SEARCH Phase 3
For Diabetes in Youth

Central Laboratory MANUAL of PROCEDURES

Specimen Collection
Processing
Shipment

UNIVERSITY OF WASHINGTON
SEATTLE, WA

NORTHWEST LIPID METABOLISM & DIABETES RESEARCH

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ABOUT THE LABORATORY

Brief History

The Northwest Lipid Metabolism and Diabetes Research Laboratories (NWRL) was established in 1971 as one of twelve laboratories involved in the Lipid Research Clinics Program, and subsequent Coronary Primary Prevention Study, funded by the National Heart, Lung, and Blood Institute. During the program, this laboratory participated in the development and standardization of methods for the separation of lipoproteins and for the chemical quantification of their components, and performance was monitored continually through the Lipoprotein Standardization Program of the Centers for Disease Control. The laboratory is directed by Santica Marcovina, PhD, ScD, Research Professor of Medicine, Division of Metabolism, Endocrinology, & Nutrition, Department of Medicine, University of Washington.

The laboratory is an Abell Kendall reference network laboratory of the National Reference System for Cholesterol, and participates in the lipid standardization programs offered by the National Heart, Lung, and Blood Institute, Centers for Disease Control, and the College of American Pathologists. In addition, the laboratory serves as the reference laboratory for the International Standardization of Apolipoproteins AI, B, and Lp(a) and is one of the five World Health Organization laboratories.

For more than 30 years, the laboratory has participated in studies to identify the prevalence of hyperlipidemia in the population and to evaluate the efficacy of intervention. Reported in 1983, results of the Coronary Primary Prevention Study demonstrated that lowering cholesterol was effective in reducing the risk of premature heart disease; this information was key in the development of treatment recommendations issued by the National Cholesterol Education Program. To maintain a high level of accuracy and consistency in results, we continue to perform the Beta Quantification procedure as outlined in the Manual of Laboratory Operations for the Lipid Research Clinics Program without introducing any technical change. The NWRL has been involved in numerous and varied multi-center investigations throughout the United States and internationally. We currently serve as the Central Laboratory for the following NIH-sponsored studies:

ACCORDION – Action to Control Cardiovascular Risk in Diabetes
AIM-HIGH -- Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes
CARDIA - Coronary Artery Risk Development in Young Adults
CIT - Clinical Islet Transplantation Consortium
DPPOS – Diabetes Prevention Program Outcomes Study
ITN (3 Protocols) – The Collaborative Network for Clinical Research in Immune Tolerance
LABS - Longitudinal Assessment of Bariatric Surgery
LITE – Lifestyle Intervention to Treat Erectile Function
Look AHEAD – Action for Health in Diabetes
NSABP – Breast Cancer Prevention Trial
SEARCH III– Search for Diabetes in Youth
SEARRCH CVD – Cardiovascular Disease in Youth
SNAP – Study of Novel Approaches for Prevention
STOPP-T2D – Studies to Treat or Prevent Pediatric Type 2 Diabetes
Teen LABS – Teen Longitudinal Assessment of Bariatric Surgery
TrialNet - Anti-CD3, CTLA4-Ig, GAD, IL-Beta 1, Natural History, NIP and Oral Insulin Protocols

Vision Statement

To be a model organization, thriving in a dynamic environment and respected as a leader in quality laboratory services with a strong commitment to continuous quality improvement.

Mission Statement

The mission of the Northwest Lipid Research Laboratories is to continuously provide the highest standards of professional and technical expertise and organizational support.

Our commitment is to not only provide the utmost in quality analytical, interpretative, advisory and consultation services, but is to offer comprehensive support as a central biochemistry laboratory for research and clinical trial studies. Our pledge is to take the steps necessary, whatever they may be, to ensure the greatest success of the studies in which we are involved.

Introduction to the Central Laboratory MOP

We have provided basic overviews in the areas where you should have already had training, such as Universal Precautions and Phlebotomy Procedures, but these are provided only as reminders and should be treated as such. If you feel you need additional training in these areas we have provided some resources for you. The sections covering specimen collection, processing and shipping is directly related to SEARCH 3, and are covered in detailed form. This detail is provided for a reason: submission of proper specimens under optimum conditions is very important. *Accurate analyses can seldom be performed on poor specimens.* Once you have familiarized yourself and have repeatedly performed these procedures, it will not be necessary for you to refer to this manual every time you collect specimens. Therefore, we have also included sections that give basic outlines easy to follow. Tables on pages 16-18 will be provided in laminated form to be posted for a quick reference.

LABORATORY CONTACTS

Should question arise, we are happy to answer them or to assist you at any time. Please feel free to contact any one of the following people. We are committed to you and to the study, and we will do what it takes to ensure our combined success!

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SUPPLY COORDINATION and SHIPPING

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SUPPLIES PROVIDED BY THE CENTRAL LABORATORY

For Blood Collection and Processing:

- Blood collection tubes for non-pediatric (expires: generally 6 to 12 months)
- Microtainers for pediatric (expires: generally 6 to 12 months)
- Portable centrifuge tubes
- Disposable plastic transfer pipettes

For Urine Collection:

- Antiseptic towlettes
- Sterile urine collection cups
- Disposable plastic transfer pipettes
- Screw-cap sample vials: 10mL transfer tubes
- Infant collection bags

For Specimen Identification:

- Specimen shipment forms (originals provided, clinics to make copies)
- Bar-coded labels for specimen tubes and shipment forms

For Specimen Shipping:

- Ziploc bags
- Cold packs
- 2” Revco freezer storage boxes with 9x9 dividers
- Polyfoam tube holders with absorbent pad and cardboard outer shell
- Polyfoam shipping containers with cardboard outer shell
- Pre-printed Federal Express air bills
- Biohazard plastic bags
- “EXEMPT HUMAN SPECIMEN” Stickers

All supplies are shipped to the main clinic sites. It is the responsibility of the five main sites to distribute the necessary supplies to their satellite clinics. Supplies are provided in bulk and should be ordered in quantities to ensure that all satellites are properly stocked at all times, while bearing in mind that some supplies have expiration dates, as noted above. Please be mindful of dated collection supplies so that you do not order too many at one time, causing expiration before their use. If you need help in determining the quantities needed and/or setting up a supply tracking system, please call our Supply and Shipping Coordinator, Marlon Ramirez, at (206) 685-3328.

To order supplies, use the ***Supply Request Form*** (attachment F).
Follow the instructions on the form and, once completed, Fax to: **206-616-4889**.
If you have questions, contact Marlon Ramirez at: **206-685-3328**.

EQUIPMENT, SUPPLIES & FACILITIES REQUIRED AT COLLECTION SITES

These are suggested supplies only; clinics may use equivalent substitutions, if desired.

For Blood Collection:

- ✓ Alcohol wipes
- ✓ Ammonia spirits ampules
- ✓ Arm boards
- ✓ Band-Aids
- ✓ Cold compresses
- ✓ Disposable gloves (powder-free, to avoid possible cross-contamination from powder)
- ✓ Finger Lancets (with tip length no greater than 2.4 mm)
- ✓ Needle (Vacutainer) holders – adult and pediatric
- ✓ Paper and/or other dermatological tape
- ✓ Sterile and non-sterile gauze pads
- ✓ Sterile, 19, 21, 23 & 25 gauge, 1" needles (multiple-sampling)
- ✓ Sterile, 21, 23, 25 gauge butterfly needles (multiple-sampling)
- ✓ Syringes, 10 cc
- ✓ Tourniquets
- ✓ Tegaderm
- ✓ Topical Anesthetic (EMLA, lidocaine/prilocaine)

For Blood Processing/Shipping/Storage:

- ✓ Tube racks
- ✓ Plastic-backed table covers
- ✓ Pipetman with disposable tips, capable of adjustment in 1µL increments
- ✓ Waterproof pens (such as laundry markers, fine-point, for scribing on labels)
- ✓ Centrifuge: refrigerated swinging-bucket type with Microtainer holding capability
- ✓ Freezer: -20°C, non-cycling for freezing of C-peptide vials and shipment cold packs
- ✓ Dry Ice: a standing order should be made so that there is always a supply on hand
- ✓ Wide (3") packing tape for sealing shipping containers

For Specimen Handling:

- ✓ Lab coat
- ✓ Goggles or face shield
- ✓ Paper towels
- ✓ Bleach decontaminant -1 part Clorox to 9 parts water, stored in a labeled bottle
- ✓ Biohazard waste containers with orange or red-plastic liners
- ✓ Sharps/biohazard containers - rigid red or orange plastic containers for sharps waste

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The ***Phlebotomy Area*** should include a chair for the subject, a table for blood collection supplies, a bed, exam table, or treatment chair that flattens out, and phone/ intercom/physical access to emergency equipment. Accommodations should ensure that the subject can sit quietly in a chair for 5 minutes prior to the venous blood draw, as recommended by NCEP guidelines, and be adjacent to or near by a lavatory which will be used for urine specimen collections.

