CIT-06 ISLET AFTER KIDNEY PROTOCOL

LABORATORY MANUAL
FOR CENTRAL LABORATORY ASSESSMENTS

VERSION 7.0

FEBRUARY 5, 2013

CONFIDENTIAL

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LIST OF APPENDICES

GLOSSARY OF TERMS

AIR_{glu}	Acute Insulin Response to Glucose
BG	Blood Glucose
CIT	Clinical Islet Transplantation
DCC	Data Coordinating Center
DI	Disposition Index
EDTA	Ethylenediaminetetraacetic acid
FSIGT	Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test
HbA1c	Hemoglobin A1c
IIT	Intensive Insulin Therapy
MMTT	Mixed Meal Tolerance Test
NIAID	National Institute of Allergy and Infectious Diseases (USA)
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases (USA)
NIH	National Institutes of Health (USA)
S_I	Insulin Sensitivity
SST	Serum Separator Tube
STS	Specimen Tracking System

1. STUDY CONTACT INFORMATION

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2. CIT-06 SPECIMEN LOGISTICS SCHEDULE

Central Laboratory Assessments					
Assessment	**Laboratory**	**Visit / Time-point**	**Volume**	**Collection Container**	**Shipping Instructions**
Albumin/Creatinine Ratio	University of Minnesota	01, 03, 10, 12, 15, 17, 19, 23 Y1	5 mL Urine	Sterile Urine Container	Ship on dry ice in batches at least weekly. Ship Monday – Thursday.
Alloantibodies	University of Pennsylvania	01 03 (Once prior to IS) 12, 13, 14, 15, 16, 17, 18, 19, 21, 23 Y1	2 mL Blood	(1) 3-mL Red-top Vacutainer	Ship on dry ice in batches at least quarterly. Ship Monday – Thursday only.
Atherogenic profile	University of Washington	03, 15, 19, 23 Y1	8.5 mL Blood	(1) 8.5 mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Autoantibodies	Barbara Davis Center	03 (Once prior to IS) 12, 13, 14, 15, 16, 17, 18, 19, 21, 23 Y1	2 mL Blood	(1) 3-mL Red-top OR (1) 3.5 mL Tiger top SST	Ship on dry ice in batches at least quarterly. Ship Monday – Wednesday only. If collected on Thursday or Friday, freeze serum at -20°C or -70°C until Monday, Tuesday, or Wednesday to ship.
Fasting serum glucose and c-peptide / serum creatinine (Note: the Fasting serum glucose and c-peptide/serum creatinine is the 0 hour sample for the MMTT)	University of Washington	01, 03 (10, 11, 12, 12a, 12b, 13, 13a, 13b, 14, 15)**q1mo** 16, 17, 18, 19, 20, 21, 22, 23 Y1	2 mL Blood	(1) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Insulin Modified FSIGT	University of Washington	03 **yrly** 12, 15, 19, 23 Y1	48 mL Total Blood 2 mL each at -10, -5, and -1 minutes pre-injection of glucose 2 mL each at 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post injection of glucose	(24) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Hemoglobin A1c (HbA1c)	University of Washington	01 (02, 03)** repeat per SOE** 10, 11, 12, 13, 14, 14b, 15, 16, 17, 18, 19, 20, 21, 22, 23 Y1, Y2, Y3	2 mL Blood	(1) 2-mL Lavender top EDTA Vacutainer	Ship on cold pack within 24 hours of collection. Ship Monday-Thursday only.

MMTT[1]: Stimulated serum Glucose and c-peptide	University of Washington	01, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 Y1	4 mL Total Blood 2 mL at 60, and 90 minutes each (Note: the Fasting serum glucose and c-peptide/serum creatinine is the 0 hour sample for the MMTT)	(2) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Plasma to Archive	NIDDK Repository	03 (Once prior to IS) 12, 13, 14, 15, 17, 19, 21, 23 Y1	4 mL Blood	(1) 4-mL Lavender top EDTA Vacutainer	Ship on dry ice in batches at least quarterly.
Serum to Archive	NIDDK Repository	03 (Once prior to IS) 12, 13, 14, 15, 17, 19, 21, 23 Y1	4 mL Blood	(1) 4-mL SST Tube	Ship on dry ice in batches at least quarterly.

[1]A Zero hour sample must always be drawn for the MMTT. If the SOE has an X by both the Fasting Serum Glucose/c-peptide & serum creatinine, this sample is used to obtain the zero hour sample. If the SOE has an X for MMTT but not for the Fasting Serum Glucose/c-peptide & creatinine

CIT-06 BLOOD VOLUME TABLE

CIT-06 MAXIMUM RESEARCH BLOOD VOLUME TABLE																										
TIME POINTS/VISITS																										
	DAYS						**WEEKS**				**MONTHS**															
TIMING OF STUDY PARTICIPATION	**SCR**	**IIT**	**WL**	**BL**	**0**	**3**	**1**	**2**	**3**	**4**	**2**	**2.5** (75d)	**6**	**9**	**12**	1 Yr post initial tx	**15**	**18**	**21**	**24**	2 Yr post initial tx	**27**	**30**	**33**	**36**	3 Yr post initial tx
VISIT	**1**	**2***	**3***	**4***	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12***	**13***	**14***	**15***	**Y1**	**16**	**17**	**18**	**19**	**Y2**	**20**	**21**	**22**	**23**	**Y3**
BLOOD VOLUMES																										
LOCAL LABORATORY ASSESSMENTS																										
Coagulation Studies (PT, PTT, INR)	5	5		5																						
Serology (Hep B, Hep C, HIV, HTLV)	7		7													7				7					7	
PRA			20													10				10					10	
CBC (WBC + Diff & Plat)	5	5	5	5	15		5	5	5	5	5	5	5	5	5	5	5	5	5	5		5	5	5	5	
Chemistries	4	4	4	4	12		4	4	4	4	4	4	12	12	12		4	4	4	4		4	4	4	4	
Thyroid Function	4		4																							
Blood type & HLA				11																						
Crossmatch				10																						
Fasting & post-prandial c-peptide						9	9																			
EBV IgG	2																									
CMV IgG, CMV IgM			4													4				4					4	
EBV by PCR			4	4																						
CMV by PCR			4	4																						
BKV by PCR	4		4	4																						
CENTRAL LABORATORY / METABOLIC ASSESSMENTS																										
HbA1c	2	8	12									2	2	2	4	2	2	2	2	2	2	2	2	2	2	2
Fasting glucose, c-pep & SCr	2		2							2	2	2	6	6	6	2	2	2	2	2		2	2	2	2	

CIT-06 MAXIMUM RESEARCH BLOOD VOLUME TABLE

TIME POINTS/VISITS																										
	DAYS						WEEKS				MONTHS															
TIMING OF STUDY PARTICIPATION	SCR	IIT	WL	BL	0	3	1	2	3	4	2	2.5 (75d)	6	9	12	1 Yr post initial tx	15	18	21	24	2 Yr post initial tx	27	30	33	36	3 Yr post initial tx
VISIT	1	2*	3*	4*	5	6	7	8	9	10	11	12*	13*	14*	15*	Y1	16	17	18	19	Y2	20	21	22	23	Y3
BLOOD VOLUMES																										
CENTRAL LABORATORY / METABOLIC ASSESSMENTS (*continued*)																										
60, 90 min c-pep/ glucose (MMTT)	4											2	2	2	2	2	2	2	2	2		2	2	2	2	
Insulin modified FSIGT			48									48			48	48										
Atherogenic profile				8.5											8.5	8.5				8.5					8.5	
IMMUNOSUPPRESSION LEVELS																										
Immunosuppression level				4	4	4	4	4	4	4	4	4	4	4	4		4	4	4	4		4	4	4	4	
MECHANISTIC ASSAYS																										
Alloantibody	2		2									2	2	2	2	2	2	2	2	2			2		2	
Autoantibody			2									2	2	2	2	2	2	2	2	2			2		2	
TAT, C3a, & C-peptide				2	8																					
ARCHIVED SAMPLES																										
Serum			4									4	4	4	4	4		4		4			4		4	
Plasma			4									4	4	4	4	4		4		4			4		4	
TOTALS (mL)	41	22	130	61.5	39	13	22	13	13	15	15	79	43	43	101.5	100.5	23	31	23	31	2	19	31	19	108.5	2

Pre-tx TOTAL (mL)	254.5
Y1 TOTAL (mL)	497.0
Y2 TOTAL (mL)	110.0
Y3 TOTAL (mL)	179.5
Cumulative (mL)	1041.0

Monthly testing required; for example, Visit 12a and 12b.

3. SPECIMEN LABELING AND COLLECTION KITS

Kits Supply

Specimen collection kits (collection tubes, labeling, and shipping containers) will be provided for each subject. Please refer to ***Appendix 1*** for a complete listing of kit components for CIT-06. ***Supplies are not provided for clinical tests performed at the local center***. Each kit and all collection tubes are labeled with a unique barcode label. Once a site is activated, an initial supply of kits (for 5 participants) and bulk materials will be sent to the site. It is the site study coordinator's responsibility to maintain an appropriate quantity of study materials at the site.

Bulk Supply

Bulk supplies are not subject specific or visit-based and are not pre-labeled with subject identification. An initial supply of bulk materials (tubes and shipping materials) will be shipped to the center upon study activation and site request. Bulk supplies are used in the following circumstances:

- When a specimen is collected as a re-draw at an unscheduled time point
- When a kit is unavailable for the specific visit
- When there is a tube breakage

Barcode Labels

Barcode labels will be provided pre-affixed to specimen collection tubes and cryovials in the kits. Additional labels will be provided in each kit. The labels include the following preprinted information: barcode symbol, barcode number, study number, visit number, site identification (ID) number, Subject ID number, specimen type (i.e., Alloantibody, Autoantibody). The label includes a space for the site to provide the date and time of collection.

How to Place an Order:

To place a supply order, the site personnel should complete either the Supply Order Form: Kit Supply ***(Appendix 2)*** or the Bulk Supply Order Forms: (Collection Containers/Materials ***- Appendix 3*** or Shipping Supplies ***– Appendix 4***, and fax or email the form to your CIT Protocol Coordinator at the DCC. Please allow at least two weeks for shipment of additional supplies. **Contact your DCC Protocol Coordinator if you do not receive email confirmation of your order within 48 hours.**

Kit and Bulk Supply Storage Conditions:

Specimen collection kits and bulk supplies should be stored at room temperature in a cool, dry location. Supplies provided have a shelf life and must be used before the expiration date. Please check kit inventory monthly for approaching expiration dates.

