PROTOCOL CIT-01:

OPEN RANDOMIZED MULTI-CENTER STUDY TO EVALUATE SAFETY AND EFFICACY OF LOW MOLECULAR WEIGHT SULFATED DEXTRAN IN ISLET TRANSPLANTATION

LABORATORY MANUAL
FOR CENTRAL LABORATORY ASSESSMENTS

VERSION 7.0

25/SEP/2012

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
CIT-01A	Islet Alone Protocol
CIT-01B	Islet After Kidney Protocol
CMV	Cytomegalovirus
CPT	Cell Preparation Tube
DCC	Data Coordinating Center
DI	Disposition Index
EBV	Epstein- Barr Virus
EDTA	ethylenediaminetetraacetic acid
FSIGT	Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test
GFR	Glomerular Filtration Rate
HbA1c	Hemoglobin A1c
ID	Identification
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)
PBL	Peripheral Blood Lymphocytes
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
S_I	Insulin Sensitivity
SST	Serum Separator Tube
STS	Specimen Tracking System
TAT	Thrombin-Antithrombin Complex

1. STUDY CONTACT INFORMATION

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2. SPECIMEN SCHEDULE (CENTRAL LABORATORY)

Central Laboratory Assessments				
Specimen Name	**Laboratory**	**Collection Container**	**Blood Volume / Specimen Type**	**Visit Numbers**
Alloantibodies	University of Pennsylvania	(1) 3-mL Red-top Vacutainer	2-mL Blood	01, 02, 10, 11, 12, Y1
Alloantibodies	The Rudbeck Laboratory	(1) 3-mL Red-top Vacutainer	2-mL Blood	01, 02
Autoantibodies	Barbara Davis Center	(1) 3-mL Red-top OR (1) 3.5-mL Gold SST	2-mL Blood	02, 10, 11, 12, Y1
Hemoglobin A1C (HbA1c)	University of Washington	(1) 2-mL Lavender top EDTA Vacutainer	2-mL Blood	01, 02, 09, 10, 11, 12, Y1
Insulin Modified FSIGT	University of Washington	(24) 3.5-mL Gold SST	48-mL Blood Total • 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	01, 10*, 12, Y1
Mixed Meal Tolerance Test • Fasting and Stimulated Serum Glucose • Fasting and Stimulated C-Peptide • Serum Creatinine	University of Washington	(6) 3.5-mL Gold SST	12-mL Blood Total • 2 mL at 0, 15, 30, 60, 90, and 120 minutes each	01
		(6) 3.5 -mL Gold SST	12-mL Blood Total • 2 mL at 0, 15,30, 60, 90 and 120	10*, 11, 12, Y1
Plasma to Archive (NIDDK)	The Rudbeck Laboratory	(4) 8.0 mL Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate	30-mL Blood	01, 10, 11, 12

Central Laboratory Assessments				
Specimen Name	**Laboratory**	**Collection Container**	**Blood Volume / Specimen Type**	**Visit Numbers**
RNA and Plasma to Archive	The Rudbeck Laboratory	(1) 10-mL EDTA tube	10-mL Blood	02, 05, 07,10, Y1
Serum to Archive (NIDDK)	The Rudbeck Laboratory	(1) 4-mL Gold SST	4-mL Blood	01, 10, 11, 12
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***DO NOT COLLECT THESE SAMPLES AT DAY 75 FOR SUBJECTS WITH CONFIRMED GRAFT FAILURE.**

3. VISIT SCHEDULE

VISIT NO.	VISIT	VISIT WINDOW
01	Enrollment / Screening	Date written informed consent is obtained
02	Baseline Visit	Within 48 hours before the islet transplantation
03	Transplant	Date of the islet transplantation
04-05	Day 1, Day 3	± 0 days
06-07	Day 7, Day 14	± 3 days
08-09	Day 21, Day 28	± 3 days
10	Day 75	± 5 days
11-12	Month 6, Month 12	± 14 days
Y1	Year 1 after first islet transplant	± 14 days

Day 75:
If needed, repeated transplant(s) will be performed more than 75 days after the first transplant.
The previous follow-up period will stop and a new follow-up period starts, beginning with the baseline visit.