UNIVERSAL PRECAUTIONS

Universal Precautions was mandated into standard December 6, 1991, by the Occupational Safety and Health Administration (OSHA) in response to increasing public concern over possible transmission of the Acquired Immune Deficiency Syndrome (AIDS) virus and Hepatitis B virus. This standard states that any health care worker who might potentially come into contact with body fluids should be educated in infection control and treat all body fluids as though they are potentially infected.

It is assumed that you have already had training in universal precautions. The following is a summary of the basic knowledge required by health care workers, and is not intended to be a complete picture of universal precautions but only the basics. For a more complete overview of universal precautions, you can visit the following web sites:

- http://www.osha.gov
- http://www.niehs.nih.gov

According to OSHA, the following is the recommended protective barrier: gloves, gown, mask and goggles, or face-shield, and they should be used when handling any body fluids.

A. Gloves

1. Wear gloves for all patient contact when body fluids are involved.
2. Change gloves between patients and when gloves are soiled or torn.
3. Wash hands thoroughly after removing gloves.
4. Remove gloves before touching telephones, charts, computers, monitors, doorknobs, refrigerator handles, food, pens/pencils, and elevator buttons. The only exception to this is telephones designated as contaminated.
5. Carry spare non-sterile vinyl exam gloves in uniform/lab coat pocket for use with unexpected contact with blood and body fluids.

B. Gowns

Wear water-repellent gowns, plastic disposable aprons, etc. when soiling with blood or body fluids is anticipated.

C. Face-Shields

Protect mucous membranes (eyes, nose, mouth) by wearing a mask and/or glasses/goggles, or use a counter-top splashguard, etc. when performing procedures where splashing of the face is likely to occur (de-capping, decanting, etc.).

COLLECTION PROCEDURES

The SEARCH study population consists of participants between the ages of 0 and 24 years. For this reason, it is imperative that there be options in specimen collection procedures which are age appropriate. The following pages provide specific instructions for two different collection procedures and provide details concerning amounts allowable for blood collection from pediatric subjects (page 12).

Collected specimens should be shipped to the laboratory freshly drawn on the day of collection or on the following morning, or frozen on dry ice, in the case of Leptin/Adipo, DAA/Cpep, IL-6 and Apo/CRP/FIB/DGUC. Outlined below are two blood collection procedures: *Venipuncture*, for older children, adolescents and young adults, and *Skin Puncture,* when venipuncture is not feasible. **It is the phlebotomist's responsibility to determine the appropriate procedure to use.**

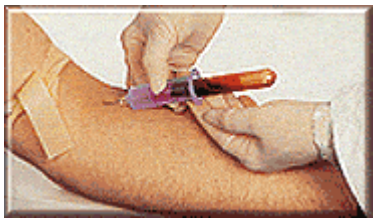
Blood Collection

As with universal precautions, it is assumed that you have already had training in blood collection and completed a phlebotomy course. This section is designed as a brief review of the basics, and also includes information specific to this study. For a more complete overview of blood collection procedures, you can visit a number of web sites. These sites are suggested only, and their usefulness must be determined individually. To choose from a list of sites, go to the following URL:

- http://phlebotomy.com/

Blood Collection for Older Children, Adolescents and Young Adults

It is understood that universal precautions will be employed during any specimen collection. The following is a suggested method of performing blood specimen collection by venipuncture.



1. Make positive patient identification.
2. Gather necessary equipment.
3. Wash your hands.
4. Don non-sterile exam gloves.
5. Explain planned procedure to patient.
6. Position patient's arm in comfortable position.
7. Select appropriate collection site.
8. Place the tourniquet above the selected collection site. Do not leave tourniquet on for longer than one minute.
9. Clean site with alcohol using circular motion from center outward; allow to air dry (using a gauze pad may re-contaminate the area).

10. Grasp arm 1-2 inches below the site to decrease vein rolling.
11. Enter the vein with the vacutainer needle bevel up at a 15 degree angle.
12. Fill necessary blood tubes and mix each specimen as required.
13. ***If venipuncture using regular size blood collection tubes does not provide a sufficient sample volume, continue specimen collection using microtainer tubes.***
14. ***For younger participants, use the pediatric microtainer system for collecting blood by venipuncture***.
15. ***Use skin puncture for blood collection only when strictly necessary.***
16. Place sharps in puncture resistant sharps container.
17. Apply gauze and tape holding pressure for 2 to 3 minutes to minimize the formation of a hematoma.
18. Remove gloves and wash hands.

Blood Collection for Younger Children and Infants

An alternate approach for specimen collection can be used for infants or those participants where it is difficult to perform a venipuncture. This procedure is referred to as a skin puncture. Capillary punctures may be performed on fingertips. These punctures may be used to obtain blood on all ages of patients although it is primarily used on infants, young children, and on others of whom it is difficult to perform a venipuncture.

Procedure

1. Select puncture site:
   Skin-puncture blood can be obtained from the:
   a) Palmar surface of the distal phalanx of a finger.

   - With older children and adults, the palmar surface of the last phalanx of a finger is most frequently used.

   When skin punctures are performed on **Child Fingers**, the following guidelines should be observed:

   - The puncture should be on the palmar surface of the distal phalanx and not at the side or tip of the finger because the tissue on the side and tip of the finger is about half as thick as the tissue in the center of the finger.
   - The fifth finger shall not be punctured because the tissue is considerably thinner than the tissue of the thumb, index, middle, and ring fingers.

2. Once it has been identified, warm the puncture site to increase the blood flow to the area. Warming the skin-puncture site can increase blood flow through the site seven-fold. It primarily increases the arterial blood flow.

   - Warm the area with a wet towel, warmed in 39-40°C water, for 3 to 5 minutes.
   - Do not leave the towel on the chosen site for longer than 5 minutes. The towel will cool the site as the towel itself begins to cool.

3. Put on gloves.

4. Cleanse the area with alcohol prep and allow to air dry.

   - *Use new alcohol preps for each successive puncture*.

5. Puncture the chosen site.

   - The use of a lancet with a tip length no greater than 2.4 mm is recommended for capillary punctures.
   - When puncturing the finger use a Free Flow lancet and puncture into the pulp of the finger, perpendicular to the puncture site. Use a new lancet for each puncture.
   - The puncture, regardless of the site, should be a quick deliberate motion, sufficiently deep so as to create a good blood return.

6. Wipe away the first drop of blood with a dry gauze pad.

7. Uncap the Microtainer, encourage blood flow to the area with moderate, intermittent pressure, and collect the blood into the appropriate tubes.

   - After puncture and wiping, a second drop of blood will form over the puncture site. When the tip of a micro-collection device touches this drop, blood will flow into the tube by capillary action. Blood flow from the puncture is enhanced by holding the puncture site downward and gently applying continuous pressure to the surrounding tissue (or proximal to the puncture site when the blood is obtained from a finger). ***Strong repetitive pressure (milking) should not be applied; it may cause hemolysis or contamination of the specimen with tissue fluid.***
   - Release pressure to allow capillary refill and obtain more blood.
   - If hard pressure is required to obtain blood, a second puncture should be performed. Squeezing too hard can result in hemolysis of the specimen necessitating a redraw.
   - Scooping the blood up from the surface of the skin should be avoided. Drops of blood should be allowed to flow freely into the collector top and down the walls of the tube. If a drop of blood becomes lodged inside the collector top, a gentle tap of

the tube on a hard surface is sufficient to move it to the bottom of the tube. When collecting an anticoagulated specimen, the specimen should be mixed well by inverting 8 to 10 times after the closure is placed on the tube. Then the tube should be given a quick shake, as if to shake down a thermometer, to remove excess blood from around the bottom of the closure.

8. At the end of the collection, apply pressure with a dry gauze pad over the puncture site until bleeding stops.

   *The use of adhesive bandages on children **under two years of age** should not be done on a routine basis for two reasons: 1) Neo-natal skin is very sensitive. 2) Young children have been known to remove the band-aid and put it in their mouth.*

9. Dispose of all the used equipment into appropriate discard container.

Maximum Allowable Blood Draw Volumes for Children

Please pay careful attention to the table below and plan your draw accordingly.

Body wt. in Pounds	Maximum drawn in one blood draw	Maximum drawn in a 30 day period
	2.5% of total blood vol.	5% of total blood vol.
2.2 lb	2.5 ml	5.0 ml
4.4 lb	4.5 ml	9.0 ml
3.3 lb	6 ml	12 ml
8.8 lb	8 ml	16 ml
11 lb	10 ml	20 ml
13.2 lb	12 ml	24 ml
15.4 lb	14 ml	28 ml
17.6 lb	16 ml	32 ml
19.8 lb	18 ml	36 ml
22 lb	20 ml	40 ml
24 thru 33 lb	22-30 ml	44-60 ml
35 thru 44 lb	32-40 ml	64-80 ml
46 thru 55 lb	42-50 ml	64-100 ml
57 thru 66 lb	52-60 ml	104-120 ml
68 thru 77 lb	62-70 ml	124-140 ml
79 thru 88 lb	72-80 ml	144-160 ml
90 thru 99 lb	82-90ml	164-180 ml
101 thru 110 lb	92-100 ml	184-200 ml
Greater than 111	100 ml	200 ml

Urine Collection

Urine collection procedures should be posted in the lavatory and explained to the participant or parent of the participant if he/she will assist with the collection. The Central Laboratory will provide laminated copies of the instructions below for this purpose.