Specimen Tracking System (STS)

Specimens will be tracked using a Specimen Tracking System (STS) from the time of collection, processing at the local site, storage at the local site, shipment to the central laboratory, storage at the central laboratory, until final disposition at the central laboratory.

4. PACKING INSTRUCTIONS

All CIT specimens shipped to participating central laboratories are classified as Category B- UN3373, in which specimens are collected for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

The packaging must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport. Packaging must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

Packaging must consist of three components:

a. a primary leak proof receptacle (Vacutainer tube or cryotube);
b. a secondary leak proof packaging (poly bag); and
c. a rigid outer packaging.

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

Secondary packaging must be secured in the 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.

The **outer packaging** external surface of the shipping container should be labeled with UN3373 and marked "Biological Substance, Category B" and shipped to the central laboratory as defined below:

CIT Packing Instructions for Non-Infectious Frozen/Dry Ice Samples

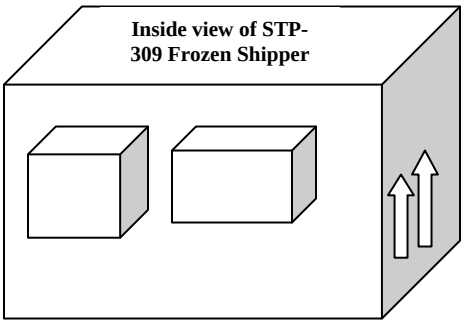
1. Insert frozen samples into provided cryoboxes. Place primary tubes in provided bubble pouches (STP-600).
2. Place the cryovial box or bubble pouches containing frozen tubes into the clear plastic bag (STP-711) containing the absorbent material. Seal the clear plastic bag by removing the white paper liner to expose the adhesive. Gently lay the adhesive covered tape over the bag opening. Gently tack together, pressing hard from the center working outward.
3. Place the clear plastic bag inside the white Tyvek envelope. Seal the Tyvek envelope by removing the white paper liner to expose the adhesive, and firmly pressing together.
4. Fill the box with dry ice by completely covering the bottom of the box (make note of the weight of dry ice being added, as this information is required to be placed on the Class "9"/UN1845 Dry Ice Label) Place the Tyvek envelope onto the dry ice (up to 2 cryoboxes may be placed into each shipper). Add more dry ice, making certain that the Tyvek envelope is completely surrounded on all sides. Gently shake to allow the dry ice to settle then add more dry ice to fill to the top.
5. Place the Styrofoam cover on the shipping box.
6. Place any shipping logs in an envelope on top of the Styrofoam cover.
7. Close the outer fiberboard box and seal the top and corners with tape.
8. Attach the following labels to the outer box:

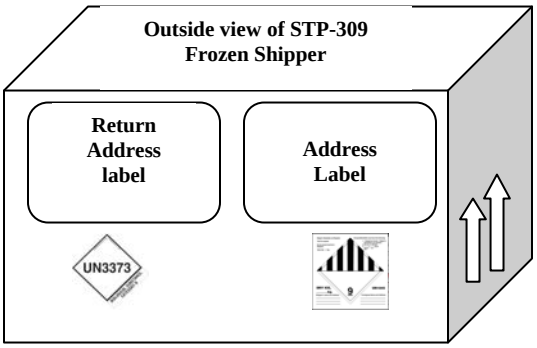
Label	Location on top of box
Return Address Label	Upper left corner
Address Label	Upper right corner
Black & White Class "9"/UN1845	Under Return Address Biological
Substance category B Label	Anywhere on front
Keep Frozen Label (optional)	Anywhere on front

9. Attach the airbill holder to the top of the package.

10. None of the labels should touch each other. Write in the amount of dry ice placed in the box on the black & white Class "9" / Dry Ice UN1845 label. This weight must be expressed in kilograms.
11. Complete the airbill (date of shipment, weight of dry ice in pounds, weight of shipment) and place in the airbill holder.
12. Ship only on Monday through Thursday by Federal Express using provided pre-printed air bills.

Please contact your CIT Protocol Coordinator with any questions regarding specimen packaging.

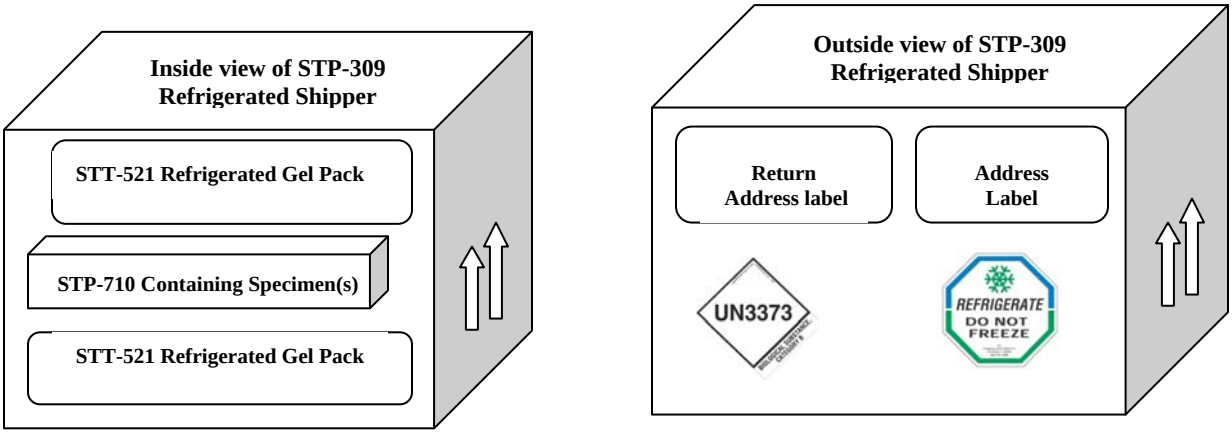




CIT Packing Instructions for Refrigerated Blood Samples

1. Pre-condition two STT-521-1000 (gel) packs to refrigerated temperature by placing in refrigerator overnight prior to use.
2. Place tube(s) in bubble wrap sleeve(s) provided.
3. Place bubble wrapped tubes inside leak-proof poly bag (STP 710 poly bag) with the sheet of absorbent paper.
4. Seal the poly bag and place inside Tyvek outer envelope.
5. Lay one gel pack flat inside the Styrofoam shipper. Press firmly into the shipping container.
6. Lay the Tyvek envelope containing the tube(s) on top of the gel pack.
7. Use the second gel pack to "sandwich" the envelope securely between the two gel packs.
8. Place the lid on the Styrofoam shipper and place any paperwork on top of lid. Seal the top and sides of the cardboard shipper with tape.
9. Attach the following labels to the front side of the box:
   Return Address Label
   Address Label
   UN3373 Biological Specimens Category B Label
   Keep Refrigerated Label
   Note: none of the labels should touch each other
10. Complete the airway bill (date of shipment, weight of shipment) and place in the airway bill holder.
11. Attach the airway bill holder to the top of the package.

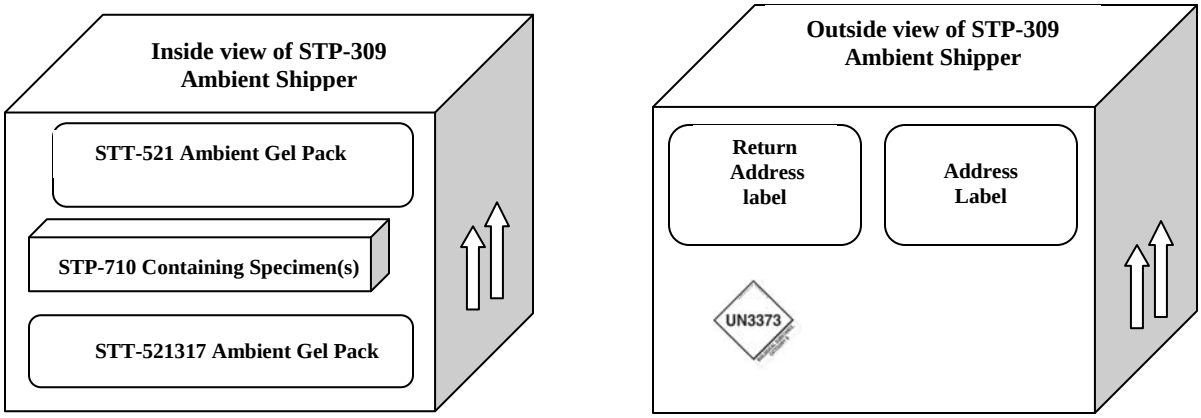
Please contact your CIT Protocol Coordinator with any questions regarding specimen packaging.



CIT Packing Instructions for Ambient Blood Samples

1. Pre-condition two STT-521-1000 (gel) packs to ambient temperature by leaving overnight at room temperature (20 -25°C) prior to use.
2. Place tube(s) in bubble wrap sleeve(s) provided.
3. Place bubble wrapped tubes inside leak-proof poly bag (STP 710 poly bag) with the sheet of absorbent paper.
4. Seal the poly bag and place inside Tyvek outer envelope.
5. Lay one gel pack flat inside the Styrofoam shipper. Press firmly into the shipping container.
6. Lay the Tyvek envelope containing the tube(s) on top of the gel pack.
7. Use the second gel pack to "sandwich" the envelope securely between the two gel packs.
8. Place the lid on the Styrofoam shipper and place any paperwork on top of lid. Seal the top and sides of the cardboard shipper with tape.
9. Attach the following labels to the top or side of the box:
   Return Address Label
   Address Label
   UN3373 Biological Specimens Category B Label (attach in designated area)
   Note: none of the labels should touch each other
10. Complete the airway bill (date of shipment, weight of shipment) and place in the airway bill holder.
11. Attach the airway bill holder to the top of the package.

Please contact your CIT Protocol Coordinator with any questions regarding specimen packaging.



5. SHIPPING INSTRUCTIONS

Federal Express is the chosen courier for the CIT studies. In general, specimens should be shipped **Monday- Thursday ONLY** and should NOT be shipped just prior to, or on holidays. Specimens that are to be batch-shipped should be shipped at least quarterly. All specimens should be shipped using overnight (next day) delivery. Some of the central labs have more specific shipping requirements. All specimens should be shipped according to the guidelines provided in the applicable Specimen Schedule.

