3. **Experiment Description:**

a. Project title and brief description of the experiment, including experiment design and data comparison.

b. Organism/species, tissue or cell lines, type of sample (e.g. DNA, gDNA, FFPE DNA, total RNA, enriched ChIP DNA, etc.), request sequencing type (e.g. mRNAseq, totalRNAseq, miRNAseq, whole exome seq, whole genome seq, etc.), specific request for sample enrichment, sample number to be submitted, , additional requests (e.g. # reads/sample, etc.).

c. If samples submitted are libraries prepared somewhere else, please fill Sequencing Format desired (e.g. 75b paired-end read, 150b single read, 28b+8b+0b+91b, etc.) as specific as your sequencing required. If there is any preference of sequencing platform, please also specify (e.g. NextSeq, NovaSeq, MiSeq, etc.).

4. Sample Information:

a. Sample type, DNA, RNA, cDNA library, gDNA library, etc.; brief description of sample isolation and purification procedure; if submitting total RNA sample, any treatment of DNase; if submitting DNA sample, any treatment of RNase; solution sample is dissolved in (e.g. H2O, TE, TAE, etc.); if any Agilent Bioanalyzer or TapeStation profile, provide the PDF file.

b. Sample information sheet: Fill the table with sample name (exact name as on tube label), sample group treatment, specify method (e.g. NanoDrop, Qubit, etc.) of concentration (ng/ul), NanoDrop spectrum if any, sample volume (ul).

c. LIBRARY INFORMATION sheet: Provide library type (e.g. cDNA library, gDNA library, ChIP library, ATAC library, etc.); library prep kit used (e.g. KAPA Stranded mRNA HyperPrep Kit, Illumina TruSeq Methyl EPIC Capture Kit, Clontech SMART-seq mRNA HT Kit, Illumina

Nextera FLEX Kit, etc.); solution the library is dissolved (e.g. H2O, TE, TAE, etc.); if any Agilent Bioanalyzer or TapeStation profile, provide the PDF file; specify method used for library concentration measurement; if libraries are pooled, specify concentration (prefer molarity, but not a must).Fill the table with library name (exact name as on tube label); library group treatment; specify method (e.g. NanoDrop, Qubit, etc.) of concentration (ng/ul); average library size, if any; library concentration in molarity, if any; total volume of library; index names and index sequences for each library.

Please list every library you are submitting in sequential order, even you are submitting them as a pool. This will be very important for bioinformatician in demultiplexing and data analysis.

5. **Data Analysis:**

It is required to specify the mode of data delivery. In addition, the form asks if you need the assistance of bioinformatics core for data processing (at additional cost). If answered "No", a person or a group who will receive data and process data analysis is required to be specified. This could be the lab contact or a bioinformatician to whom the PI is designated for data analysis. If only certain parts of data process are desired, e.g. mapping QC only, gene expression analysis or complete analysis, please specify in the area provided.

GENERAL FEEDBACK

After receiving RNA/DNA sequencing request from DHART SPORE participants, the work flow is:

IU Biorepository (sample intake) → **CMG** (QC, library prep and sequencing) → **Bioinformatics** (sequencing analysis, data disposition)

Project status can be found under "status" column under respective submission within iLab. It is updated regularly by the core personnel. A CMG representative will try to provide regular status reports at various critical stages of the work flow processes. For example, the first status report will usually come after the completion of sample QC of DNA and RNA. Sometimes decisions will need to be made at this point as to the best type of library prep process to employ based on the quantity and/or quality of the DNA and RNA samples.

The second status report usually will come when the sequencing is complete. After that, you will be advised or asked to discuss your study design and data analysis strategy once the sequencing data is transferred to Bioinformatics.

The time required to complete various stages of a project vary. CMG does our best to move each project through the various work flows as quickly as feasible, however, we emphasize accuracy and achieving the goals of the project over speed. We will do our best to keep you informed of the progress of your project.

For kinome profiling, Dr. Angus or an Angus lab member will return normalized data as soon as possible after receipt to the project investigator or team member for discussion and interpretation. Status updates will be provided or in response to inquiry. Processing includes tissue handling, protein quantification, affinity chromatography with kinase inhibitor beads, trypsin digestion and sample preparation, each individual sample run on a 2 h gradient, raw file searches, and data analysis. For samples with both kinome profiling and sequencing, additional analysis and integration will involve the IU Bioinformatics team, Dr. Zhang and Dr. Huang.