Confirm that participants understand the following procedure:

ENGLISH

Female: Holding the labial folds apart with one hand, wipe once with the first wipe from front to back down the left fold and discard wipe; wipe once with the second wipe from front to back down the right fold and discard wipe; wipe once down the center from front to back and discard wipe. Void a small amount of urine into the toilet. Void urine into the sample collection cup without allowing the cup to contact anything but the flow of urine. Cap quickly.

Male: Wipe the tip of the penis and discard wipe. Void a small amount of urine into the toilet. Void urine into sample collection cup without allowing the cup to contact anything but the flow of urine. Cap quickly.

SPANISH

Hembra: Sujetando el pliegue labial, apártelo con una mano, límpiese una vez con el primer paño desde la parte delantera hacia la parte trasera y hacia la izquierda del pliegue y deseche el paño, límpiese una vez con el segundo paño desde la parte de delante hacia la parte trasera y hacia la derecha del pliegue y deseche el paño, límpiese una vez hacia el centro desde la parte delantera y hacia la parte trasera y deseche el paño. Vacíe una cantidad pequeña de orina en el inodoro. Vacíe la orina en el vaso de recolección de muestras sin dejar el vaso en contacto con ningún objeto excepto el fluido de la orina. Tápelo rápidamente.

Macho: Límpiese la punta del pene y deseche el paño. Vacíe una cantidad pequeña de orina en el inodoro. Vacíe la orina en el vaso de recolección de muestras sin dejar el vaso en contacto con ningún objeto excepto el fluido de la orina. Tápelo rápidamente.

Urine Collection for Infants

Urine collection for infants should be clearly explained to the parent of the participant. The Central Laboratory will provide laminated copies of the instructions below for this purpose. The instructions are also provided as an attachment (Attachment H) to be given to parents to take home with them. You may also want to personalize this attachment with site information and make multiple copies of it for distribution.

Supplies Provided

- Pediatric Urine Collection Bag
- Sterile Collection Cup with Lid

Procedure

1. Thoroughly wash the area around the urethra.

2. Open the pediatric urine collection bag, and place it on your infant.

3. For males, the entire penis can be placed in the bag and the adhesive attached to the skin. For females, the bag is placed over the labia.

4. Place a diaper over the infant (bag and all).

5. Check your baby frequently and remove the bag after the infant has urinated into it.

6. The urine should be drained into the provided collection cup and refrigerated until transport back to the clinic.

For active infants, this procedure may take a couple of attempts — lively infants can displace the bag, causing an inability to obtain the specimen.

First Morning Void Collections

Special Supplies required The 10mL transfer containers must be used to ship the 1st AM VOIDS specimens. Do not ship urine to the CBL using the standard urine collection cup. The urine cup caps are not designed for shipping and will leak, resulting in the sample being rejected.

Female Participants:
1 - Urine collection cup (transfer urine into 10mL collection tube at clinic)
3 - Antiseptic wipes

Male Participants:
1 - Urine collection cup (transfer urine into 10mL collection tube at clinic)
1 – Antiseptic wipe

Specimen Collection

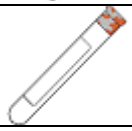
Instruct the participant to void the first void of the morning into their collection containers. The container should be kept refrigerated until it is returned to the clinic for processing.

Verify that the participant understands the instructions.

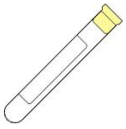
Instruct female participants that specimens should not be collected during menstruation.

Ask the participant if they have recently had a urinary tract infection. If the answer is yes, ask if they have completed medication for the infection. If they are still taking medication for the infection the collection should be rescheduled.

Collection Chart - COHORT Study Visit

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Clinic Instructions
HbA1c	Non-Fasting Acceptable	2.0mL EDTA purple-top		**Label: HbA1c** 1. Refrigerate 2. Do **NOT** centrifuge 3. Ship fresh
Lipid, Glucose, Creatinine Leptin, Adiponectin Cystatin C	Fasting	8.5mL SST tiger-top		**Label: Cystatin C** 1. Room temp 20-30 min. 2. Centrifuge 3. Transfer 0.5mL into 2mL blue top. Freeze and ship on dry ice. **Label: Leptin/Adipo** 4. Transfer 0.5mL into 2mL vial blue top. Freeze and ship on dry ice. **Label: Lipid/Glu/Creat.** 5. Transfer remaining volume into 10.0mL transfer tube and ship fresh.
DAA (GAD65, IA2, ZnT8) C-peptide IL-6	Fasting (If the subject is non-fasting, a specimen should still be collected for DAA and IL-6 analysis, which does not require fasting.)	3.5mL SST gold-top		**Label: DAA/Cpep** 1. Room temp 20-30 min. 2. Centrifuge 3. Transfer 1.0mL into a 2mL blue top. Freeze and ship on dry ice. **Label: IL-6** 4. Transfer 0.5mL into a 2mL blue top. Freeze and ship on dry ice.
ApoB hsCRP Fibrinogen DGUC Rf	Fasting	3.0mL EDTA purple-top		**Label: Apo/CRP/FIB/DGUC** 1. Ice for 20-30 min. 2. Centrifuge 3. Transfer all plasma into 2mL blue top. Freeze and ship on dry ice.
DNA Storage	Non-Fasting Acceptable	8.5mL ACD yellow-top		**Label: DNA** 1. Refrigerate 2. **Do Not** centrifuge and ship fresh.
Serum Storage	Fasting	8.5mL SST tiger-top		**Label: Serum Storage** 1. Room temp 20-30 min. 2. Centrifuge and ship fresh.
Plasma Storage	Fasting	2 – 5.0mL PPT pearl-top		**Label: Plasma Storage** 1. Ice for 20 – 30 min. 2. Centrifuge and ship fresh.
SPOT Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: SPOT Urine** 1. Completely fill the 10ml tube and ship fresh.
1st AM VOID Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: AM VOID Urine** 1. Completely fill the 10ml tube and ship fresh.

Collection Chart - REGISTRY Study Visit

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Clinic Instructions
DAA (GAD65, IA2, ZnT8) C-peptide	Fasting (If the subject is non-fasting, a specimen should still be collected for DAA analysis, which does not require fasting.)	3.5mL SST gold-top		**Label: DAA/Cpep** 1. Room temp 20-30 min. 2. Centrifuge 3. Transfer 1.0mL into a 2mL blue top and freeze.
HbA1c	Non-Fasting Acceptable	2.0mL EDTA purple-top		**Label: HbA1c** 1. Refrigerate 2. Do Not centrifuge 3. Ship fresh
Lipid, Glucose, Creatinine	Fasting	8.5mL SST tiger-top		**Label: Lipid/Glu** 1. Room temp 20-30 min. 2. Centrifuge 3. Ship fresh
DNA Storage	Non-Fasting Acceptable	8.5mL ACD yellow-top		**Label: DNA** 1. Refrigerate 2. **Do Not** centrifuge 3. Ship fresh
Serum Storage	Fasting	8.5mL SST tiger-top		**Label: Serum Storage** 1. Room temp 20-30 min. 2. Centrifuge 3. Ship fresh
Plasma Storage	Fasting	2 – 5.0mL PPT pearl-top		**Label: Plasma Storage** 1. Ice for 20 – 30 min. 2. Centrifuge 3. Ship fresh
SPOT Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: SPOT Urine** 1. Completely fill the 10ml tube and ship fresh.
1st AM VOID Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: AM VOID Urine** 2. Completely fill the 10ml tube and ship fresh.

Microtainer Collection

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Clinic Instructions
HbA1c	Non-Fasting Acceptable	EDTA Microtainer purple-top		**Label: HbA1c** 1. Refrigerate 2. Do Not centrifuge 3. Ship fresh
Lipid, Glucose	Fasting	2 x SST Microtainer Yellow-top		**Label: Lipid/Glu** 1. Room temp 20-30 min. 2. Centrifuge 3. Ship fresh
DAA (GAD65, IA2, ZnT8) C-peptide	Fasting (If the subject is non-fasting, a specimen should still be collected for DAA analysis, which does not require fasting.)	SST Microtainer Yellow-top		**Label: DAA/Cpep** 1. Room temp 20-30 min. 2. Centrifuge 3. Transfer 0.5 mL into a 2mL blue top and freeze.
ApoB hsCRP Fibrinogen	Fasting	EDTA Microtainer purple-top		**Label: ApoB/CRP/FIB** 1. Chill 20-30 min. 2. Centrifuge 3. Transfer 0.5mL of plasma to transfer vial and freeze 4. Ship on dry ice
SPOT Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: SPOT Urine** 1. Completely fill the 10ml tube and ship fresh.
1st AM VOID Urine Alb/Creat & Storage	Non-Fasting Acceptable	Urine collection cup and 10mL transfer vial		**Label: AM VOID Urine** 3. Completely fill the 10ml tube and ship fresh.

DETAILED INSTRUCTIONS

It is the phlebotomist's responsibility to determine the appropriate procedure to use for blood collection. Once determined, strict adherence to the instructions outlined below should be followed, referencing the appropriate table above for guidance.