- Federal Express Account Number: **4261-1614-6**
- Contact the appropriate laboratory personnel prior to specimen shipments. Please provide the FedEx tracking number to the appropriate laboratory personnel prior to specimen shipments.
- The top copy of the FedEx Airbill (Sender's Copy) should be retained to track the package. All shipments can be tracked using Federal Express on-line tracking (http://www.fedex.com) or by calling the toll-free number 1-800-Go-FedEx, Monday-Friday from 8:00AM-6:00PM.
- Be certain to include a completed Specimen Submission Form, date the specimen was obtained, and the name of the sending institution.

FOR U.S. TO U.S. SHIPMENTS:

Pick-up request: Prepare a shipment and schedule a courier online with FedEx Ship Manager at www.fedex.com/se_english. Access FedEx Ship Manager at fedex.com by clicking on "Ship" at the top of the screen from any page on fedex.com or **Call Federal Express @ 1-800-463-3339**. Give them the account number in section 5, payment of the FedEx Air bill, and the zip code of YOUR pickup address. FedEx will dispatch a courier to pick up the package.

FOR U.S. TO CANADA / SWEDEN OR CANADA / SWEDEN TO U.S.:

DO NOT USE THE FedEx US AIRBILL. You will need to complete the Expanded Service International Air Waybill. Please refer to ***Appendix 7*** and ***8*** for proper completion instructions. You will need to include 3 copies of the Commercial Invoice with each shipment**.** See ***Appendix 9*** for proper completion instructions).

For International Pick up call Federal Express @ 1-800-247-4747. Give them the account number, the FedEx Air bill number, and the zip/postal code of YOUR pickup address. FedEx will dispatch a courier to pick up the package.

Note: If you need assistance with International shipments, Federal Express International Customer Service can be reached at: 1-800-247-4747.

6. SPECIMEN COLLECTION, PROCESSING, AND SHIPPING PROCEDURES

For each of the tests listed below, please refer to the Specimen Logistics Schedule, located in Section 2 of this manual, for details regarding time-points, total blood volumes, and the central laboratory performing the analysis on the sample, as well as a summary of the collection tubes needed.

7.1 Albumin/Creatinine Procedure (First morning spot urine)

- The subject should be asked to do a ***first morning void*** midstream urine sample collection. This should be done at home the morning of their clinic appointment. The subject should be provided with explicit instructions and a ***sterile*** urine cup for the collection. The cup containing the specimen should be kept tightly closed and refrigerated, or on ice, until it is delivered to the clinic.
- Urine Collection Criteria
  1) The subjects should be asymptomatic for acute intercurrent illness, UTI, menstruation, and gross hematuria
  2) Confirm that the subject has discontinued non-steroidal anti-inflammatory medications (e.g., NSAIDs) for at least 48 hours prior to urine collection. Aspirin is acceptable.
  3) The subject should avoid strenuous exercise for at least 24 hours prior to the urine collection.
- At the clinic the specimen should be aliquoted into one pre-labeled 4.0 ml cryovial. Fill to approximately **75% of the volume.** Dipstick the urine using a Multistix. If the Multistix is positive for nitrites and leukocytes a clean catch specimen should be sent to the lab for urinalysis and urine culture to rule out a urinary tract infection (UTI). If the subject does have a UTI the specimen should be discarded and recollected at least one week after the subject has completed antibiotic therapy.
- Tube should be frozen at -70°C and shipped weekly.
- Follow packing instructions outlined in section 5 of this manual.
- Batch ship weekly, **frozen,** on dry ice. Specimens should be shipped on Monday-Thursday. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7).***

  University of Minnesota Medical Center
  Central Biochemical laboratory
  Room L-275 Mayo
  420 Delaware Street S.E.
  Minneapolis, MN 55455
  Phone: 612-273-3391

- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. An automatic email will be sent to jbucksa1@fairview.org.
- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-612-273-3489.

7.2 Alloantibody

- Collect 2.0 mL blood sample in a plain red top Vacutainer® tube.
- After a firm clot has formed, spin the Vacutainer® tube in the centrifuge at 2000-3000 RPM for 10 minutes.
- Aliquot a minimum of 1 mL of serum into the 1.8 mL cryovial.
- Freeze the aliquot at -70°C.
- Follow packing instructions outlined in section 5 of this manual.
- Ship samples on dry ice.
- Batch ship at least quarterly. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).
- ***Note: Shipments to this laboratory must be made Monday – Thursday.***

  Hospital of the University of Pennsylvania
  HLA Laboratory, Attention: Jane Kearns
  7 Founders Pavilion
  3400 Spruce Street
  Philadelphia, PA USA 19104
  Phone: +1-215-662-6010
- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. An automatic email will be sent to kearnsj@uphs.upenn.edu.
- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-215-662-3538.

7.3 Atherogenic Profile

- Subjects should fast for at least 10 hours prior to sample collection (Fasting recommended by the National Cholesterol Education program is 12 hours).
- Collect blood into an 8.5 mL SST Vacutainer®.
- Immediately after blood collection, the Vacutainer® should be inverted gently 5-6 times to allow the silica on the tube wall to mix with the blood thus activating the clot formation.
- Position the Vacutainers® in a tube holder and let the samples stand at room temperature for no less than 20 minutes but no longer than 30-45 minutes.
- Centrifuge (using a refrigerated centrifuge) the Vacutainer® at 1200-1500 RCF(g) [about 3500 RPM] for 10 minutes at 4˚C.
- Aliquot the following volumes of serum into labeled 1.8 mL cryovials as follows:

  1) Lipid Panel – 1.5 mL
  2) C-reactive protein – 0.5 mL
  3) Serum Amyloid A – 0.5 mL
  4) ApoAI/ApoB – 0.5 mL

- The MINIMUM amount of serum needed is 1.0 mL for the lipid profile and 0.35 mL for each of the other tests.
- Freeze samples immediately by placing the cryovials in a -20 or (ideally) -70 C freezer until ready to ship.

- Follow packing instructions outlined in section 5 of this manual.
- Batch ship weekly. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).
- Note: *It is recommended that shipments to this laboratory be made Monday - Thursday*. Do not ship on Friday or any day preceding national holidays. When a holiday falls on Friday, the last day to ship should be Wednesday. Due to the length of the Thanksgiving holiday and taking into account possible shipping delays, it is strongly recommended to NOT ship samples after Monday of Thanksgiving week.

  Northwest Lipid Metabolism and Diabetes Research Laboratories
  University of Washington
  Attention: Receiving Area
  401 Queen Anne Avenue North
  Seattle, WA USA 98109
  Phone: +1-206-685-3331

- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. An automatic email will be sent to smm@u.washington.edu.
- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.

7.4 Autoantibody

- Collect 2.0 mL blood sample plain red top (no additives) or Gold SST tube.
- The blood sample should be allowed to clot.
- Once the blood has clotted the tube should be centrifuged. Spin the tube for 10 minutes at 1500g (approximately 3000 RPM).
- After removing the tube from the centrifuge the serum should be removed and transferred to a 1.8 mL cryovial with a screw cover.
- Freeze the tubes in a -20°C or -70°C freezer.
- Follow packing instructions outlined in section 5 of this manual.
- Batch ship at least quarterly on dry ice. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (with dry ice) (***Appendix 7***)
- ***Note: Shipments to this laboratory must be made Monday – Wednesday.*** If sample is collected on Thursday or Friday, freeze serum at -20°C or -70°C until Monday, Tuesday, or Wednesday and ship in dry ice.

  Barbara Davis Center
  Attn: Dr. Liping Yu
  M20-4201E
  1775 Aurora Ct, UC Denver, AMC
  Aurora, CO 80045
  Phone: 303-274-6809

- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. An automatic email will be sent to Liping.yu@ucdenver.edu.

- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-303-724-6839.

7.5 Fasting Serum Glucose, C-Peptide, and Serum Creatinine

- Collect 2 mL blood in a 3.5 mL Gold SST. (Note: this sample is also the 0 hour serum glucose & c-peptide for the MMTT.)
- Allow blood to clot at room temperature a minimum of 20 minutes but no longer than 40 minutes, in a vertical position.
- Centrifuge (using a refrigerated centrifuge) the Vacutainer® at 1000-1300 RCF (g) [about 3,000 RPM] for 10-15 minutes.
- Prepare (2) 1.8 mL cryovials, with the specimen type labeled as follows:
  - Fasting Serum Glucose and Serum Creatinine
  - Fasting C-Peptide
- Using a disposable plastic pipette, transfer about 0.5 mL of serum into each cryovial.
- Freeze the samples immediately by placing the cryovial in a -20°C or -70°C freezer.
- Follow packing instructions outlined in section 5 of this manual.
- Batch ship at least weekly. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).
- ***Note: It is recommended that shipments to this laboratory be made Monday – Thursday.***

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- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.
- Per the SOE, the fasting serum glucose, creatinine and c-peptide may be drawn locally at some visits. See Section 8 for instructions for local draws and shipping from local lab to site.

7.6 FSIGT (Insulin-Modified Frequently-Sampled Intravenous Glucose Tolerance Test)

- Subjects may require basal insulin overnight prior to testing in order to meet fasting glucose criteria. The subject may be hospitalized overnight and placed on intravenous insulin, if necessary. If the subject is not hospitalized overnight, the

subject should arrive to the hospital in the early morning for a short-term insulin infusion to achieve glucose criteria, if necessary.