Appendix I: TRIB Protocol for Plasma Preparation for Cytokine Assays

Appendix I: Protocol for Plasma Preparation for Cytokine Analysis

Collection

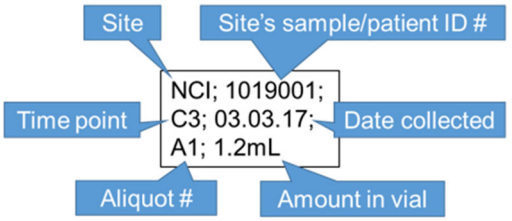
1. Collect (6mL) peripheral blood into an 8 mL EDTA purple top tube.
2. Centrifuge at 2200 rpm for 15 minutes at 4˚ C.
3. Collect plasma and distribute/aliquot 1-1.5 mL into each 2mL cryovial/*tubes (1-5 cryotubes).

   **NOTE: Microcentrifuge tubes, 2.0 mL, with screw cap clear, sterile from Thermo Scientific (Catalog#: 3469-11) are the best.*

4. Fix a ^**printed** label on each tube with: Site, site's sample/patient ID number, sample time point, date, a # and amount/vial (i.e. volume). ***See Figure 1.***

   ^NOTE: Cryo Babies, Cryotags, Size 9187-1700 White are the most reliable for frozen storage.

Figure 1: Printed Label Example



5. Store samples at -80° C until being shipped as a batch on dry ice to the address provided below.

Shipping Address:

DHART SPORE
Indiana University School of Medicine
351 W. 10th St., TK-217
Indianapolis, IN 46202

6. Complete the **Sample Submission e-Form/Manifest *(Figure 2)***.

Appendix I (CONTINUED): Protocol for Plasma Preparation for Cytokine Analysis

7. Label and organize samples in box for shipping/storage ***(Figure 3 [A-D])***.

Figure 2: *Indiana University - CYTOKINE Pharmacodynamic Studies*

Children's Clinical Research Center/Translational Research and Integrated Biology Laboratory at Indiana University (Renbarger/Bijangi-Vishehsaraei)

DHART SPORE Sample Submission e-Form (Rev. 05/11/2022)

Pharmacodynamic Studies: Plasma biomarker (cytokine) assays

BACKGROUND: Plasma biomarker (cytokine) assays will be performed using multiplex simultaneous quantification of soluble cytokines and growth factors. Fix a printed label on each tube with: Date, Timepoint, Volume size, Aliquot #, Site and Site's Sample/Patient ID number (See Figure 1 - Printed Label Example). ***PLEASE SEE IMPORTANT NOTE BELOW.***

I. PROJECT DESCRIPTION AND SUBMITTING SITE CONTACT INFORMATION

Required Fields

Study/Project# (including Clinical Protocol #):				
Submitting Site Investigator	Name.:		Phone:	
	Submitting Site:		Email:	
Submitting Site Address				IRB Number, if applicable
	Dept. Name or No.			
Contact:*	Name:	Email:		Phone:

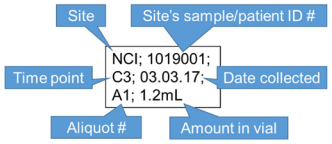
* PERSON TO CONTACT IF THERE ARE QUESTIONS ABOUT THE SAMPLES OR THE REQUESTED SERVICES.

Please ship samples to:
Children Clinical Research Center/TRIB Lab
Attn: Khadijeh (Sholeh) Bijangi-Vishehsaraei, PhD, MS
705 Riley Hospital Drive, Room 2641
Indianapolis, IN 46202

Fix a printed label on each tube with: Site ID, site's sample/patient ID, time point, date, tube # and amount/vial (Cryo Babies, Cryotags, Size 9187-1700 White are the most reliable). See Figure 1.

PLEASE DO NOT SHIP FOR WEEKEND DELIVERY!! Ship samples by Federal Express Priority Overnight Monday through Thursday only.

Figure 1: Printed Label Example



Please include any additional information pertinent to successful competion of the services requested.