Briefly, the steps to take are as follows:

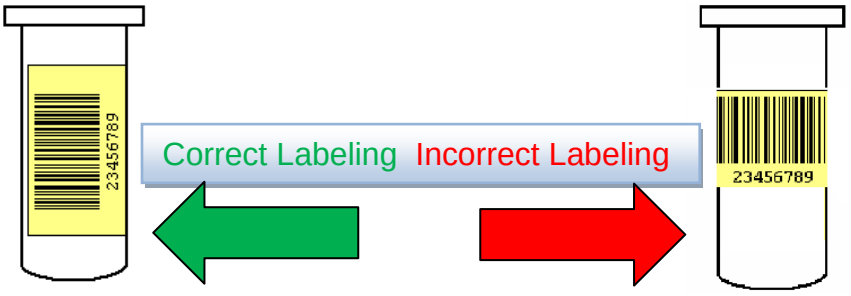
1. Label specimen tubes and vials.
2. Verify that the participant has reported as fasting: **8 hours minimum**, 10 hours recommended *(if noncompliant, if you choose to draw blood anyway, make sure to indicate actual number of hours in appropriate section of the specimen shipment form).*
3. Collect specimens.
4. Centrifuge and process.
5. Store vials to be frozen.
6. Ship fresh specimens to the laboratory.

How should the specimens be labeled?

The Central Laboratory provides labels to clinics. Participant ID and draw date information must be hand-written on the labels, as indicated. Do this before removing labels from their page, and use an indelible pen.

Affix the appropriate label (as denoted on the label) to each of the blood tubes, urine collection cup, and sample vials according to the instructions provided in the Visit Tables.

Label orientation is important for proper scanning of the barcode. Please affix labels to the collection tube and transfer tubes as shown here, with the barcode number running vertically.



In which order do I collect specimens?

Specimens should be collected in the order they are listed in the tables provided on pages 16-18. This order was developed to ensure that the most crucial specimens are collected with priority. Additionally, the following rules should be adhered to when collecting specimens:

As you draw blood, remember to:

- Mix each plasma blood tube (purple-tops, yellow-top and pearl-top) **8-10 times immediately** after collection by inverting the tube gently and evenly. This assures adequate mixing with the anticoagulant.

- The same needs to be performed with the serum tiger-top tubes, gold top tubes and yellow-top **microtainers** to assure adequate mixing of silica particles with the blood, which is required to activate clot formation. Gently invert these tubes **5 times**.

- **Avoid under-filling** the collection tubes. Purple-top collection tubes containing EDTA must be filled to at least 30% of the fill volume of the tube. If the tube is not filled to at least 30% of fill volume, there will be a dilutional effect from the anticoagulant and the specimen will be unsatisfactory for testing. For yellow-top DNA tubes, a minimum of 5 mL of blood should be collected by filling the tube to 60% of the fill level. Sites should not send any under-filled blood collection tubes to the Central Laboratory.

Once blood has been collected and mixed:

- Transfer plasma purple-tops, yellow-top, and pearl-top vacutainers© to an ice bucket (or refrigerator), leaving them **no longer than 30 minutes** prior to centrifugation. The purple-top tube *labeled for HbA1c* and the yellow-top *labeled for DNA* should be transferred to the refrigerator and remain there until shipment – they should **not** be centrifuged.

- To allow clot formation prior to their transfer to the centrifuge, the serum tiger-top tubes and yellow-top **microtainers** must stand upright at **room temperature for at least 20 minutes, but no longer than 30 minutes**. If there is a deviation of +/- 10 minutes, this fact should be noted on the specimen requisition form under notes.

Urine Specimen:

- Instruct the study participant, or parent of the participant if they will assist with collection, in the proper procedure for urine collection, and provide him/her with the necessary collection materials. *PLEASE NOTE:* Due to the possibility of blood contamination during collection, participants who are menstruating should not be asked to provide a urine sample.

- Transfer the filled urine collection cup to the refrigerator until later processing.

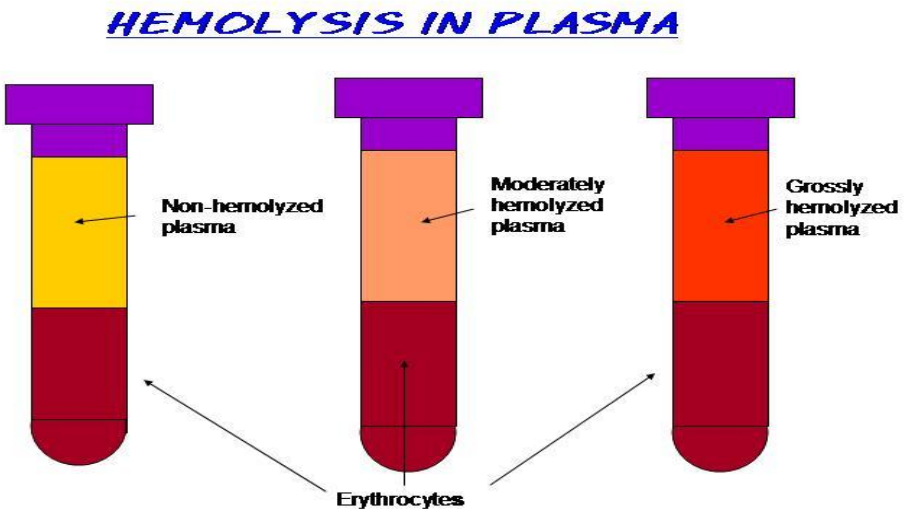
What causes hemolysis?

Causes of Hemolysis and/or Red Blood Cell Contamination

Please note that the accuracy of analysis depends on the quality of the samples. **Hemolysis can interfere with many of the laboratory analysis. To reduce the rate of hemolyzed or contaminated specimens, please review your specimen handling procedures with the rules shown below in mind. Please ensure that all centrifuged specimens are inspected for erythrocyte contamination. If erythrocytes (red blood cells) are observed floating in the serum or plasma layer, re-centrifugation is required. If the specimen is grossly hemolyzed (as shown below), collect a fresh specimen, and discard the old one. If there is an indication of sample hemolysis, this should be noted on the Specimen Shipment form.**

The usual causes of hemolysis and or erythrocyte contamination are as follows:

1. ***Specimen collection with needles smaller than 21 gauge***
2. ***Prolonged tourniquet application***
3. ***Improper centrifuge speed—all centrifuges need to be inspected annually to verify proper RPM ~ 3,000 +/-300 or 3500 RPM for free rotor centrifuges***
4. ***Improper or non- balancing of the centrifuge***
5. ***Prolonged centrifugation time which can lead to a heat build-up***
6. ***Centrifugation prior to clotting of the specimen***



How do I process specimens?

Centrifuge blood tubes (except the purple-top for HbA1c & yellow-top vacutainer for DNA):

After a strictly followed ***20 to 30 minutes*** standing at room temperature and chilling on ice/refrigerator, transfer the SST tiger-top, gold-top, PPT pearl-top and/or yellow-top microtainers to the centrifuge, loading the centrifuge according to the manufacturer's instructions.

Centrifuge at 1200 - 1500 RCF (g) [~3500 RPM] for 10 minutes.

Refrigerated Centrifuge

If using a refrigerated centrifuge, set the temperature to 4°C. Following centrifugation, transfer the tubes to a refrigerator set at 4°C or prepare the appropriate tubes for immediate shipment (as described in the next section).

Non-refrigerated Centrifuge

If using a non-refrigerated centrifuge, it is *imperative* that blood tubes not be allowed to sit unattended after rotation has ceased. This process generates heat and tubes must be immediately transferred to the refrigerator once spinning has stopped. It is recommended that a timer pinned to the lab coat be used to alert you when tubes will need to be transferred. ***Leaving tubes in a non-refrigerated centrifuge or at room temperature will compromise the accuracy of the analyses***.

Prepare frozen vials soon after centrifugation is complete:

To avoid degradation in C-peptide levels, the entire procedure of collecting, processing and solidly freezing specimens (at -20°C, -70°C or buried in dry ice) must be completed ideally within 1 hour but never exceeding 2 hours.

Serum Vacutainer/Microtainer Processing for DAA, C-peptide and IL-6

Obtain the SST tiger-top tube, gold-top tubes and/or the yellow-top microtainer after centrifugation and twist off the cap, ensuring that the last movement opens the stopper *away* from the face. **To avoid aerosol or possible splashing that may occur while uncapping the tubes, we strongly recommend using a face-shield or counter-top splashguard during this procedure.** Hold the tube upright in one hand. Obtain a disposable transfer pipette and decant **1.0mL** of serum into a 2mL blue-cap cryovial labeled DAA/Cpep and **0.5mL** into a 2mL blue-cap cryovial labeled IL-6.

Plasma Vacutainer/Microtainer Processing for ApoB, hsCRP, Fibrinogen and DGUC

Obtain the EDTA purple-top vacutainer or microtainer after centrifugation and twist off the cap, ensuring that the last movement opens the stopper *away* from the face. **To avoid aerosol or possible splashing that may occur while uncapping the tubes, we strongly recommend using a face-shield or counter-top splashguard during this procedure.** Hold the tube upright in one

hand. Obtain a disposable transfer pipette and decant **ALL** of plasma into a 2mL blue-cap cryovial labeled **ApoB/CRP/FIB/DGUC.**

Be extremely careful to not disturb the cell border with the pipette tip as this may allow red cells to contaminate the serum and plasma.