- Because of the inherent variability in each individual subject's response to insulin (and to different insulin preparations), the following **guidelines are suggested** to achieve as close to a steady state in regards to both insulin and glucose levels at the time glucose criteria are met and testing proceeds:
- Fasting should begin at 2100. the evening before testing, and any evening long-acting insulin should be administered at this time: NPH may be given at its regular dose; pre-transplant glargine may be given at its regular dose, but the dose of glargine should be reduced by at least 50% in subjects post-transplant; further dose reductions or dose eliminations can be performed at the discretion of the transplant endocrinologist.
- Rapid-acting insulin (lispro or aspart) may be administered via injection or pump bolus prior to 3 a.m. if necessary to correct hyperglycemia (capillary glucose > 140 mg/dl); basal lispro or aspart administered via a pump may be continued until 6 a.m. when the rate should be reduced by 50%; basal pump insulin should be turned off 30-min prior to baseline blood sampling.
- Intravenous regular insulin may be administered as needed prior to testing to achieve fasting glucose criteria in addition to (for early morning arrivals) or in place of the above (for overnight hospitalizations): an intravenous bolus of insulin may be administered up to 30-min prior to baseline blood sampling; basal intravenous insulin should be turned off 20-min prior to baseline blood sampling. See ***Appendix 5*** for the Insulin Infusion Protocol.
- Time = 0 should occur at 9:00 a.m. for the FSIGT (suggested allowable range 8:00 a.m. – 10:00 a.m.).
- Intravenous catheters for the FSIGT should be in place 30-min prior to the collection of baseline blood samples. The blood sampling catheter should be placed in the contra-lateral hand or arm from the infusion catheter, which should be placed in a high flow antecubital vein. The site of the blood sampling catheter should be warmed with a heating pad to promote arterialization of the venous blood. If absolutely necessary, the blood sampling catheter can be placed in the ipsilateral hand distal to the infusion catheter. Both the infusion and blood sampling catheter should be kept patent with slow infusions of 0.9% saline. The blood sampling catheter should be flushed with saline between each sample using a 3-way stop cock to ensure the precision of each sample.

Prior to the start of testing the capillary or serum glucose results should be:

- The pre-transplant BG value should be between 70-140 mg/dL (3.89-7.77 mmol/L).
- The post-transplant BG value should be between 70 – 115 mg/dl (3.89 – 6.38 mmol/L).

Specimen Collection

The acute insulin response to glucose (AIR_{glu}), insulin sensitivity (S_I), and disposition index (DI) will be determined using the FSIGT test.

- Time points for blood collection:
  - baseline (t= -10, -5, and -1 minute)
  - t= 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post-injection of glucose (0.3 g/kg over 1 minute starting at -30 sec) with an injection of insulin (0.03 U/kg over 30 seconds) at t=20 minutes.

- At each of the 24 time points listed above, draw 1 mL blood to be discarded as waste. Because the blood is sampled from an IV line that should be flushed between samples using a 3-way stop cock, it is necessary to draw the 1 mL discard prior to each FSIGT sample.
- After the 1 mL discard, draw an additional 2 mL blood into Gold SST at the time points listed above. An FSIGT worksheet can be located in ***Appendix 6***.
- Total blood collected should be 72 mL (2mL sample + 1mL waste each at 24 time points= 72 mL total). Total blood shipped for the FSIGT test is 48 mL (2 mL sample for each 24 time points = 48 mL total).
- Allow blood to clot a minimum of 20 minutes but no longer than 40 minutes, in a vertical position.
- Centrifuge (using a refrigerated centrifuge) the Vacutainer® at 1000-1300 RCF (g) [about 3,000 RPM] for 10-15 minutes at 4°C.
- Aliquot the following volumes of **serum** into labeled specimen tubes
  - Glucose- 150 µL
  - C-peptide- 300 µL
  - Insulin- 300 µL
- **Pre-transplant** - prepare 2 cryovials (1 labeled glucose and 1 labeled insulin) for each of the time point identified below (Total 48 cryovials).
  - baseline (t= -10, -5, and -1 minute) pre-injection of glucose.
  - t= 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post-injection of glucose.
- **Post-transplant** - prepare 3 cryovials (1 labeled glucose, 1 labeled c-peptide, and 1 labeled insulin) for each of the time point identified below (Total 30 cryovials).
  - baseline (t= -10, -5, and -1 minute) pre-injection of glucose.
  - t= 1, 2, 3, 4, 5, 7, 10, minutes post-injection of glucose.
- **Post-transplant** - prepare 2 cryovials (1 labeled glucose and 1 labeled insulin) for each of the time point identified below (Total 28 cryovials).
  - t= 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post-injection of glucose.
- Freeze the sample immediately by placing the cryovial in a -80°C freezer within 1 hour of blood draw.
- Follow packing instructions outlined in section 5 of this manual.
- Batch ship weekly. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***)
- ***Note: It is recommended that shipments to this laboratory be made Monday – Thursday.***

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- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.

7.7 Hemoglobin A1c

- Collect 2 mL blood into lavender top Vacutainer® tube (EDTA), and gently invert the Vacutainer® 8-10 times. The tube should be filled to capacity, due to the need to have an appropriate proportion of EDTA and blood.
- Refrigerate the sample immediately and ship on cold pack.
- Follow packing instructions outlined in Section 5 of this manual. Pack on cold pack (do not use dry ice) in the shipping container outside the leak-proof plastic container holding the tubes.
- In order for the results to be reported within 72 hours, the specimens should be shipped within 24 hours of collection, using overnight delivery. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (Without Dry Ice) (***Appendix 8***)
- ***Note: Shipments to this laboratory must be made Monday – Thursday.***

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- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.
- Per the SOE, the Hemoglobin A1c may be drawn locally at some visits. See Section 8 for instructions for local draws and shipping from local lab to site.

7.8 Mixed Meal Tolerance Test (MMTT): Stimulated Serum Glucose and C-Peptide

Insulin Management

- Subjects may require basal insulin overnight prior to testing in order to meet fasting glucose criteria. The subject may be hospitalized overnight and placed on intravenous insulin, if necessary. If the subject is not hospitalized overnight, the subject should arrive to the hospital in the early morning for a short-term insulin infusion to achieve glucose criteria, if necessary.
- Because of the inherent variability in each individual subject's response to insulin (and to different insulin preparations), the following **guidelines are suggested** to achieve as close to a steady state in regards to both insulin and glucose levels at the time glucose criteria are met and testing proceeds:
- Fasting should begin at 20:00 the evening before testing, and any evening long-acting insulin should be administered at this time: NPH may be given at its regular dose; pre-transplant glargine may be given at its regular dose, but the dose of glargine

should be reduced by at least 50% in subjects post-transplant; further dose reductions or dose eliminations can be performed at the discretion of the transplant endocrinologist.

- Rapid-acting insulin (lispro or aspart) may be administered via injection or pump bolus prior to 3 a.m. if necessary to correct hyperglycemia (capillary glucose >140 mg/dl (7.77 mmol/L); basal lispro or aspart administered via a pump may be continued until 6 a.m. when the rate should be reduced by 50%; basal pump insulin should be turned off 30-min prior to baseline blood sampling.
- Intravenous regular insulin may be administered as needed prior to testing to achieve fasting glucose criteria in addition to (for early morning arrivals) or in place of the above (for overnight hospitalizations): an intravenous bolus of insulin may be administered up to 30-min prior to baseline blood sampling; basal intravenous insulin should be turned off 20-min prior to baseline blood sampling. See ***Appendix 5*** for the Insulin Infusion Protocol.
- Time = 0 should occur at 9:00 a.m. (suggested allowable range 8:00 a.m. – 12:00 p.m.) The MMTT lab kits do not have a vacutainer or aliquot for the 0 hour sample. The glucose/c-peptide & serum creatinine sample is the 0 hour MMTT sample.
- If the BG value is between 70-180 mg/dL (3.89-10.0 mmol/L) prior to the MMTT at the local center, then fasting serum glucose and C-peptide levels will be drawn.
- If BG is <70 mg/dL (3.89 mmol/L) or >180 mg/dL (10.00 mmol/L) at the local center, the test will be rescheduled for the next possible day.
- The subject will receive 6 mL per kg body weight (maximum 360 mL) of Boost®, High Protein Drink, or a nutritionally equivalent substitute, consumed in 5 minutes starting at time 0.

Specimen Collection

- Collect 2 mL blood in a Gold SST for stimulated serum glucose and C-peptide at each time-point listed on the Specimen Schedule for the applicable visit.
- Allow blood to clot at room temperature for a minimum of 20 minutes but no longer than 40 minutes, in a vertical position.
- Centrifuge (using a refrigerated centrifuge) the Vacutainer® at 1000-1300 RCF (g) [about 3,000 RPM] for 10-15 minutes.
- Prepare (2) 1.8 mL cryovials for each time point and label the specimen with the time-point of collection and the specimen type (i.e. 60 minute serum glucose).
- Using a disposable plastic pipette, aliquot about 0.5 mL serum into each of 2 cryovials.
- Freeze the samples immediately by placing the cryovial in a -20°C or -70°C freezer.

TO AVOID DEGRADATION OF SPECIMEN, THE ENTIRE PROCEDURE OF COLLECTING, PROCESSING AND FREEZING THE SAMPLES MUST BE COMPLETED WITHIN 1 HOUR. THEREFORE, SAMPLES FOR 60 AND 90 MINUTES ANALYSES SHOULD BE PROCESSED SEPARATELY.

- Follow packing instructions outlined in section 5 of this manual.
- Batch ship at least weekly. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).

- ***Note: It is recommended that shipments to this laboratory be made Monday – Thursday.***

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7.9 Plasma to Archive

- Draw (1) 4-mL of blood directly into an EDTA Vacutainer® (lavender top tube), Centrifuge at 2,500 rpm for 10 minutes and aliquot 1.5 mL of plasma into a 1.8 mL sterilized cryovial.
- Follow packing instructions outlined in section 5 of this manual.
- Batch ship at least quarterly on dry ice. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).

  Fisher BioServices Corporation
  Attention: Sandra Ke or Heather Higgins
  20301 Century Blvd, Bldg 6, Suite 400
  Germantown, MD USA 20874
  Phone: +1-240-686-4702
- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. An automatic email will be sent to heather.higgins@FisherSci.com and sandra.ke@FisherSci.com.
- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form (***Appendix 10***) and fax a copy of the form including the airbill tracking number to the laboratory at +1-301-515-4049.

7.10 Serum to Archive

- Collect 4-mL of blood in a Gold SST tube. Allow the vacuum to be exhausted.
- Gently invert tube 5 times, ensuring mixing of clot activator with blood.
- Allow blood to clot for a minimum of 30 minutes but no longer than 60 minutes in a vertical position.
- Centrifuge between 1100 and 1300 g (about 2500 – 3000 RPM) for 10 minutes in a swing-head unit, or for 15 minutes in a fixed angle unit. Be sure to balance the centrifuge.
- A barrier will form, separating serum specimen from clot.

- Aliquot specimen equally into 3 – 1.8mL cyrovials and freeze at -70°C.
- Follow the packing instructions outlined in Section 5 of this manual.
- If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (***Appendix 7***).
- Batch ship at least quarterly on dry ice. Ship Monday – Thursday.