Year 1 Post- First Transplant:
Y1 visit to be completed ONLY if a subsequent transplant were to occur. If the subject does not receive a second or third transplant, then the 1-year post first transplant visit will be visit 12

For a Second or Third Transplant:
If 1-year post first transplant (Visit Y1) falls within a visit window of a scheduled re-transplant study visit, complete only the CRFs for the 1-year post initial transplant visit.

If Y1 visit does not fall within a re-transplant visit, complete the Y1 CRFs.

4. 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. ***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 10 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.

Each kit contains a Requisition Form. The Central Labs only need one copy and you should keep a copy for your records. It does not matter which copy (white, yellow, or pink) goes to the lab or you keep. You may need to make additional copies at your site so you have enough Requisition Forms to cover all the samples being sent to each lab.

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. 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, site id number, subject id number, tube type, specimen type (i.e., Alloantibody, Autoantibody). The label includes a time point (if applicable) and space for the site to provide the date of collection.

How to Place an Order:

To place a supply order, the site personnel should complete either the Supply Order Fax: 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 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. Details regarding the Specimen Tracking System are provided in the CIT STS Users Guide.

5. PACKING INSTRUCTIONS

All CIT-01 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:

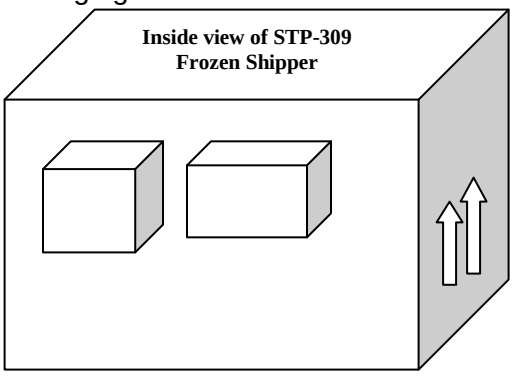
CIT Packing Instructions for Non-Infectious Frozen/Dry Ice Samples

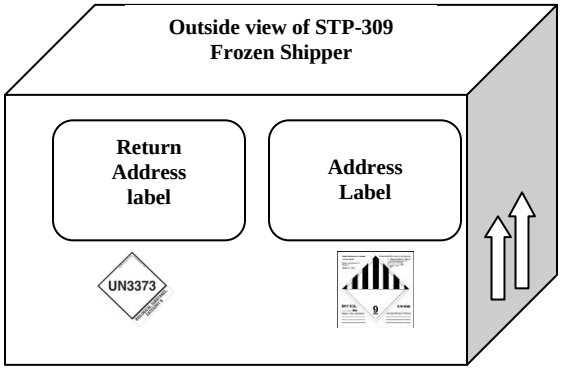
1. Insert frozen samples into provided cryoboxes.
2. Place the cryovial box containing frozen tubes into the clear plastic bag (STP-711) containing the absorbent material. Seal the clear plastic bag by removing the white paper liner to expose the adhesive. Gently lay the adhesive covered tape over the 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.