Recap the cryovial tightly; gently invert it several times to mix the sample, and place it in a Revco freezer storage box (vials from multiple participants should be kept in the same Revco box to maximize efficiency). The Revco box should be immediately placed at -20°C, -70°C or buried in dry ice, until its shipment to the Central Laboratory. Re-cap vacutainer and discard in a biohazard waste container.

Process Urine

SPOT Urines – Aliquot 10.5mL of urine into 10mL transfer vial, label and ship fresh on cold packs.

1st AM VOID Urines – Female and male participants will bring in their first morning void in a standard urine collection cup. Aliquot at 10.5mL of urine into the 10mL urine transfer vial, label and ship on cold packs.

Preparing Fresh Shipments

- Obtain the participant's label sheet used during specimen collection.
- Obtain a *Fresh Specimen Shipment Form* applicable to the participant's visit (attachment A or C).
- Use a "Fresh Specimen Shipment Form" and affix the "Central Laboratory Shipment Form Barcode Label." Neatly write participant ID and visit information in the spaces provided on the form.
- Obtain a polyfoam tube holder (with cardboard outer shell and absorbent pad) and place it open on the work surface.
- Obtain the blood and urine tubes from the refrigerator and place them on the work surface, preferably in a tube rack.
- Check the specimen tubes and verify the draw date and ID information. Complete the shipment form, checking-off the space corresponding to each specimen present. Should there be any missing vials, indicate the reason for this in the 'comments' section of the form.
- As the vials are being tallied and verified, place them on their sides in the open polyfoam tube holder in the slots provided (double-up the smaller tubes to save space), until all vials for a single participant set have been accounted for. ***Double-check that the urine tube is securely tightened***. Once a participant set is complete within the tube holder, place the absorbent pad on top of the vials, secure the top-half by properly aligning the slots and place the entire set into a cardboard outer shell.

- 1st AM VOID Urine Specimens should be <u>double bagged</u> in biohazard bags with an absorbent pad placed in the in the primary bag that contains the 120mL urine transfer tube.
- Place each tube holder into a biohazard bag and seal.
- Participant sets should then be transferred into shipping containers as outlined in the following section.

Preparing Frozen Shipments

Serum and plasma vials being stored in Revco boxes at -20°C or -70°C should be shipped to the Central Laboratory on dry ice. If the vials have been stored in dry ice only, due to no freezer access, they should be shipped **on the day of collection**. When possible, avoid shipping frozen specimens on Friday. **<u>NEVER SHIP SAMPLES ON SATURDAY</u>**

Please follow this procedure to prepare frozen specimens for shipment:

- On an on-going basis, the *Frozen Specimen Shipment Forms* (attachment B or D) should be filled-in as specimens are processed and transferred to the freezer. This way, when it is time to ship to the Central Laboratory, all that needs to be done is to verify the completed shipment forms with the vials included in the shipment. Do this by removing the lid of the Revco box, keeping the bottom half on dry ice, and quickly comparing the box contents to the shipment forms. Once the contents have been confirmed, replace the lid, keeping the Revco box on dry ice, and ready the shipping container as described in the next section.

SHIPPING INSTRUCTIONS
General Procedures

Blood tubes and urine specimens are shipped fresh with cold packs as coolant, within 24 hours of collection, via ***Priority Overnight*** *Federal Express courier service*. Frozen vials are shipped at least weekly on dry ice. In-the-field collection sites that do not have access to a -20°C freezer should place the processed frozen vials in dry ice and ship on the day of collection.

Shipment Forms

Master copies of the Specimen Shipment Forms are provided in the *attachments* section of this manual. Make multiple copies for your use. Specimen Shipment Forms include: **Fresh Shipment Form** & **Frozen Shipment Form**

These forms are used to indicate the number and types of vials included in the shipment, the identity of the samples, and pertinent clinic and visit information. The forms are organized so that one form must be filled out per subject per shipment for the fresh samples, and another per subject per visit for the frozen samples. When a shipment is made, a photocopy of the form(s) should be produced and retained at the clinic. The original(s) are placed in a ziploc bag and included with the specimens.

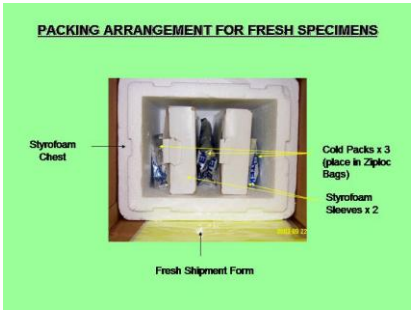
Shipping Containers and Coolant

Polyfoam shipping containers and cold-packs are provided by the Central Laboratory. ***Please freeze cold-packs in a -20°C freezer, as opposed to a -70°C freezer, as excessive freezing of the cold-packs may cause partial freezing of the specimens during transport, compromising the accuracy of analysis.***

To ship FRESH specimen sets, follow these steps:

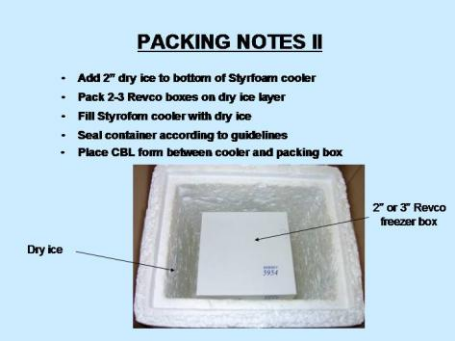
- Obtain a polyfoam shipping container and place up to 3 tube holders (with cardboard outer shells and containing the participant tubes sets) upright in the container, with each tube holder placed into a biohazard bag first. Fill with 2–3 cold-packs frozen at least overnight, preferably longer. To avoid possible leakage, place each cold pack in a plastic bag. Fit the polyfoam lid on tightly.

- Make a copy of each of the shipment forms and retain at the clinic. Place the originals in a ziploc bag, positioning them **inside** the polyfoam shipping container. Close the cardboard exterior shell around the polyfoam container and tape shut. Affix completed air bill and "Exempt Human Specimen" label to the container, choose **Priority Overnight**, and call FedEx for pickup.



To ship FROZEN specimen vials, follow these steps:

- Obtain a polyfoam shipping container and place one to two Revco freezer storage boxes containing the specimen vials inside (the Revco boxes should be placed inside biohazard bags first), fill the shipping container with dry ice to capacity, and fit the polyfoam lid on tightly. Label the shipping box with dry ice labels and "Exempt Human Specimen" label prior to shipping.



- Make a copy of the *Frozen Shipment Form(s)* and retain at the clinic. Place the original(s) in a ziploc bag, positioning it inside the polyfoam shipping container. Close the cardboard exterior shell around the polyfoam container and tape shut. Affix completed air bill to the container, choose **Priority Overnight**, and call FedEx for pickup.

Shipments should be addressed to:

SEARCH 3 Central Laboratory
Northwest Lipid Metabolism & Diabetes Research Laboratories
401 Queen Anne Avenue N.
Seattle, WA 98109
Phone: (206) 616-6474

FAX the Central Laboratory with the FedEx tracking number(s):

Any day a shipment is made, FAX the Central Laboratory, using a copy of the *Shipment Notification Fax* form (attachment E), to alert of a shipment's pending arrival. This will allow laboratory personnel to investigate and track packages if there are delays or problems with the courier. Make multiple copies of this form for your use since one will be used each day a shipment is made.

Fax the completed form to: 206-685-6880.

Shipping Schedule

Shipments may be made **Monday through Thursday** of the week. If a Friday shipment of fresh samples is required, follow the instructions below:

- Only ship one participant sample set per shipping container.
- Place as many frozen cold packs into the container as possible.
- Check **'Saturday Delivery'** on the air bill.
- Alert the laboratory of the shipment and its tracking # via fax.

Do not ship frozen samples on a Friday, unless there is no access to a freezer.
NEVER SHIP SAMPLES ON SATURDAY.

If fresh samples cannot be shipped on Friday, transfer serum or plasma from the blood collection tubes to transfer tubes and place them, along with the whole blood for HbA1c, in the refrigerator and ship the samples on Monday. In this circumstance DO NOT collect blood for DNA. **Follow the same procedure for samples collected during Saturday visits.**

Holiday Schedule

The CCL is officially closed on all US federal holidays and, more importantly, **FedEx will NOT deliver on these days.** Therefore, avoid shipping on any day *preceding* a US federal holiday (see calendar below).

When a holiday falls on a Monday or Tuesday, the last day to ship samples is the Thursday of the preceding week. The samples are expected to be delivered on Friday, but if there is a delay we will receive the samples on Saturday. When a holiday falls on a Friday, the last day to ship samples is the Wednesday of that week. The samples are expected to be delivered on Thursday, but this allows for receipt on Saturday if there are FedEx delays.