  Fisher BioServices Corporation
  Attention: Sandra Ke or Heather Higgins
  20301 Century Blvd, Bldg 6, Suite 400
  Germantown, MD USA 20874
  Phone: +1-240-686-4702
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7. SPECIMEN COLLECTION AND SHIPPING PROCEDURES FROM LOCAL LABS TO CLINICAL SITE

Some of the central lab samples can be drawn locally at a lab convenient to the study subject. The samples that can be drawn locally are identified in each protocol's Schedule of Events. Samples that are drawn locally must still be scanned through the CIT Specimen Tracking System. Therefore, these samples must be shipped from the lab where they were collected to the clinical site. After scanning and processing the samples, the clinical site will ship them to the central laboratory.

Tips for Local Sample Collection and Shipping to the Clinical Site

- While the subject is in the clinic for a visit prior to one of the visits that allows for local sample collection, discuss whether the subject will be having the samples drawn at the clinical site or locally.
- For local draws, prepare the appropriate lab kit.
  - Enter the Subject ID on the requisition form, the tube(s) and aliquots
  - Complete the "Prepare for Visit" step in the CIT Specimen Tracking System to assign the kit to the subject
- Send the subject home with all of the appropriate shipping supplies (see Section 4, Packing Instructions)
  - Include cold packs, biohazard bags and all required labels
  - Complete the Fed Ex airbill addressed to your clinical site
    - If sample will be shipped frozen, make sure the dry ice section is completed (FedEx will not ship the sample if the amount of dry ice is not clear)

  - If dry ice is needed, a local vendor for dry ice can be identified by searching Google for dry ice and the subject's zip code.
- Complete and send the "Subject Instructions" sheet home with subject (Appendix 11)
- Complete and send "Local Lab Instructions" on how to draw, process and ship the sample to the clinical site (appendix 12).
- It is helpful to talk to the local lab manager prior to the first collection to discuss logistics and arrange payment.
- When the samples are received back at the clinical site, scan and complete the "Receive Specimens in Lab", "Process and Aliquot Specimens", and "Ship Specimens from the Lab" steps in Specimen Tracking and ship samples to the appropriate central lab for processing. Note: HbA1c samples should be received at the central lab within 1-2 days of the time of draw. So, if these samples are to be drawn locally, they must be processed and shipped in a timely manner in order to assure accurate results.

APPENDIX 1: CIT-06 KIT COMPONENTS

Note: Kits for REPEAT procedures are listed at the end of Appendix 1.

VISIT 01 **Screening**	**KIT # 1**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial
VISIT 02 **IIT**	**KIT # 2**	**HbA1C** *****repeat per SOE***** (1) 2-mL EDTA Vacutainer Tube	
Visit 03 **Wait List** **(Once prior to induction immunosuppression)**	**Kit # 3**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **FSIGT** (24) 3.5-mL Gold SST (48) 1.8-mL Cryogenic Vials **Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive (NIDDK)** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials ~~**DNA**~~ ~~(1) 4 mL Lavender top EDTA Vacutainer~~
VISIT 03 **Wait List –** **Repeat monthly**	**KIT # 2**	**HbA1C** *****repeat per SOE***** (1) 2-mL EDTA Vacutainer Tube	
VISIT 03 **Wait List –** **Repeat yearly**	**KIT # 4**	**HBA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube	**FSIGT** (24) 3.5-mL Gold SST (48) 1.8-mL Cryogenic Vials

VISIT 04 & 05 **Day -2** **through** **Day 0**	**KIT # 5**	~~**IMMEDIATELY PRIOR TO INDUCTION IMMUNOSUPRESSION**~~	
		~~**TAT, C3a, C-Peptide**~~ ~~(1) 2-mL Lavender top EDTA Vacutainer Tubes~~ ~~(1) 1.8-mL Cryogenic Tubes~~	
		~~**ADDITIONAL Timepoints on Day 0**~~	
		~~**TAT, C3a, C-Peptide**~~ ~~(4) 2-mL Lavender top EDTA Vacutainer Tubes~~ ~~(4) 1.8-mL Cryogenic Tubes~~	
VISIT 10 **(Day 28/Month 1)**	**KIT # 6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube
VISIT 11 **(Day 56/Month 2)**	**KIT #7**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube
VISIT 12 **Day 75** **Month 2.5** ***Secondary Endpoint***	**KIT # 8**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube ***Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials ***MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	***FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive (NIDDK)** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials

VISITS **12a (Month 4)** **12b (Month 5)**	**KIT # 9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	
VISIT 13 **Month 6**	**KIT # 10**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISITS **13a (Month 7)** **13b (Month 8)**	**KIT # 9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	
VISIT 14 **Month 9**	**KIT # 10**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISIT 14a **Month 10**	**KIT # 9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	

VISIT 14b **Month11**	**KIT # 7**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube
VISIT 15 **Month 12** ***Secondary Endpoints*** **OR** **VISIT Y1** **(1 year post-initial transplant)** ***Primary & Secondary Endpoints***	**KIT # 11**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials	**Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISITS **16 (Month 15)** **18 (Month 21)**	**KIT # 12**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial

VISITS **17 (Month 18)**	**KIT # 13**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
19 (Month 24)	**KIT # 11**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mLCryogenic Vials **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials	**Atherogenic Profile** (1) 8.5mL Gold SST (4) 1.8 mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISIT Y2 **(2 years post initial transplant)**	**KIT # 2**	**HBA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube	
VISIT 20 **Month 27**	**KIT # 14**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials

VISIT 21 **Month 30**	**KIT # 10**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISIT 22 **Month 33**	**KIT # 14**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vial	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials
VISIT 23 **Month 36**	**KIT # 11**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT** (2) 3.5-mL Gold SST (4) 1.8-mLCryogenic Vials **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials	**Atherogenic Profile** (1) 8.5mL Gold SST (4) 1.8 mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **Plasma to Archive** (1) 4-mL Lavender top EDTA Vacutainer (1) 1.8-mL Cryogenic Vials
VISIT Y3 **(3 years post initial transplant)**	**KIT # 2**	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube	

Reduced Follow-up (Year 1, 2, and 3 post-final transplant)	**KIT # 51**	**Serum Creatinine** (1) 3.5-mL Gold SST (1) 1.8-mL Cryogenic Vial	**HbA1C** (1) 2-mL Lavender top EDTA Vacutainer Tube **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial
Reduced Follow-up (6 months after premature discontinuation	**Kit # 51x**	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial	
Suspected Graft Failure	**Kit # 50z**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8mL cryovial

* Do not collect these samples at Day 75 for subjects with confirmed graft failure.

SEE APPENDIX 1 FOR KIT SUPPLY ORDER FORM

Kit #8 (Visit 12), Kit #10 (Visits 13, 14, and 21), Kit #11 (Visits 15, Y1, 19, and 23), Kit #12 (Visits 16 and 18), Kit #13 (Visit 17), and Kit #14 (Visits 20 and 22), contain two 3.5-mL Gold SST tubes for the MMTT. Only one of these two tubes will be filled (at 90 minutes), unless it is suspected that the participant has suffered graft failure (in which case, the second tube should be filled at 60 minutes). If there is no suspicion of graft failure, one of the two 3.5-mL Gold SST tubes can be discarded.

APPENDIX 2: SUPPLY ORDER FORM: KIT SUPPLY

Protocol CIT-__ __: Please complete form and fax to University of Iowa @+1-319-353-3960

Site Name & #:	_____	CIT Protocol:	_____
Shipping Address:			
Order Date:		Due Date @ Site:	_____
Requested By:	_____	Requestor's phone:	_____
Requestor's FAX:	_____	Requestor's email:	_____

VISIT NUMBER	KIT NUMBER	QUANTITY ORDERED
	Kit(s) #	
	Kit(s) #	
	Kit(s) #	
	Kit(s) #	
	Kit(s) #	

You will receive an initial supply of kits #1 and #2 for 5 participants upon notice of your site activation.

Please check your kits' expiration dates and DO NOT order more than a 6 month supply of kits.

APPENDIX 3: SUPPLY ORDER FORM: BULK SUPPLY (COLLECTION CONTAINERS/MATERIALS)

Protocol CIT-__ __ **:** Please complete form and fax to University of Iowa @**+1-319-353-3960**

Site Name & #: _____ CIT Protocol: _____

Shipping Address:

_____

_____

_____

_____

Order Date: _____ Due Date @ Site: _____

Requested By: _____ Requestor's phone: _____

Requestor's FAX: _____ Requestor's email: _____

* Indicate the Laboratory

Reference No.	Description	Quantity / Units
COLLECTION CONTAINERS / MATERIALS		
Fisher # 02-683-99A	2-mL Lavender-top (EDTA) Vacutainer® -Small Volume Pediatric Tube	_____/100 Tubes
Fisher # 02-683-99C	4-mL Lavender-top (EDTA) Vacutainer® Tubes	_____/100 Tubes
Fisher # 02-657-27	3-mL Red Top Vacutainer® Tubes	_____/100 Tubes
Fisher # 02-683-93A	4-mL Gold Top SST Vacutainer Tubes	_____/100 Tubes
Fisher # 02-683-97B	3.5-mL Gold Top SST Vacutainer® Tubes	_____/100 Tubes
Fisher # 02-683-96	8.5-mL SST	_____/100 Tubes
Fisher # 12-565-171N	1.8-mL Cryogenic Vials	_____/100 Vials
Fisher # 05-402-90	Eppendorf Pipette (adjustable 100 mcL-1000 mcL)	_____ 1 each
Fisher # 05-403-113	Pipette Tips	_____/960 Tips
Fisher # 13-711-61	Sterile Urine Container	_____/1 each
Fisher # 12-565-162N	4-mL Cyrovials	_____/400 tubes
Fisher #23-111-253	Multi-Stix	__/100 Test strips

APPENDIX 4: SUPPLY ORDER FORM: BULK SUPPLY (SHIPPING SUPPLIES)

Protocol CIT-__ __: Please complete form and fax to University of Iowa @+1-319-353-3960

Site Name & #:	_____	CIT Protocol:	_____
Shipping Address:			
Order Date:		Due Date @ Site:	_____
Requested By:	_____	Requestor's phone:	_____
Requestor's FAX:	_____	Requestor's email:	_____

SHIPPING SUPPLIES	
Shipping Container – ambient kit	boxes
Shipping Container – refrigerated kit	boxes
Shipping Container – frozen kit	boxes
STT-711 ambient gel packs	each
FedEx Airbills to University of Washington—DRY ICE	airbills
FedEx Airbills to University of Washington—COLD PACK	airbills
FedEx Airbills to University of Pennsylvania	airbills
FedEx Airbills to Barbara Davis Center	airbills
FedEx Airbills to NIDDK Repository	airbills
FedEx Airbills to University of Minnesota	airbills

APPENDIX 5: INSULIN INFUSION PROTOCOL

I Starting Insulin Infusion (as piggyback into a saline kvo)

If FSBG >250 (13.89 mmol/L), start regular insulin (0.5 U/mL) gtt @ 2 U/hr, and check FSBG in 60 min

If FSBG 181 – 250 (10.06 – 13.89 mmol/L), start regular insulin (0.5 U/mL) gtt @ 1.5 U/hr, and check FSBG in 60 min

If FSBG 151 – 180 (8.39 – 10.0 mmol/L), start regular insulin (0.5 U/mL) gtt @ 1.0 U/hr, and check FSBG in 60 min

If FSBG 116 – 150 (6.44 – 8.33 mmol/L), start regular insulin (0.5 U/mL) gtt @ 0.5 U/hr, and check FSBG in 60 min

If FSBG 70 – 115 (3.89 – 6.38 mmol/L), do nothing, and check FSBG in 60 min

If FSBG 60 – 69 (3.33 – 3.83 mmol/L), check FSBG in 15 min, then phone M.D.