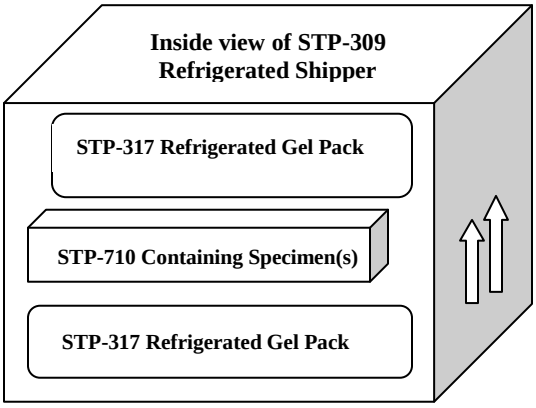


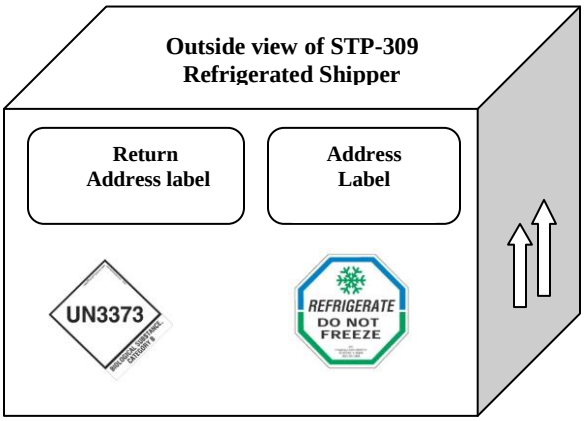


CIT Packing Instructions for Refrigerated Blood Samples

1. Pre-condition two STP-317 PCM STT-521-1000(gel) packs to refrigerated temperature by placing in refrigerator overnight prior to use.
2. Place tubes inside leak-proof poly bag (STP 710 poly bag) with the sheet of absorbent paper.
3. Seal the poly bag and place inside Tyvek outer envelope.
4. Lay one gel pack flat inside the Styrofoam shipper. Press firmly into the shipping container.
5. Lay the Tyvek envelope containing the tube(s) on top of the gel pack.
6. Use the second gel pack to “sandwich” the envelope securely between the two gel packs.
7. 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.
8. 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
9. Complete the airway bill (date of shipment, weight of shipment) and place in the airway bill holder.
10. Attach the airway bill holder to the top of the package.

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



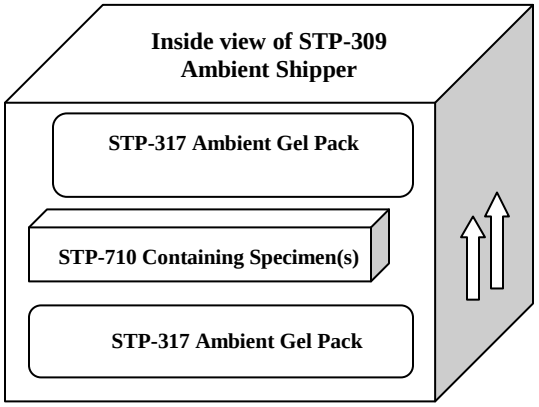


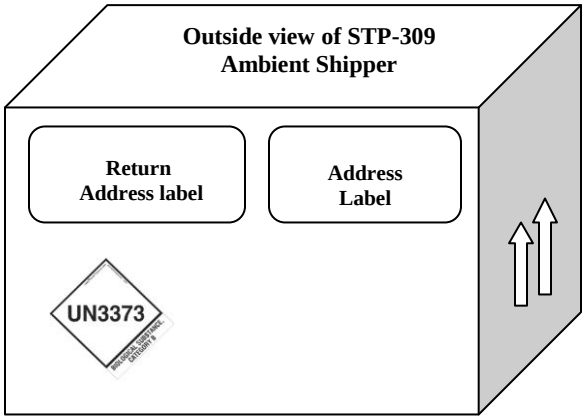
CIT Packing Instructions for Ambient Blood Samples

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 tubes inside leak-proof poly bag (STP 710 poly bag) with the sheet of absorbent paper.
3. Seal the poly bag and place inside Tyvek outer envelope.
4. Lay one gel pack flat inside the Styrofoam shipper. Press firmly into the shipping container.
5. Lay the Tyvek envelope containing the tube(s) on top of the gel pack.
6. Use the second gel pack to “sandwich” the envelope securely between the two gel packs.
7. 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.

8. 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
9. Complete the airway bill (date of shipment, weight of shipment) and place in the airway bill holder.
10. Attach the airway bill holder to the top of the package.

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





6. SHIPPING INSTRUCTIONS

Federal Express is the chosen courier for the CIT-01 study, however DHL or Jetpak is the chosen carrier for shipments within Sweden. In general, specimens should be shipped Monday- Thursday ONLY and should NOT be shipped 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.

- For shipments to US or Canada: Federal Express Account Number: **4261-1614-6**
- For shipments from Norway/Sweden to Sweden: DHL Account Number: 950367837 or Jetpack Account Number: 659111
- 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/se/) or by calling the International Customer Service Number at 1-800-247-4747, Monday-Friday from 8:00AM-6:00PM. If you have any further inquiries, please call **Customer Service**: **0200 252 252** If you can't access the toll free number call: +46 8 797 99 60.Be certain to include with the shipment the transplant recipients unique identification number, date the specimen was obtained, and the name of the sending institution.

7. SWEDEN or NORWAY TO U.S.:

Pick-up request: Prepare a shipment and schedule a courier online with FedEx Ship Manager at www.fedex.com/se **. You can acces**s **FedEx Ship M**anager at fedex.com by clicking on "Ship" at the top of the screen from any page on fedex.com. Or **Call Federal Express** @ 1-800-247-4747. 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.