Due to the length of the **Thanksgiving holiday**, the last day to ship samples is the Monday of Thanksgiving week. The samples are expected to be delivered on Tuesday, but if there is a delay we will receive the samples on Wednesday.

Federal Holiday	2011	2012	2013
New Year's Day	Friday, December 31	Monday, January 2	Tuesday, January 1
MLK Jr's Birthday	Monday, January 17	Monday, January 16	Monday, January 21
President's Day	Monday, Feruary 21	Monday, February 20	Monday, February 18
Memorial Day	Monday, May 30	Monday, May 28	Monday, May 27
Independence Day	Monday, July 4	Wednesday, July 4	Thursday, July 4
Labor Day	Monday, Septmenber 5	Monday, September 3	Monday, September 2
Veterans Day	Friday, November 11	Monday, November 12	Monday, Nov. 11
Thanksgiving	Thursday, November 24 Friday, November 25	Thursday, November 22 Friday, November 23	Thursday, November 28 Friday, November 29
Christmas Day	Monday, December 26	Tuesday, December 25	Wednesday December 25

ATTACHMENTS - FORMS

A: COHORT - Fresh Shipment Form
B: COHORT - Frozen Shipment Form
C: REGISTRY - Fresh Shipment Form
D REGISTRY - Frozen Shipment Form
E: Fax Notification Form
F: Supply Request Form
G: Destruction of Specimen Request
H: Urine Collection for Infants
I: First Morning Urine Void Participant Instruction Handout
J: GUIDELINES FOR SHIPPING PATIENT SPECIMENS

The SEARCH 3 Central Laboratory

Northwest Lipid Metabolism and Diabetes Research Laboratories – University of Washington
401 Queen Anne Avenue North, Seattle, WA 98109-4517
Phone: (206) 616-6474 FAX: (206) 685-6880

COHORT STUDY - FRESH SPECIMEN SHIPMENT FORM

This form is used to accompany specimens drawn for a single subject and shipped to the Central Laboratory for analysis and storage. Refer to the Laboratory Manual of Procedures for detailed instructions – **This form is not an instructional sheet**. Once completed, photocopy this form and retain at the clinic. The original should be placed into a Ziploc bag, inside the polyfoam shipping container.

Shipment Form Barcode Label

Participant Information:

Patient ID Number ☐ (Site) ☐☐ (Sub-site) ☐☐☐☐☐ (Sequential ID)

Is this patient 18 years of age or older?

YES NO

Circle one

Type of Visit:

❑ COHORT Visit ❑ Redraw for Visit _____

Date of Visit:

☐☐ Month ☐☐ Day ☐☐ Year

Number of Hours Fasting:

☐☐.☐

Check ✓ Items Included in Shipment:

HbA1c	Whole blood	❑ EDTA tube 2.0mL purple-top	❑ EDTA Microtainer purple-top	❑ Missed
Lipid/Glu/Creat	Serum	❑ 10 ml transfer tube	❑ SST Microtainer yellow-top	❑ Missed
Serum Storage	Serum	❑ SST tube 8.5mL tiger-top	NA	❑ Missed
Plasma Storage	Plasma	❑ 2 PPT tubes 5.0mL pearl-top	NA	❑ Missed
DNA storage	Whole blood	❑ ACD tube 8.5mL yellow-top	NA	❑ Missed
SPOT Alb/Creatinine & Storage	Urine	❑ 10 mL transfer tube	NA	❑ Missed
1st AM VOID Alb/Creatinine & Storage	Urine	❑ 10mL transfer tube	NA	❑ Missed

It is important that the laboratory be able to contact the person who performed this visit and completed this form:

Contact: _____

Phone: _____

Email: _____

Comments: _____

_____

The SEARCH 3 Central Laboratory

Northwest Lipid Metabolism and Diabetes Research Laboratories – University of Washington
401 Queen Anne Avenue North, Seattle, WA 98109-4517
Phone: (206) 616-6474 FAX: (206) 685-6880

COHORT STUDY- FROZEN SPECIMEN SHIPMENT FORM

This form is used to accompany specimen(s) drawn from a single subject and shipped to the Central Laboratory for analysis. Refer to the Laboratory Manual of Procedures for detailed instructions – **This form is not an instructional sheet**. Once completed, photocopy this form and retain at the clinic. The original should be placed into a Ziploc bag, inside the polyfoam shipping container.

Shipment Form Barcode Label

Participant Information:

Patient ID Number ☐ (Site) ☐☐ (Sub-site) ☐☐☐☐☐ (Sequential ID)

Is this patient 18 years of age or older?

YES NO

Circle one

Type of Visit:

❑ COHORT Visit ❑ Redraw for Visit _____

Date of Visit:

☐☐ (Month) ☐☐ (Day) ☐☐ (Year)

Number of Hours Fasting:

☐☐.☐

Check ✓ Items Included in Shipment:

DAA (GAD/IA2/ZnT8) C-Peptide (SST 3.5mL gold top)	Serum	❑ 2.0mL Blue Cap Cryovial	❑ Missed
IL-6 (SST 3.5mL gold top)	Serum	❑ 2.0mL Blue Cap Cryovial	❑ Missed
Cystatin C (SST 8.5mL tiger top)	Serum	❑ 2.0mL Blue Cap Cryovial	❑ Missed
Leptin/Adiponectin (8.5mL SST tiger-top)	Serum	❑ 2.0mL Blue Cap Cryovial	❑ Missed
ApoB/CRP/FIB/ DGUC RF (EDTA 2.0mL purple)	Plasma	❑ 2.0mL Blue Cap Cryovial	❑ Missed

Comments:

_____

_____

_____

_____

It is important that the laboratory be able to contact the person who performed this visit and completed this form:

Contact: _____

Phone: _____

Email: _____

The SEARCH 3 Central Laboratory

Northwest Lipid Metabolism and Diabetes Research Laboratories – University of Washington
401 Queen Anne Avenue North, Seattle, WA 98109-4517
Phone: (206) 616-6474 FAX: (206) 685-6880

REGISTRY STUDY - FRESH SPECIMEN SHIPMENT FORM

This form is used to accompany specimens drawn for a single subject and shipped to the Central Laboratory for analysis and storage. Refer to the Laboratory Manual of Procedures for detailed instructions – **This form is not an instructional sheet**. Once completed, photocopy this form and retain at the clinic. The original should be placed into a Ziploc bag, inside the polyfoam shipping container.

Shipment Form Barcode Label

Participant Information:

Patient ID Number ☐ (Site) ☐☐ (Sub-site) ☐☐☐☐☐ (Sequential ID)

Is this patient 18 years of age or older?

YES NO

Circle one

Type of Visit:

❑ REGISTRY Visit ❑ Redraw for Visit _____

Date of Visit:

☐☐ Month ☐☐ Day ☐☐ Year

Number of Hours Fasting:

☐☐.☐

Check ✓ Items Included in Shipment:

HbA1c	Whole blood	❑ EDTA tube 2.0mL purple-top	❑ EDTA Microtainer purple-top	❑ Missed
Lipid/Glu/ Creat	Serum	❑ SST tube 8.5mL tiger-top	❑ SST Microtainer yellow-top	❑ Missed
Serum Storage	Serum	❑ SST tube 8.5mL tiger-top	NA	❑ Missed
Plasma Storage	Plasma	❑ 2 PPT tubes 5.0mL pearl-top	NA	❑ Missed
DNA storage	Whole blood	❑ ACD tube 8.5mL yellow-top	NA	❑ Missed
SPOT Alb/Creatinine & Storage	Urine	❑ 10 mL transfer tube	NA	❑ Missed
1st AM VOID Alb/Creatinine & Storage	Urine	❑ 10 mL transfer tube	NA	❑ Missed

Comments:

_____

_____

_____

_____

It is important that the laboratory be able to contact the person who performed this visit and completed this form:

Contact: _____

Phone: _____

Email: _____

The SEARCH3 Central Laboratory

Northwest Lipid Metabolism and Diabetes Research Laboratories – University of Washington
401 Queen Anne Avenue North, Seattle, WA 98109-4517
Phone: (206) 616-6474 FAX: (206) 685-6880

REGISTRY STUDY - FROZEN SPECIMEN SHIPMENT FORM

This form is used to accompany specimen(s) drawn from a single subject and shipped to the Central Laboratory for analysis. Refer to the Laboratory Manual of Procedures for detailed instructions – **This form is not an instructional sheet**. Once completed, photocopy this form and retain at the clinic. The original should be placed into a Ziploc bag, inside the polyfoam shipping container.

Shipment Form Barcode Label

Participant Information:

Patient ID Number ☐ ☐☐ ☐☐☐☐☐
Site Sub-site Sequential ID

Is this patient 18 years of age or older?

