

**Primary Immune Deficiency Treatment Consortium (PIDTC)
Laboratory Procedures:
6907 Prospective and Longitudinal SCID Study
Mechanistic Studies**

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General Information

I. Purpose: The “PIDTC Laboratory Procedures: 6907 Prospective and Longitudinal SCID Study: Mechanistic Studies” is a guide to the collection, processing (if applicable) and shipping of the Mechanistic Studies for the PIDTC Prospective and Longitudinal SCID Study.

II. Safety Procedures:

- Human derived specimens are potentially infectious.
- Practice Universal Precautions.
- Wear gloves and other personal protective equipment (PPE) to protect from potential exposure to blood borne pathogens and other pathogens (from saliva, stool, etc.) when working with human biological specimens.

III. Labeling and Tracking of Specimens:

1. Labeling of specimen:
 - a. Record the study number (i.e., 6907), the subject’s PIDTC Participant ID, the cycle number (i.e., C1), the study visit (i.e., 6 mth), date of collection (i.e., MM/DD/YY), and a sequential two-digit number if there is more than one tube for patient. Each specimen must be labeled with a unique identifier/“barcode” number.
 - b. Example: Participant ID 123456, 3 specimens collected at 6 month visit on July 26, 2022, would be labeled as follows:

6907 123456 C1 6mth 07/26/22 - 01
6907 123456 C1 6mth 07/26/22 - 02
6907 123456 C1 6mth 07/26/22 - 03
2. For each specimen complete the Specimen Collection REDCap form in the PIDTC 6907 database.
3. Please contact the appropriate receiving laboratory at least one week prior to the planned collection/shipping date to confirm the laboratory’s ability to receive the sample.
 - a. The Long-Boyle lab will announce when the frozen pharmacokinetic/ pharmacogenomic (PK/PG) samples can be shipped to their lab. Do not send anything unless requested.

For questions regarding collection, processing, or shipping of specimens please contact:

- The Program Management Team is
 - Rafael Ricon, PIDTC Program Coordinator: rafael.ricon@ucsf.edu
 - Lisa Lim, PIDTC Senior Project Manager: lisa.lim@ucsf.edu

- Jessica Ni, PIDTC Project Manager: Jessica.ni@ucsf.edu
- The laboratory contact for the research specimen in question if not resolved via Program Management. See the contact information in the table below.

PIDTC FedEx Account Number	Usage
344797331	This FedEx Account number can be used to ship specimens for any of the 6907 Protocol Mechanistic studies except for the PK and PG studies.

PIDTC Lab Contact and Shipping Information

PIDTC Laboratory	Shipping Address
<p>Dr. Elie Haddad</p> <p>Intrinsic (HSC) Function Studies of Blood</p>	<p>CHU Sainte-Justine Attn: Kathie Béland 3175 chemin Cote Sainte-Catherine Room 6.17.100 Montréal Québec H3T 1C5 Canada</p> <p>*The following PO number must appear on the waybill for appropriate in-house delivery: contract #3600.</p> <p>**Call Cole International at (450) 476-0888 between 8 AM and 5 PM ET on the date of shipping for customs pre-clearance. Do not send an email or leave a message.</p> <p>For questions contact Lucie at Cole International: Lucie.Guillet@coleintl.com</p>
<p>Dr. Janel R. Long-Boyle</p> <p>Pharmacokinetic and Pharmacogenomic Studies</p>	<p>ATTN: Liusheng Huang Drug Research Unit Dept of Clinical Pharmacy UCSF Pride Hall, Room 2100 2540 23rd st. San Francisco, CA 94110 Phone: (415)335-2971(cell) 415-476-5454 (Lab)-</p> <p>Phone: (415)502-2594 (Liusheng) Email: Liusheng.Huang@ucsf.edu</p> <p>Phone: (415)514-1142 (Florence) Email: Florence.Marzan@ucsf.edu</p>
<p>Dr. Luigi D. Notarangelo</p> <p>Extrinsic (Thymic) Function Studies of Blood</p>	<p>Dr. Luigi D. Notarangelo Lab National Institute of Allergy and Infectious Diseases National Institutes of Health Building 10, room 5-3840 10 Center Drive – MSC Bethesda, MD 20892</p> <p>Email: Marita Bosticardo (marita.bosticardo@nih.gov) Phone: (301)761-7550</p>