DO NOT USE THE FedEx US AIRBILL. You will need to complete the ***Expanded Service International Air Waybill***. Please refer to ***Appendix 6 and 7*** for proper completion instructions. You will need to include 3 copies of the Commercial Invoice with each shipment ***(Appendix 8).***

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

8. SPECIMEN COLLECTION, PROCESSING, AND SHIPPING PROCEDURES

For each of the tests listed below, please refer to the Specimen 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.

8.1 Hemoglobin A1c

Specimen Collection and Processing

Hemoglobin A1c will be performed at the University of Washington (Seattle, Washington, USA) at screening, waitlist/baseline, day 28, day 75, and month 6 and 12 months following the islet transplant. If a subsequent transplant occurs then the previous follow-up schedule ends and a new visit schedule starts, beginning with the baseline visit. If a subsequent transplant occurs, then the HbA1c will also be collected at 1 year post the initial islet transplantation.

- Collect 2 mL blood into a 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.

Packing and Shipping Instructions

- 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.
- Follow the shipping instructions outlined in Section 6 of this manual.
- In order for the results to be reported within 72 hours, the specimens should be shipped within 24 hours of collection, using overnight delivery.
- Please complete an Expanded Service International Air Waybill (Without Dry Ice) – Appendix 7 and the Commercial Invoice form (Appendix 8).

Note: Shipments to this laboratory must be made Monday – Wednesday.

- Ship the samples to the laboratory using overnight delivery (preferably FedEx Courier), Monday through Wednesday. Do not ship just prior to holidays.

  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 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.

8.2 Mixed Meal Tolerance Test (MMTT): Stimulated Serum Glucose, C-Peptide, and Creatinine

Insulin Management

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:

- Subjects will be instructed to keep a stable diet (e.g., 200-300 g carbohydrates/ day) and avoid alcohol for three days prior to the test. They will also be instructed not to eat or inject insulin s.c. after 8 PM the night before the test. Indicate subject compliance on the CIT-01 MMTT-FSIGT Weight Maintenance Source Documentation, which is a study Master Document.
- The recommendation for patients on intermittent sc injections of insulin is that before MMTT and FSIGT they are hospitalized and kept euglyemic with an IV insulin infusion overnight according to local algorithm. IV insulin should be turned off 30 min prior baseline blood sampling.
- Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Basal lispro or aspart administered via pump should be reduced by at least 50 % at 06 00 a.m. the day of the test and turned off 30 min prior to baseline blood sampling. These and "insulin independent" subjects may arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked.
- If the BG is < 70 mg/dL (3.9 mmol/L) or > 136 mg/dL (8.0 mmol/L), the test will be rescheduled for the next possible day. If the BG is 70 – 136 mg/dl (3.9-8.0 mmol/L), basal serum glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 ml per kg body weight (to a maximum of 360 mL) of Boost® High Protein Drink or Resource Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 15, 30, 60, 90, and 120 minutes, stimulated serum glucose and c-peptide levels will again be drawn.
- Time = 0 should occur at 9:00 a.m. for the MMTT (suggested allowable range 8:00 a.m. – 10:00 a.m.).

Specimen Collection and Processing

Fasting and stimulated serum glucose, c-peptide levels, and creatinine will be determined at screening,day 75, month 6, and month 12 following the islet transplantation. If a subsequent islet transplant occurs then the previous follow-up schedule ends and a new visit schedule starts, beginning with the baseline visit. If a

subsequent transplant occurs, then the MMTT will also be performed at 1 year following the initial islet transplant.

- Collect 2 mL blood in a Gold SST for fasting and 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 1100-1300 RCF (g) [about 2,000 -3,000 RPM] for 10 minutes.
- Prepare (2) 1.8 mL cryovials for each time point (total 6 cryovials), 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 FASTING, 60 MINUTES AND 90 MINUTES ANALYSES SHOULD BE PROCESSED SEPARATELY.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- We recommend shipping Monday-Wednesdays to avoid lost shipments due to dry ice evaporation with any delayed arrivals. Do not ship just prior to holidays.
- Batch ship at least weekly.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  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 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. NOTE: you will need to expand the columns in the specimen list to show all the data before printing. 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.