YES NO
Circle one

Type of Visit:

❑ REGISTRY Visit ❑ Redraw for Visit: _____

Date of Visit:

☐☐ ☐☐ ☐☐
Month Day Year

Number of Hours Fasting:

☐☐.☐

Check ✓ Items Included in Shipment:

DAA (GAD/IA2/ZnT8) C-Peptide (SST 3.5mL gold)	Serum	❑ 2.0mL Blue Cap Cryovial	❑ Missed

Comments:

_____

_____

_____

_____

_____

It is important that the laboratory be able to contact the person who performed this visit and completed this form:

Contact: _____

Phone: _____

Email: _____

FAX

The SEARCH 3 Central Laboratory

Northwest Lipid Metabolism and Diabetes Research Laboratories – University of Washington

TO: **Specimen Processing**
FAX: **(206) 685-6880**
Phone: (206) 616-6474

FROM: _____

Phone: _____

FAX: _____

PLEASE BE ADVISED OF THE FOLLOWING SHIPMENT ARRIVAL:

Clinical Site: _____ Number of boxes shipped: _____

Date specimens shipped: _____

Fed Ex Tracking Numbers: _____

_____

_____

_____

Remarks:

The SEARCH 3 Central Laboratory

SUPPLY REQUEST FORM

✸ALL REQUESTS FOR SUPPLIES SHOULD BE MADE AT LEAST✸
***TWO WEEKS* PRIOR TO THEIR ANTICIPATED NEED**

Clinic: _____Order Completed by: _____ Phone: _____

Date Ordered: _____/_____/_____ (MM DD YY) Date Needed: _____/_____/_____ (MM DD YY)

Date Request Received by Lab: ___/___/___ (MM DD YY) Date Supplies Shipped from Lab: ___/___/___ (MM DD YY)

ITEM	QUANTITY DESIRED	QUANTITY SHIPPED	QUANTITY PENDING
2.0mL Purple-top Vac.			
3.0mL Purple-top Vac.			
7.5mL Tiger SST Vac.			
8.5mL Tiger SST Vac.			
8.5mL Yellow-top Vac.			
5.0mL Pearl-top Vac.			
3.5mL Gold-top Vac.			
Purple-top Microtainer			
Yellow SST Microtainer			
Urine Collection Cup			
10mL Urine Tube			
Anitseptic Wipe			
Collection Bag for Infant			
Transfer Pipette			
2mL blue-cap Cryo Vial			
Polyfoam Tube Holders			
Cardboard Shell Holder			
Shipping Container			
Cardboard Shipping Box			
2” Revco Freezer Box			
Cold Packs			
Ziploc Bags			
Biohazard Adhesive Bags			
Biohazard Ziploc Bags			
COHORT Barcode Labels			
REGISTRY Barcode Labels			
FedEx Air bills			

Questions? Please call:
Marlon Ramirez at 206-685-3328

-Fill in the amount of each item desired on the table to the left.

-Fax the completed form to the lab:

206-685-6880

-Your order will be processed and shipped to you with a copy of this form enclosed.

Insure that contents exactly match the supplies specified on this form.

-If there are no discrepancies, sign the form and fax back to the lab.

If there are problems, please call:
206-685-3328

Comments:_____
_____
_____
_____

I have reviewed the contents of my shipment and confirm that all supplies listed have been received.

Signed: _____ Date: _____

Request for Sample Destruction

At any time, a participant (or their parent/legal guardian), who have given written consent for the collection and storage of DNA, serum, and/or plasma, may decide to withdraw their consent. They have the right to request that their stored specimens be retrieved and destroyed. The participant and/or their parent/legal guardian are entitled to written confirmation that the stored specimens have been destroyed.

The Request for Sample Destruction is used to communicate the participant's and/or the parent/legal guardian's request for stored specimens to be destroyed. The clinical site is responsible for conveying this request to the Central Chemistry Laboratory (CCL) where the specimens are being stored.

The Request for Sample Destruction form is divided into three sections. Section 1 is the request for stored specimen destruction and is completed by the clinical site. Sections 2 and 3 are completed at the CCL verifying that stored specimens have been retrieved and destroyed.

Upon receipt of a Request for Sample Destruction form from the clinical site, the CCL will destroy all stored specimens specified by the request. Destruction of stored specimens will be done in accordance with standard procedures for decontamination and removal of human specimens.

Section1 must be completed by the clinical site identifying the study participant and type of stored specimens to be destroyed. It is required that this section be signed by the Principal Investigator or Study Coordinator. After completion, the Request for Sample Destruction form is transmitted via FAX (206-616-4889 or 206-685-6880) or sent by regular mail to the CCL.

Section 2 is completed at the CCL. After receipt of the signed Request for Sample Destruction, the Database Administrator will identify the sample ids for the stored specimens involved. The sample id's along with the Request for Sample Destruction form will be forwarded to Specimen Management. Specimen Management will pull and deliver the identified samples to the Autoclave Technician. Specimen Management will sign their part of Section 2 after samples are retrieved from storage and delivered to autoclaving. Section 2 is also signed by the Autoclave Technician, who personally autoclaves and disposes of the samples, attesting that samples provided to him have been properly destroyed. Notation regarding storage status of the samples is then updated by the appropriate staff person at the CCL.

Section 3 is completed at the CCL. This section is signed by the Laboratory Director as final confirmation that removal and destruction of the samples has been properly performed and documented.

The completed Request for Sample Destruction is then faxed to the clinical site to confirm that the destruction of stored specimens has been completed. The Request for Sample Destruction is then filed at the CCL as confirmation that the destruction has been completed.

Request for Sample Destruction

SEARCH for Diabetes in Youth

CCL - Northwest Lipid Metabolism & Diabetes Research Laboratories
University of Washington
401 Queen Anne Avenue North
Seattle, WA 98109-4517

FAX 206-616-4889 or 206-685-6880

Section 1

Clinical Site: _____ Participant ID: _____ Collection Date: _____

I formally request that the vial(s) obtained from the above study participant containing the stored specimens checked below be disposed of and not retained for use in any research activities.

❑ DNA ❑ Plasma ❑ Urine Pellet **(check which is applicable)**
❑ Serum ❑ Urine

Signature: _____ Date: _____
Principal Investigator or Study Coordinator

Section 2

I attest that the samples requested for disposal have been identified, retrieved, and provided to the autoclave technician for destruction, and status of these samples updated in the database.

Signature: _____ Date: _____
Specimen Management

I attest that the samples delivered to me by Specimen Management have been destroyed in accordance with standard procedures for decontamination and destruction of human specimens.

Signature: _____ Date: _____
Autoclave Technician

Section 3

As requested by the Clinical Site on behalf of the study participant listed above, I confirm that all samples requested for disposal have been completely and properly destroyed.

Signature: _____ Date: _____
Santica M. Marcovina, PhD, ScD
Laboratory Director and PI

Section 1: to be completed by the Clinical Site, then fax or mail to the CCL.

Sections 2 & 3: to be completed by the CCL, then faxed or mailed back to the Clinical Site.

URINE INSTUCTIONS FOR INFANTS

Supplies Provided

- Pediatric Urine Collection Bag
- Sterile Collection Cup with Lid

Procedure

1. Thoroughly wash the area around the urethra.

2. Open the pediatric urine collection bag, and place it on your infant.

3. For males, the entire penis can be placed in the bag and the adhesive attached to the skin. For females, the bag is placed over the labia.

4. Place a diaper over the infant (bag and all).

5. Check your baby frequently and remove the bag after the infant has urinated into it.

6. The urine should be drained into the provided collection cup and refrigerated until transport back to the clinic.

For active infants, this procedure may take a couple of attempts — lively infants can displace the bag, causing an inability to obtain the specimen.

IF YOU HAVE QUESTIONS
PLEASE CONTACT:

_____

_____

_____

PATIENT INSTRUCTIONS FOR COLLECTING FIRST MORNING VOID

Female:

Females should not collect urine specimens during menstruation.

Holding the labial folds apart with one hand, wipe once with the first wipe from front to back down the left fold and discard wipe; wipe once with the second wipe from front to back down the right fold and discard wipe; wipe once down the center from front to back and discard wipe. Void urine into the sample collection cup without allowing the cup to contact anything but the flow of urine. Cap quickly. The container should be kept refrigerated until you bring the container with the urine to the clinic at your study visit. A fasting blood sample will also be collected.

Male:

Wipe the tip of the penis and discard wipe. Void urine into sample collection tube without allowing the tube to contact anything but the flow of urine. Cap quickly. The container should be kept refrigerated until you bring the container with the urine to the clinic at your study visit. A fasting blood sample will also be collected.

Guidelines FOR SHIPPING PATIENT SPECIMENS

GENERAL INFORMATION

Summary of Patient Specimen Exemptions: Under IATA DGR 2007, Section 3.6.2.2.3.6 permits certain types of patient specimens to be shipped with reduced documentation, labeling, and packaging if the specimens meet the standards for the exemption. Specimens that meet the following definitions and other criteria are qualified for the exemption; specimens that fail to meet the definition and other criteria must continue to be meet the 2007 rules:

1. Specimen must meet the following definition:

Specimens are those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components …being transported for purposes such as research, diagnosis, investigational activities, disease treatment, and prevention.

2. Minimal likelihood that the specimen contains a pathogen:

A patient …specimen is considered exempt if there is a minimal likelihood that pathogens are present. In determining whether a patient …specimen has a minimal likelihood that pathogens are present, an element of professional judgment is required to determine if a substance is exempt. This judgment should be based on the known medical history, symptoms, and individual circumstances of the source …and endemic local conditions.