If FSBG < 60 (3.33 mmol/L), give juice, check FSBG in 15 min, then phone M.D.

II Titrating Insulin Infusion

1) First, act on a low blood glucose as follows:

If FSBG <60 (3.33 mmol/L), stop infusion, give juice, check FSBG in 15 min, then phone M.D.

If FSBG 60 - 69 (3.33 – 3.83 mmol/L), stop infusion, check FSBG in 15 min, then phone M.D.

2) If blood glucose is not low, next act on a decreasing blood glucose as follows:

If FSBG decreases by >40 (2.22 mmol/L), decrease rate by 1 U/hr, and check FSBG in 30 min

If FSBG decreases by >80 (4.44 mmol/L), decrease rate by 2 U/hr, and check FSBG in 30 min

3) If blood glucose is not low, and has not decreased by > 40 (2.22 mmol/L), then:

If FSBG 70 – 84 (3.89 – 4.66 mmol/L), decrease rate by 0.5 U/hr, and check FSBG in 30 min

If FSBG 85 – 115 (4.72 – 638 mmol/L), no change, and check FSBG in 60 min

If FSBG 116 – 180 (6.44 – 10.0 mmol/L), increase rate by 0.5 U/hr, and check FSBG in 60 min

If FSBG 181 – 250 (10.06 – 13.89 mmol/L), increase rate by 1.0 U/hr, and check FSBG in 60 min

If FSBG >250 (13.89 mmol/L), increase rate by 2.0 U/hr, and check FSBG in 60 min

If a titration step results in turning off the insulin infusion, then the next FSBG should be acted on as presented in I: Starting Insulin Infusion

APPENDIX 6: FSIGT WORKSHEET

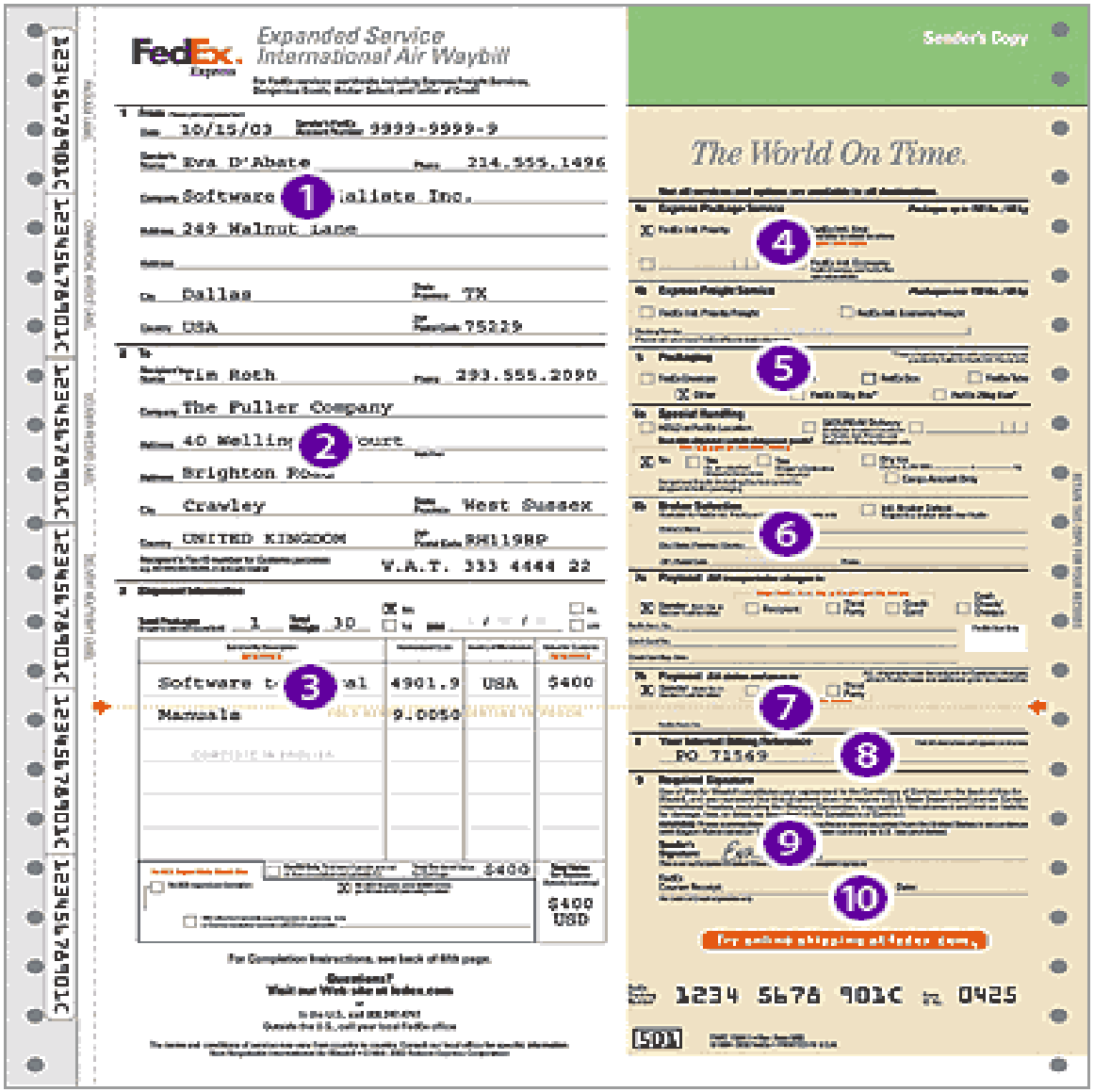
STUDY#:_____

SUBJECT ID: _____ DATE: _____

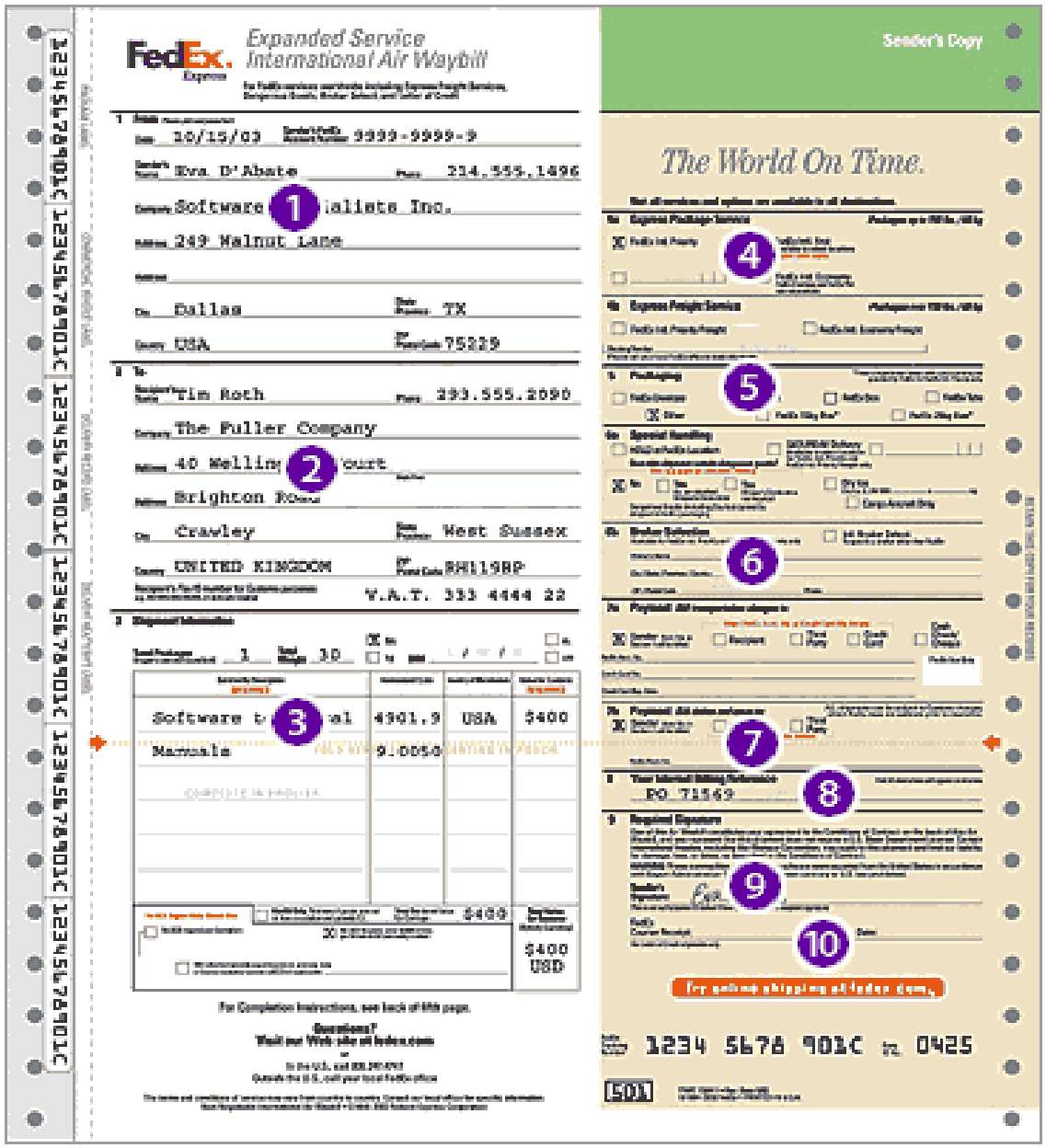
FSIGT PROTOCOL				
Relative Time (minutes)	**Exact Time (hours)**	**Sample #**	**2 ml SST Vacutainer®**	**Notes/BG**
-30				IVs placed
-10		1	Yes	
-5		2	Yes	
-1		3	Yes	
0				**Dextrose (begin pushing dextrose @ - 30 sec, push over 1 minute)**
1		4	Yes	
2		5	Yes	
3		6	Yes	
4		7	Yes	
5		8	Yes	
7		9	Yes	
10		10	Yes	
12		11	Yes	
14		12	Yes	
16		13	Yes	
18		14	Yes	
20		15	Yes	**Insulin (begin pushing insulin @ 20 minutes, push over 30 sec)**
22		16	Yes	
25		17	Yes	
30		18	Yes	
40		19	Yes	
50		20	Yes	
70		21	Yes	
100		22	Yes	
140		23	Yes	
180		24	Yes	