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

Insulin Management

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:

- Subjects will be instructed to keep a stable diet (e.g., 200-300 g carbohydrates/ day) and avoid alcohol for three days prior to the test. They will also be instructed not to eat or inject insulin s.c. after 8 PM the night before the test. Indicate subject compliance on the CIT-01 MMTT-FSIGT Weight Maintenance Source Documentation, which is a study Master Document.
- The recommendation for patients on intermittent sc injections of insulin is that before MMTT and FSIGT they are hospitalized and kept euglyemic with an IV. insulin infusion overnight according to local algorithm. IV insulin should be turned off 30 min prior baseline blood sampling.
    - Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Basal lispro or aspart administered via pump should be reduced by at least 50 % at 06 00 a.m. the day of the test and turned off 30 min prior to baseline blood sampling. These and "insulin independent" subjects may arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked.
- Prior to the start of testing the capillary or plasma glucose results should be:
    - The pre-transplant BG value should be between 3.9-7.8 mmol/L.
    - The post-transplant BG values should be between 3.9-6.4 mmol/L.
    - A BG reading with a glucometer should be done at -10 to make sure subject BG level meets criteria.
    - 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 could 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.
    -

Specimen Collection and Processing

The acute insulin response to glucose (AIR_{glu}), insulin sensitivity (S_I), and disposition index (DI) will be determined using the FSIGT test, at screening, and day 75 after the first islet transplant, and month 12 following the islet transplant. If a subsequent transplant occurs then the previous follow-up schedule ends and a new visit schedule starts, beginning with the baseline visit. If a subsequent transplant, then FSIGT test will also be performed at 1 year following the initial transplant.

- Time points for blood collection:

- Draw baseline samples (t= -10, -5, and -1 minute)
- The -1 blood sample must be drawn prior to the infusion of glucose Glucose (o.3 g/kg) infusion. The goal is to have the glucose infusion completed prior to the 1 minute sample.
- t= 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20 (the 20 minutes sample must be drawn prior to the infusion of insulin) begin to draw the 20 minutes sample at 19 minutes and 45 seconds.
- An injection of insulin (0.03 U/kg over 30 seconds should be given at the 20 minute time point after the 20 minute blood sample is drawn
- Continue drawing samples at t= 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post-injection of glucose.

- At each of the 24 time points listed above, draw 1 mL blood to be discarded as waste to avoid dilution of sample. 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 a Gold SST at the time points listed above. An FSIGT worksheet can be located in **Appendix** 5.
- Document any problems with the test (difficult blood draw, sample hemolyzed, etc.) on the FSIGT worksheet and in STS.
- Given time most subjects' blood sugar will rebound on its own. However, if subject becomes symptomatic and requires treatment the test should be stopped and no further samples drawn.
- 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 1100-1300 RCF (g) [about 2,000 - 3,000 RPM] for 10 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 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 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 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.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- We recommend shipping Monday-Wednesdays to avoid lost shipments due to dry ice evaporation with any delayed arrivals. Do not ship just prior to holidays.
- Batch ship weekly.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  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 shipment 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 9)and fax a copy of the form including the airbill tracking number to the laboratory at +1-206-685-6880.

8.4 Alloantibody (University of Pennsylvania)

Specimen Collection and Processing

Alloantibody testing will be performed at screening, baseline, day 75, month 6, and month 12 following the islet transplant. If a subsequent transplant occurs then the previous follow-up schedule ends and a new visit schedule starts, beginning with the baseline visit. If a subsequent transplant occurs, then the Alloantibody test will also be performed at 1 year following the initial transplant.

- 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 a 1.8 mL cryovial.
- Freeze the aliquots at -70°C.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- Ship the samples on dry ice.
- Batch ship at least quarterly.
- Shipments to this laboratory must be made Monday through Wednesday. The laboratory is not staffed on weekends or holidays.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  Hospital of the University of Pennsylvania
  HLA Laboratory, Attention: Jane Kearns

7 Founders Pavilion
3400 Spruce Street
Philadelphia, PA USA 19104
Tel: +1-215-662-6010

- On the day of shipment 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +1-215-662-3538.

8.5 Alloantibody (The Rudbeck Laboratory)

Specimen Collection and Processing

Alloantibody testing will be performed at screening and baseline.