Examples of specimens which MAY be transported under the exemption include the blood or urine tests to monitor cholesterol levels, glucose levels, or hormone levels, …tests required to monitor organ function such as heart, liver, or kidney function for humans…and antibody detection in humans…

Patient …specimens, for which there is minimal likelihood that pathogens are present may utilize the exemption, provided the specimen is in a packaging which will prevent any leakage. The packaging must meet the following conditions:

1. The packaging must consist of three components:

(a) a leak-proof primary receptacle (s);
(b) a leak-proof secondary packaging, and
(c) an outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm x 100 mm.

2. For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging so that during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the

cushioning material.

3. When multiple fragile primary receptacles are placed in a single secondary packaging, they must be individually wrapped or separated to prevent contact between them.

DOCUMENTATION

1. If dry ice is used as a refrigerant, mark "Dry ice, 9, UN1845, III on the air bill (check the dry ice checkbox on the FedEx air bill).

2. Check the "no" checkbox on the FedEx air bill in response to the question: "Does this shipment contain Dangerous Goods"

PACKAGING and LABELING

1. Place the "Exempt Human Specimen" label on the outside of the shipping box if the specimen contains no known pathogen.

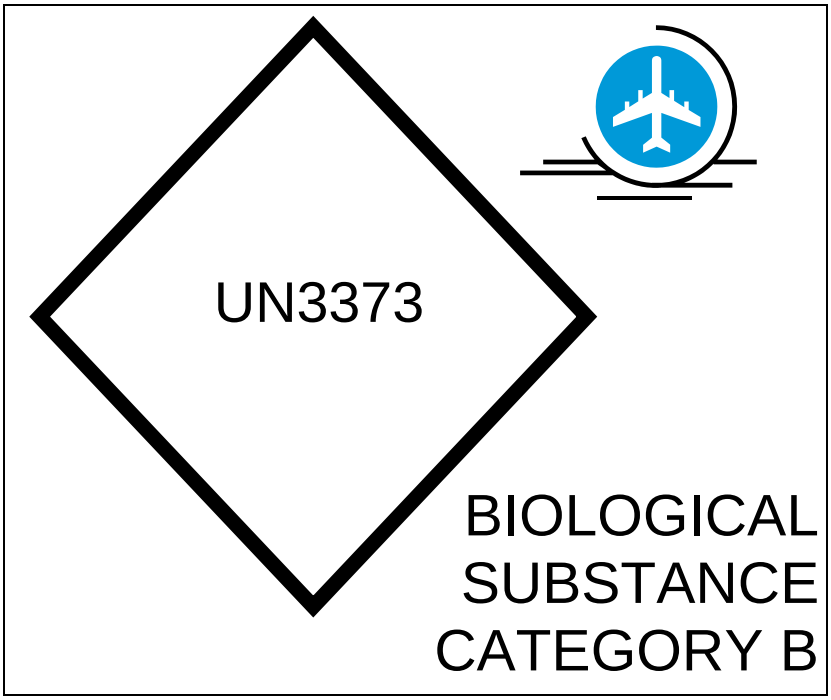
2. DO NOT use the "Biological Substance, Category B UN3373" label on the outer container unless you ARE aware the specimen contains a pathogen.

3. If dry ice is used as a refrigerant place the standard Dry Ice label on the outside of the shipping box and complete the required information on it.

Be certain to review these policies with your institution to assure compliance with your local policies or determinations. The information provided here is our recommendation for clinical sites to expedite shipments in the most efficient manner while maintaining compliance with IATA regulations.

Affix "Exempt Human Specimen" label on all shipments that have NO KNOWN PATHOGENS.

Affix this label to the outside box ONLY when you are AWARE you are sending specimens that contain **known** pathogens.



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SHIPPING DRY ICE REFRIGERATING A NON-DANGEROUS COMMODITY[1]

Step 1 Understand that Dry ice is a listed Dangerous Good. “Dry Ice” appears in bold print and is therefore a Proper Shipping Name. (“Carbon dioxide, solid” may also be used.)

UN/ ID No.	Proper Shipping Name/Description	Class or Div.	Sub Risk	Hazard Label(s)	PG	Passenger and Cargo Aircraft				Cargo Aircraft Only		S.P. see 4.4	ERG Code
						Ltd Qty							
						Pkg Inst	Max Qty per Pkg	Pkg Inst	Max Qty per Pkg	Pkg Inst	Max Qty per Pkg		
A	B	C		E	F	G	H	I	J	K	L	M	N
1845	Dry ice†	9		Miscel-laneous	III	—	—	904	200 Kg	904	200 Kg	A48	9L

Step 2 As a listed dangerous good packaging must conform to Packing Instruction #904. The Special Provision in Column M (“A48”) states that packaging tests are not considered necessary. (No UN packaging is required.)

Step 3 The General Packing Requirements (See Below) must be followed, but we may use any good, strong non-spec outer packaging designed to allow the outflow of dry ice vapors. The Shipper’s Declaration is not required for Dry ice and non-dangerous goods. Dry ice may be included in an over pack, provided the over pack meets the requirements of Packing Instruction 904.

IATA Dangerous Goods Regulations

PACKING INSTRUCTION 904

STATE VARIATIONS: USG-13

OPERATOR VARIATIONS: HP-02, IC-08, VN-11

☐ This instruction applies to UN 1845 on passenger and cargo aircraft and CAO.

The General Packing Requirements of 5.0.2 must be met.

Carbon dioxide, solid (dry ice), when offered for transport by air, must be in packaging designed and constructed to permi the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging.

Arrangements between shipper and operator(s) must be made for each shipment, to ensure ventilation safety procedures are followed.

The Shipper's Declaration requirements of Subsections 8.1 and 10.8.1 are only applicable when the Carbon dioxide, solic (dry ice) is used as a refrigerant for dangerous goods that require a Shipper's Declaration.

△ When a Shipper's Declaration is not required, the following information, as required by 8.2.3 for the Carbon dioxide, solic (dry ice), must be contained in the "Nature and Quantity of Goods" box on the air waybill:

- proper shipping name (**Dry ice** or **Carbon dioxide, solid**);
- UN 1845;
- the number of packages; and
- the net quantity of dry ice in each package.

The net weight of the Carbon dioxide, solid (dry ice) must be marked on the outside of the package.

Note 1: *Refer to the relevant airline's loading procedures for Carbon dioxide, solid (dry ice) limitations.*

Note 2: *For Air Waybill requirements see 8.2.3. For loading instructions see 9.3.12.*

Note 3: *For cooling purposes, an overpack may contain Carbon dioxide, solid (dry ice), provided that the overpack meets the requirements of Packing Instruction 904.*

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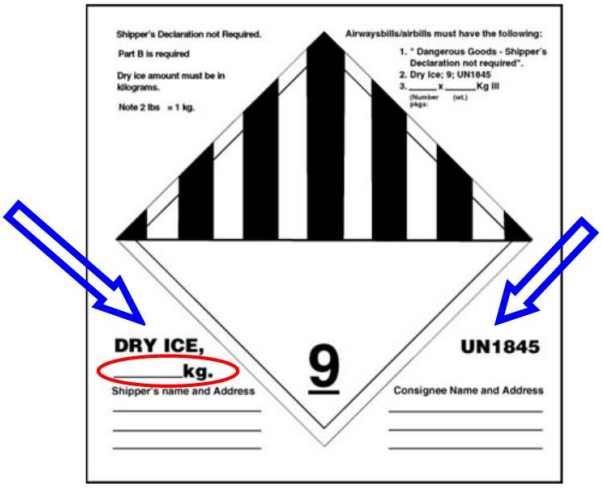
904

[1] This information is intended to promote safe shipping and handling by the University of Washington and those entities that conduct business with the University of Washington. It is not intended to meet any training requirements or to constitute a determination of compliance with the law. Any non-University of Washington entity must make an independent determination of compliance with the law.

Step 4 Mark and label the package. While you don't need a UN specification package or a DDG, the package must be marked and labeled. Mark the outside of the outer package with the gross weight of dry ice inside.

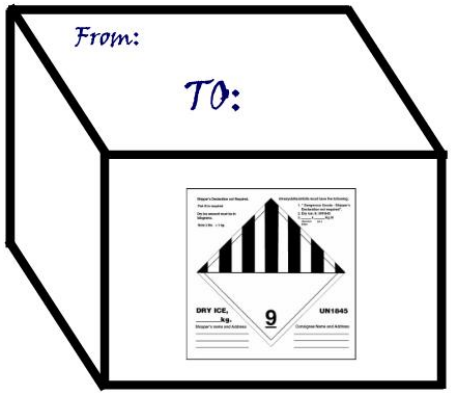
If you have one of these labels that has the proper shipping name and ID number pre-printed on it (See the arrows) then all you need to do is fill in the weight in Kg of the dry ice inside and list it in the encircled area.



If you use a regular class 9 label you will need to mark the box with the proper shipping name, UN number and the weight of dry ice in kilograms.

"Dry Ice"
"UN1845"
"___ Kg"

The Finished box should look like this:



In the "Special Handling" area of the waybill, you need to check the "Dry Ice" box and list the number of packages and net quantity of dry ice per package.