IMPORTANT NOTE: BEFORE SENDING YOUR SAMPLES, PLEASE COMPLETE THIS FORM AND EMAIL A COPY TO KHADIJEH (SHOLEH) BIJANGI-VISHEHSARAEI (khbijang@iupui.edu). PLEASE ALSO INCLUDE A HARD COPY OF THE COMPLETED FORM WITH THE SAMPLE SHIPMENT. SHOULD YOU HAVE QUESTIONS ABOUT COMPLETING THE FORM, CONTACT KHADIJEH (SHOLEH): (317) 278-3050

II. SAMPLE SUBMISSIONS:

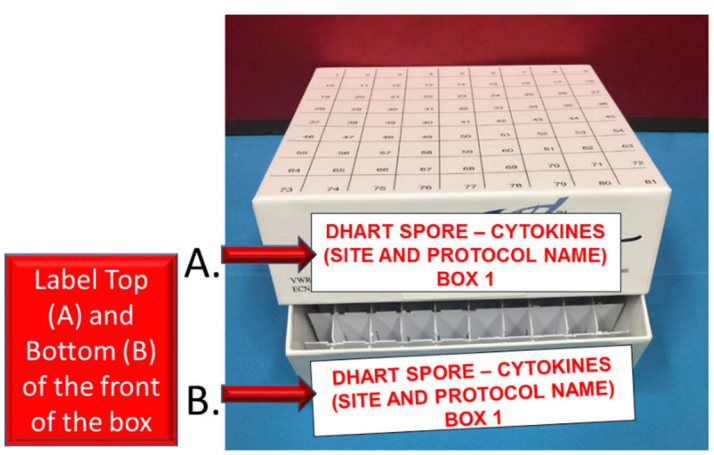
*This ID should appear on the tube label.

(A) Entry #	(B) DE-IDENTIFIED Subject/Patient ID	(C) Investigator-Assigned Unique ID*	(D) Material Supplied As:	(E) Aliquot/Tube Number	(F) Actual volume of the sample/vial	(G) Unit (ul, ml)	(H) Timepoint	(I) Species (H, M, O)	Comments
Example 1	3019001	PA076/CHOP	Plasma	A1	1	mL	Pretreatment	Human	
Example 2	3019001	PA076/CHOP	Plasma	A2	1	mL	Pretreatment	Human	
Example 3	3019001	PA076/CHOP	Plasma	A3	1	mL	Pretreatment	Human	
Example 4	3019001	PA076/CHOP	Plasma	A4	0.5	mL	Pretreatment	Human	
Example 5	1019001	PL-0505/NIH	Plasma	A1	1	mL	Cycle 3	Human	
Example 6	1019001	PL-0505/NIH	Plasma	A2	0.8	mL	Cycle 3	Human	
3									
4									

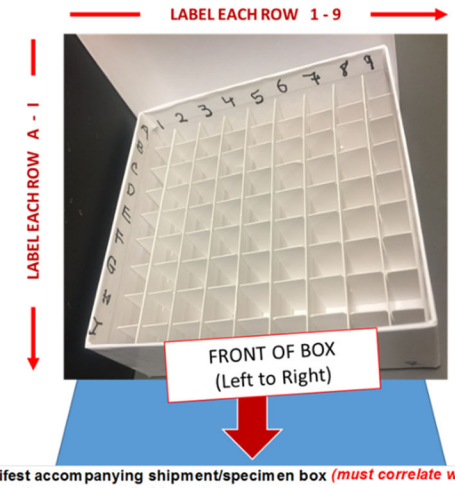
Appendix I (CONTINUED): Protocol for Plasma Preparation for Cytokine Analysis

8. Label and organize samples in box for shipping/storage ***(Figure 3 [A-D])***.

Figure 3 (A-D): Labeling and organizing box for shipment.



C.



D.

EXAMPLE: Manifest accompanying shipment/specimen box ***(must correlate with the location of the tubes in the box and accompany all shipments)***

Box #	1	2	3	4	5	6	7	8	9
A	NCI; 1019001; Pre-Tx; Day/Mo/Yr; Aliquot 1; 1.2mL	NCI; 1019001; Pre-Tx; Day/Mo/Yr; Aliquot 2; 0.5mL	NCI; 1019001; C3; Day/Mo/Yr; Aliquot 1; 2mL						
B									
C									
D									
E									
F									
G									
H									
I									

9. Ship collection of samples (frozen on dry ice and overnight express) to the DHART SPORE Biorepository Core at Indiana University (Monday to Thursday) at the following address:

   DHART SPORE
   Indiana University School of Medicine
   351 W. 10th St., TK-217
   Indianapolis, IN 46202

   IMPORTANT! Please include a hard copy of the submission form (Figure 2 and Figure 3D) in the shipment

10. Notify the recipient/contact of the laboratory (khbijang@iupui.edu) and the DHART SPORE repository Core (dhartbio@iupui.edu) via email **prior to shipping** –AND- include an electronic copy of submission form ***(Figure 2 and Figure 3D)***.