Procedures – Mechanistic Studies

PRIMARY IMMUNE DEFICIENCY TREATMENT CONSORTIUM

PROCEDURE NAME: Intrinsic HSC Function Studies of Blood
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KEY INFORMATION	
Study type	Mechanistic Study
Specimen	Peripheral blood
Lab performing the test	Haddad Lab
Timepoint	Pre-DT Period: Time of enrollment NOTE: Collected ONLY if patient has an unknown SCID mutation

I. Purpose

To collect blood and send to the Haddad Lab to describe the proportion of patients without known mutation who have intrinsic (HSC) vs. extrinsic (thymic) HSC defects.

II. Materials Required

Sodium heparin green top tube (green top/5-10mL)

III. Details of Procedure

Part A: Collect Blood Sample

1. Contact the Haddad Lab **as soon as possible before the specimen is collected** (at least one week before) to discuss specimen collection and scheduling.
2. Follow your institution and laboratory's procedures for collecting 3-5mL of peripheral blood into a sodium heparin tube.
3. Keep at ambient temperature.
4. If there will be several hours before sample is shipped, put the blood sample on a rocking platform at ambient temperature.

Part B: Ship for Processing and Storage

1. Ship peripheral blood specimen at ambient temperature as soon as possible – within 24 hours of draw is acceptable.
2. Due to custom delays, it is safer to send samples on Mondays and Tuesdays. Wednesday is an option but is at higher risk of delay into the weekend.
3. Contact custom broker's number to talk to someone there to clear the package.
4. After the pick-up send the tracking number to the contact e-mail.

PRIMARY IMMUNE DEFICIENCY TREATMENT CONSORTIUM

PROCEDURE NAME: Extrinsic (Thymic) Function Studies of Blood

KEY INFORMATION	
Study type	Mechanistic Study
Specimen	Peripheral blood
Lab performing the test	Notarangelo Lab
Timepoint	Pre-DT Period: Time of enrollment Note: Collected ONLY if patient has an unknown SCID mutation

I. Purpose

To collect blood and send to the Notarangelo Lab to describe the proportion of patients without known mutation who have intrinsic (HSC) vs. extrinsic (thymic) HSC defects.

II. Materials Required

EDTA tube (purple top)

III. Details of Procedure

Part A: Collect Blood Sample

1. Contact the Notarangelo Lab **as soon as possible before the specimen is collected** (at least one week before) to discuss specimen collection and scheduling.
2. Follow your institution and laboratory's procedures for collecting 5-7mL of peripheral blood into a EDTA tube.
3. Keep at ambient temperature.
4. If there will be several hours before sample is shipped, put the blood sample on a rocking platform at ambient temperature.

Part B: Ship for Processing and Storage

1. Ship **overnight at ambient temperature** to the Notarangelo Lab following the shipper's and your institution's guidelines for shipping diagnostic specimens.
2. The Notarangelo Lab accept samples Monday to Thursday

PRIMARY IMMUNE DEFICIENCY TREATMENT CONSORTIUM

PROCEDURE NAME: Pharmacokinetic (PK) and Pharmacogenomic (PG) Studies

KEY INFORMATION

Study type	Mechanistic Study
Specimen	Peripheral blood sample
Lab performing the test	Long-Boyle Lab
Timepoint	Any Day of Drug Administration

I. Purpose

To enhance the predictability of the current models and contribute to pharmacodynamic (PD) analysis to identify improved exposure targets for subgroups of patients with differential forms of SCID.

Note: PK samples will be used to run the PG Study at the Long-Boyle Lab. No need to collect a separate sample for the PG Study.

II. Materials Required and Collection Information

Table 1: Collection times for individual conditioning agents

If there would be multiple infusions of the same drug, PK/PG samples can be collected after any of those infusions so long as the infusion number is recorded.