Observations/Notes: none ☐
Dextrose Administered: D50 _____ D 25 _____ Amount given _____ml
Dextrose start time _____ Dextrose Stop time _____
Insulin _____Units (give over 30 seconds begin push at 20 minutes)

APPENDIX 7: EXPANDED SERVICE INTERNATIONAL AIR WAY BILL COMPLETION INSTRUCTIONS (WITH DRY ICE)



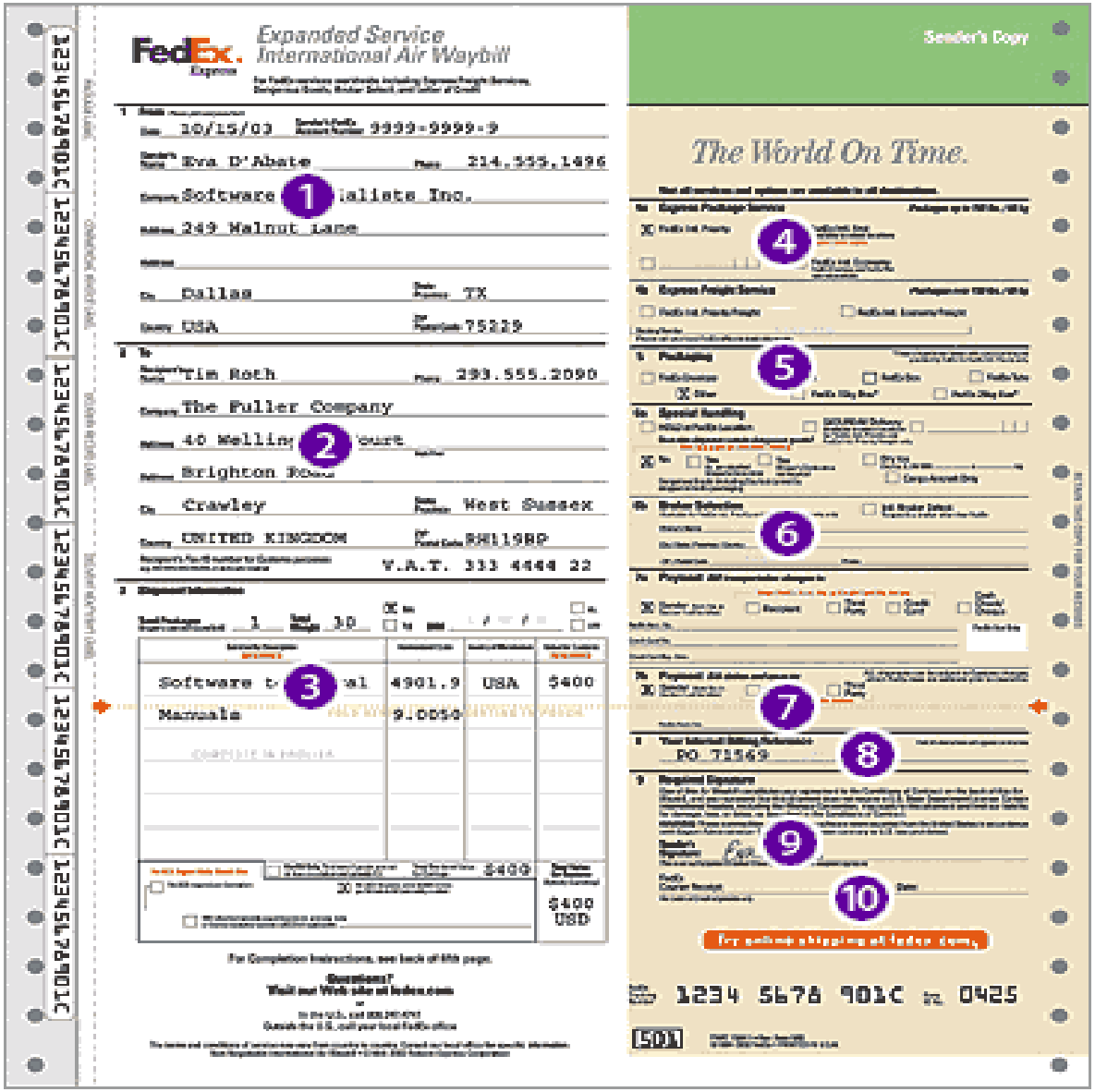
1. **Sender Information**
   Enter your shipping information.
   This includes the address you are shipping from, your name, your phone number, the FedEx account number should already be pre-printed on the airbill. *If the FedEx account number is not pre-printed on the airbill you may enter this information,* ***4261-1614-6***
2. **Recipient Information**
   Enter the recipients shipping information.
   This includes the address you are shipping to, the company name (central laboratory), the recipients name, the recipients phone number, and country.
3. **Shipment Information**
   - **Total Packages**
     1
   - **Total Weight**
     4 kg
   - **DIM Points VOL**
     (8 LONG, 8 larg, 14); check inches
   - **Commodity Description.**
     Perishable, Non-Infectious, Non-Hazardous, Human Biological Substance – (Blood) UN 3373
     Dry Ice, Class 9
     UN 1845 1 x 4 kg
   - **Harmonized Code.**
     Leave Blank
   - Country of Manufacture
     Leave Blank
   - **Total Value for Customs.**
     US $10.00
   - **Total Declared Value for Carriage.**
     US $10.00



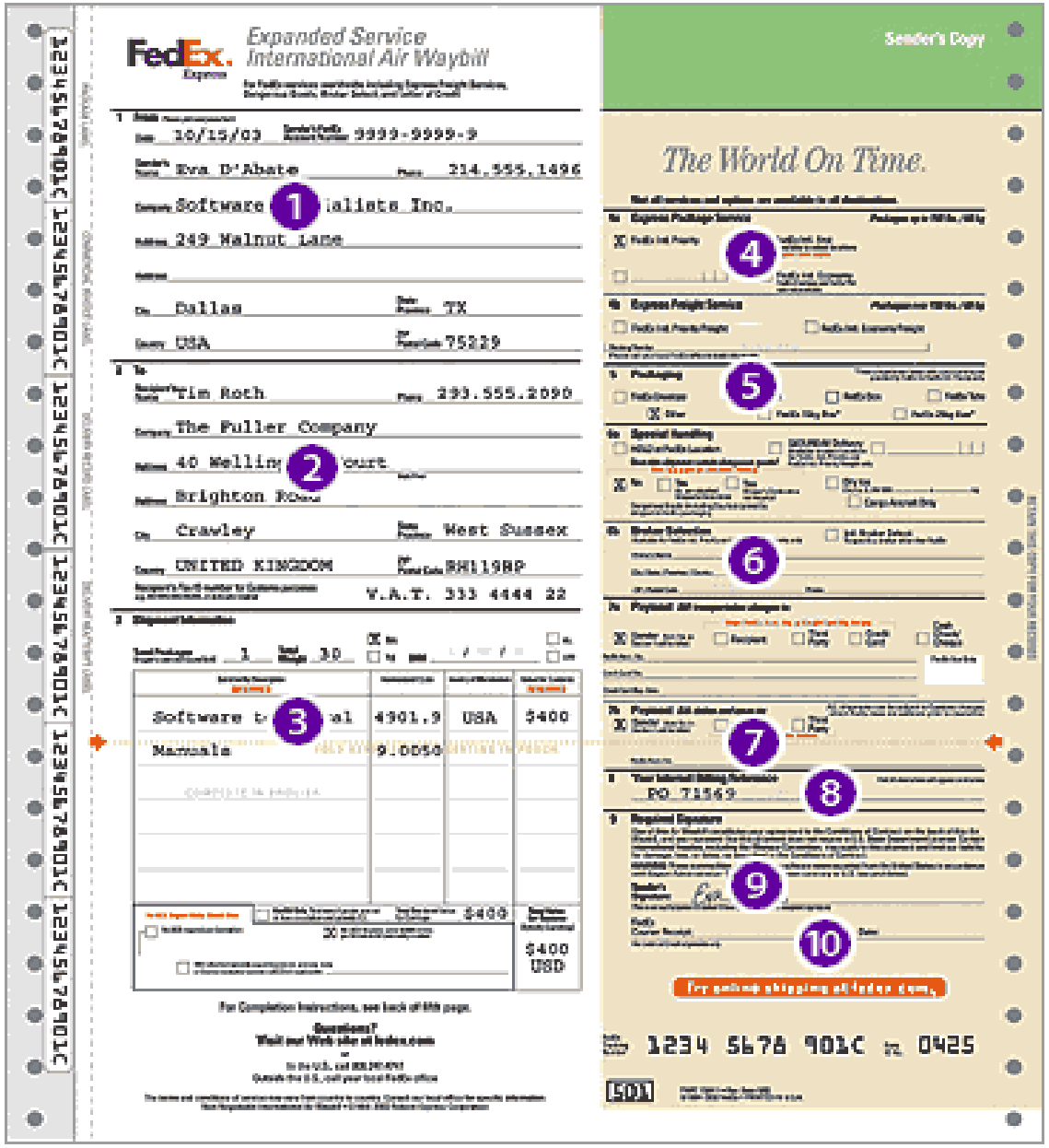
4. **Express Package Service**
   4a. Indicate FedEx International Priority®.
   4b. Leave Blank
5. **Packaging**
   Indicate the type of FedEx Express packaging you are using, or mark "other" if you are using your own packaging.
6. **Special Handling**
   Does the shipment contain dangerous goods?
   Check "Yes, Shippers declaration not required"
   Dry Ice: Indicate 1 x 4 kg
7. **Payment**
   Select Third Party as your method of payment and provide the appropriate FedEx account number, 2729-9789-6.

NOTE: 3 copies of the commercial invoice (FedEx FACTURE PRO FORMA/ PROFORMA INVOICE) should be included with all shipments.

APPENDIX 8: EXPANDED SERVICE INTERNATIONAL AIR WAY BILL COMPLETION INSTRUCTIONS (WITHOUT DRY ICE)



1. **Sender Information**
   Enter your shipping information.
   This includes the address you are shipping from, your name, your phone number, the FedEx account number should already be pre-printed on the airbill. *If the FedEx account number is not pre-printed on the airbill you may enter this information,* ***4261-1614-6***
2. **Recipient Information**
   Enter the recipients shipping information.
   This includes the address you are shipping to, the company name (central laboratory), the recipients name, the recipient's phone number, and country.
3. **Shipment Information**
   - **Total Packages**
     1
   - **Total Weight**
     1 kg
   - **DIM Points VOL**
     (8 LONG, 8 larg, 14); check inches
   - **Commodity Description.**
     Perishable, Non-Infectious, Non-Hazardous, Human Biological Substance – (Blood) UN 3373
   - **Harmonized Code.**
     Leave Blank
   - Country of Manufacture
     Leave Blank
   - **Total Value for Customs.**
     US $10.00
   - **Total Declared Value for Carriage.**
     US $10.00



4. **Express Package Service**
   4a. Indicate FedEx International Priority®.
   4b. Leave Blank
5. **Packaging**
   Indicate the type of FedEx Express packaging you are using, or mark "other" if you are using your own packaging.
6. **Special Handling**
   Does the shipment contain dangerous goods?
   Check "No"
7. **Payment**
   Select Third Party as your method of payment and provide the appropriate FedEx account number, 2729-9789-6.

NOTE: 3 copies of the commercial invoice (FedEx FACTURE PRO FORMA/ PROFORMA INVOICE) should be included with all shipments.

APPENDIX 9: FEDERAL EXPRESS COMMERCIAL INVOICE

COMPANY NAME

COMPANY ADDRESS

COMMERCIAL INVOICE

INTERNATIONAL AIR WAYBILL NO.

(NOTE: All shipments must be accompanied by a Federal Express International Air Waybill.)

DATE OF EXPORTATION

EXPORT REFERENCES (i.e., order no., invoice no.)

SHIPPER/EXPORTER (complete name and address)

CONSIGNEE (complete name and address)

COUNTRY OF EXPORT

COUNTRY OF MANUFACTURE

COUNTRY OF ULTIMATE DESTINATION

IMPORTER — IF OTHER THAN CONSIGNEE (complete name and address)

MARKS/NOS.	NO. OF PKGS.	TYPE OF PACKAGING	FULL DESCRIPTION OF GOODS	QTY.	UNIT OF MEASURE	WEIGHT	UNIT VALUE	TOTAL VALUE
	TOTAL NO. OF PKGS.					TOTAL WEIGHT		TOTAL INVOICE VALUE

SEE REVERSE SIDE FOR HELP WITH THE ABOVE SECTION

Check one
☐ F.O.B.
☐ C & F
☐ C.I.F.

FOR U.S. EXPORT ONLY: THESE COMMODITIES, TECHNOLOGY, OR SOFTWARE WERE EXPORTED FROM THE UNITED STATES IN ACCORDANCE WITH THE EXPORT ADMINISTRATION REGULATIONS. DIVERSION CONTRARY TO UNITED STATES LAW IS PROHIBITED.

I DECLARE ALL THE INFORMATION CONTAINED IN THIS INVOICE TO BE TRUE AND CORRECT.

SIGNATURE OF SHIPPER/EXPORTER (Type name and title and sign.) DATE

M-1054 REV 04/03 LOGOS 116547 WCS

INSTRUCTIONS FOR COMPLETING THE COMMERCIAL INVOICE

The Commercial Invoice is the primary document required by customs officials in international locations.
The form on the reverse side should only be used if your company does not have its own corporate invoice form. If you use your own corporate invoice form, it must contain the following information:

ALL REQUESTED INFORMATION MUST BE SUPPLIED and the goods being shipped must be described in full as follows:

MARKS/NOS.	Any identifying marks or number used on packaging
NO. OF PKGS.	Total number of packages described on each line
TYPE OF PACKAGING	Type of packaging being used, i.e., roll, tube, carton
FULL DESCRIPTION OF GOODS	Complete details of the item(s) being shipped, including name, part numbers, serial numbers, and H.S. numbers, if available

THE FOLLOWING GUIDELINES APPLY TO SPECIFIC CATEGORIES OF SHIPMENTS:

On items being shipped for repair. Use model (or part) name and serial numbers if available. Describe the part accurately and the reason for shipment (e.g., "used steel fly wheel for lathe returned for repair").

On parts of machinery or equipment. Write in specific part name and part numbers for every different part. Describe each part in simple language (e. g., "fuel pump Model D-Serial Number 811256 for ABC 3-ton truck Model 7").

On nontextile samples. Fully describe each sample and purpose (e.g., "seven assorted and differently colored samples of plastic laminate described in contract bid. Not for resale.")

All textiles. (Includes finished goods, bolts of cloth, samples or swatches.) Textiles MUST be described completely, including composition of fabric, type of assembly, and identity of user and ornamentation, if any (e.g., "Lady's short sleeved 100% cotton sewn blouse with pearl buttons, Man's knitted 100% wool sweater, Girl's long sleeved 65% polyester/35% cotton crocheted sweater, Knitted fabric swatch dyed 65% cotton/35% rayon 12 inches X 12 inches, Woven fabric sample bleached 100% cotton 7 yards X 45 inches").

QTY.	Quantity of items described on each line
UNIT OF MEASURE	Lb, kg, pieces, sets, pairs, yards
WEIGHT	Weight of items described on each line
UNIT OF MEASURE	$ Value of each unit
TOTAL VALUE	$ Value of items described on each line
TOTAL INVOICE VALUE	$ Value of all items listed on the invoice

FOR FURTHER INFORMATION OR ASSISTANCE, CONSULT THE FEDEX SERVICE GUIDE, OR CALL CUSTOMER SERVICE AT 1 800 GO FEDEX (800-463-3339)

APPENDIX 10: Specimen Submission Form

Specimen Submission Form

Instructions: Please fax a copy of this completed form to the contact at the specimen destination prior to shipping specimens. Sections 1 & 2 must be completed.

Section 1 - SPECIMEN DESTINATION:

Contact Name at Delivery Point: _____

Phone at Delivery Point: ( ) _____

FAX at Delivery Point: ( )_____

Section 2 - SHIPPER INFORMATION:

Site ID #: _____

Contact Name: _____

Phone: ( ) _____

FAX: ( ) _____

E-mail _____

Shipping Condition: _____ Ambient _____ Frozen _____Cold Pack

Number of Patients: _____ Number of vials: _____

Date Shipped: _____/_____/_____

Courier Service: (Circle one)

a) Federal Express
b) Airborne Express
c) World Courier
d) DHL
e) Other: _____
f)

AIRBILL #: _____

APPENDIX 11: Instructions to Subject regarding Local Labs

Date _____

Re: Subject Instructions for blood specimen collection and shipping for islet transplantation clinical trials

We hope to save you time and travel by having blood specimens for the islet transplant clinical trial collected and shipped from your local laboratory.

Please call your local laboratory and make an appointment to have your blood collected on a Monday, Tuesday or Wednesday.

In order to meet the study schedule, please have the laboratory collect the specimens between (date) _____ and (date) _____ and ship them immediately using the pre-paid FedEx shipping label and shipping materials that accompany this request.

If we have included a cold pack, place it in the refrigerator until your appointment. Do not freeze the cold pack.

If we have included a freezer pack, place it in the freezer until your appointment.

_____ Fast from 11 pm the evening before the blood collection. You may continue to drink water. On the morning of the blood collection, take only those medications that we have discussed with you to take until your blood is collected. Make an early morning appointment at your local laboratory.

_____ Fasting is not required before blood collection. Make an appointment in the morning at your local laboratory. The laboratory needs time to ship the sample the same day that it is drawn.

On the appointed day, take the enclosed "Local Lab Instructions", the cold and/or freezer pack, the shipping box and the FedEx label to your local laboratory and have your blood collected. Instructions to the laboratory to prepare and ship the specimens are on the "Local Lab Instructions" sheet.

Please contact us when your blood has been drawn so we know to look for the samples.

If you have questions, call us any time.
Phone number: _____
Sincerely,

APPENDIX 12: Instructions to Local Lab for Drawing, Processing and Shipping labs to sites.

Date _____

Re: Local Lab Instructions for blood specimen collection and shipping for islet transplantation clinical trials

_____ Instructions for drawing and shipping Hemoglobin A1c

Please draw 2 mL of blood into the provided lavender top vacutainer. The tube should be filled to capacity, due to the need to have an appropriate proportion of EDTA and blood.

Gently invert the tube 8 – 10 times.

Enter the date and time of draw on the requisition and tube.

Ship the sample immediately on cold pack, using the pre-paid FedEx shipping label and shipping materials that accompany this request using overnight delivery.

_____ Instructions for drawing and shipping Serum glucose, creatinine, and c-peptide

Patient must be fasting.

Please draw 2 mL of blood into the Gold SST tube provided.

Enter the date and time of draw on the requisition and tube.

Allow blood to clot at room temperature for a minimum of 20 minutes but no longer than 40 minutes.

Centrifuge at 3000 rpm for 10 – 15 minutes.

Aliquot serum into the 2 aliquots provided and freeze immediately.

Once the aliquots are frozen, ship them using the pre-paid FedEx shipping label and shipping materials that accompany this request.

If you have questions, call us any time.
Phone number: _____
Sincerely,