Drug	1 st PK collection interval	2 nd PK collection interval	Volume of whole blood collected per sample	Collection tube*
Fludarabine	0-2 hours post end of infusion	4-24 hours post end of infusion	1.0 mL	2.0 mL K ₂ EDTA (lavender top)
Clofarabine	0-2 hours post end of infusion	4-24 hours post end of infusion	1.0 mL	2.0 mL K ₂ EDTA (lavender top)
Melphalan	0-30 minutes post end of infusion	2-6 hours post end of infusion	0.5 mL	2.0 mL Lithium Heparin (light green top)
Thiotepa	0-2 hours post end of infusion	2-24 hours post end of infusion	0.5 mL	2.0 mL K ₂ EDTA (lavender top)
Cyclophosphamide	0-2 hours post end of infusion	2-24 hours post end of infusion	1.0 mL	2.0 mL K ₂ EDTA (lavender top)

*Collection tubes – spray-coated tubes that do not have a serum separating gel should be used to allow collection of both plasma and cell matrix (red blood cells/buffy coat).

Table 2: Collection times for individual immune modulators (serotherapy)

Drug	1 st PK collection interval	2 nd PK collection interval	Volume of whole blood collected per sample	Collection tube*
Alemtuzumab (Campath)	0-60 minutes post end of infusion	18-24 hours post end of infusion	1.0 mL	2.0 mL lithium heparin (light green top)
Antithymocyte globulin (ATG)	0-60 minutes post end of infusion	18-24 hours post end of infusion	1.0 mL	2.0 mL lithium heparin (light green top)

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*Collection tubes – spray-coated tubes that do not have a serum separating gel should be used to allow collecting both plasma and cell matrix (red blood cells/buffy coat).

III. Details of Procedure

Part A: Collect Blood Sample, Spin, and Collect Plasma, Freeze

1. Collect blood samples at the times listed above in the indicated blood collection tubes.
2. Record the sample collection information corresponding to the drug (collection time and volume, in the corresponding Nursing PK Paper Sheet).
3. All pharmacogenomic samples should be collected through a central line and follow the pediatric BMT central line drawing protocol for pharmacogenomic studies.
4. At each sample collection time, collect the indicated volume of whole blood into designated tube and place immediately on ice. Do not collect less than the volume indicated in Table 1 and Table 2.
 - i. **Plasma samples containing all drugs EXCEPT cyclophosphamide**
Whole blood should be immediately placed on wet ice and sent to the central lab for processing. Specimens must be centrifuged, aliquoted and frozen within 30 minutes of drawing. The vacutainer tube should be centrifuged at 3000-4000 rpm for 10 minutes in a refrigerated centrifuge at 4°C. Then, the plasma should be transferred into a 2mL labeled cryovial storage tube and stored at -70°C until samples are shipped to the core study lab.
 - ii. **Plasma samples containing CYCLOPHOSPHAMIDE**
Given the stability of cyclophosphamide and metabolites a derivatizing agent should be added to the plasma as soon as possible.
Whole blood should be immediately placed on wet ice and sent to the central lab for processing. Specimens must be centrifuged, aliquoted and frozen within 20 minutes of drawing. The vacutainer tube should be centrifuged at 3000-4000 rpm for 10 minutes in a refrigerated centrifuge at 4°C.
 - a. Then, the plasma should be transferred into a 2mL labeled cryovial storage tube, mixed with 2M semicarbazide at 10:1 ratio, and stored at -70°C until samples are shipped to the core study lab. To obtain semicarbazide contact Rafael Ricon (contact information above).

Part B: Ship for Processing and Testing at Long-Boyle Lab

1. Reach out to the Long-Boyle Lab personnel (contact information above) for instructions on batching and when to ship.

2. E-mail to Dr. Janel Long-Boyle (janel.long-boyle@ucsf.edu) or send with specimen the Nursing PK Paper Sheet. Keep a copy of the Nursing PK Paper Sheet.
3. Ship frozen tube(s) in a leak-proof secondary container with absorbent; pack in protective material in a Styrofoam box containing dry ice. Frozen tubes are subject to breakage upon impact. To reduce the risk of breakage during shipment, frozen tubes should be treated in the same manner as glass tubes. (If you do not have a Styrofoam box, contact the Long-Boyle Lab.)
4. Ship the tube(s) to the Long-Boyle Lab following the shipper's and your institution's guidelines for shipping diagnostic specimens.