- Collect 2.0 mL blood sample in a plain red top (no additives) Vacutainer® tube.
- After a firm clot has formed, spin the Vacutainer® tube in the centrifuge at 2000-3000 RPM or 1100-1300 RCF(g) for 10 minutes.
- Aliquot a minimum of 1 mL of serum into a 1.8 ml cryovial.
- Freeze the aliquot at -70°C.

Packing and Shipping Instructions

- Follow packing instructions outlined in section 5 of this manual.
- Follow shipping instructions outlined in section 6 of this manual.
- Ship the samples on dry ice to the HLA laboratory using overnight delivery, Monday through Wednesday. The laboratory is not staffed on weekends or holidays.
- Batch ship quarterly.

Elisabeth Wijkström
Clinical immunology
Rudbeck laboratory (C5), University Hospital
751 85 Uppsala, Sweden
Tel: +46-18-6114063

- On the day of shipment 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 Elisabeth.wijkstrom@klinimm.uu.se and anna.andersson@klinimm.uu.se.
- 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +46-18-553149.

8.6 Autoantibody (Barbara Davis Center, Denver Colorado)

Specimen Collection and Processing

Autoantibody testing will be performed at baseline, day 75, month 6, and month 12 following the islet transplantation. If a subsequent transplant occurs then the previous follow-up schedule ends and a new visit schedule starts, beginning with the baseline visit. If a subsequent transplant occurs, then the Autoantibody test will also be performed at 1 year following the initial transplant.

- 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 1100 – 1300 RCF (approximately 2000-3000 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.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- Batch ship at least quarterly on dry ice.
- 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.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  Barbara Davis Center
  Attn: Dr. Liping Yu
  M20-4201E
  1775 Aurora Ct. UC Denver, AMC
  Aurora, CO USA 80045
  Tel: +1-303-724-6809
  Liping.yu@ucdenver.edu

- On the day of shipment 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@uchsc.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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +1-303-724-6893.

8.7

8.8 Serum to Archive

Specimen Collection and Processing

Serum to archive should be collected, processed, and shipped to the Rudbeck Laboratory where they will be stored until all archived specimens are collected for the subject. Once all specim***e***ns are collected for a subject, the specimens will be batch shipped to the NIDDK repository. Please refer to Section 9, De-Identification of Archived Specimens. Specimens should be collected at the screening visit, day 75, month 6, and month 12 following the islet transplantation.

- Collect 4.0 mL of blood in a Gold SST tube. Allow the vacuum to be exhausted.
- Gently invert the tube 5 times, ensuring mixing of clot activator with blood.
- Allow the 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.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- Batch ship at least quarterly on dry ice using overnight delivery Monday through Wednesday. The laboratory is not staffed on weekends or holidays.

  Elisabeth Wijkström
  Clinical immunology
  Rudbeck laboratory (C5), University Hospital
  751 85 Uppsala, Sweden
  Tel: +46-18-6114063

- On the day of shipment Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. NOTE: you will need to expand the columns in the specimen list to show all the data before printing. An automatic email will be sent to Elisabeth.wijkstrom@klinimm.uu.se and anna.andersson@klinimm.uu.se.
- 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +46-18-553149.

The samples will be registered in the biobank, re-labeled and shipped from Uppsala to Fisher BioServices when all of the samples are collected for a subject.

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  Fisher BioServices Corporation
  Attention: Sandra Ke or Heather Higgins
  20301 Century Blvd, Bldg 6, Suite 400
  Germantown, MD USA 20874
  Office: +1-240-686-4702

- On the day of the shipment Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. NOTE: you will need to expand the columns in the specimen list to show all the

data before printing. 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 9)and fax a copy of the form including the airbill tracking number to the laboratory at +1-301-515-4049.

8.9 Plasma to Archive

Specimen Collection and Processing

Once all specimens are collected for a subject, the specimens will be batch shipped to the NIDDK repository. Please refer to section 9, De-Identification of Archived Specimens. Specimens should be collected at the screening visit, day 75, month 6, and month 12 following the islet transplant. Collect 30 mL of blood (4 CPT tubes for isolation of PBL) from each individual into a BD ® Vacutainer Mononuclear Cell Preparation Tube (CPT) blue/black closure top tube. Each tube should be labeled with the subject's unique identification number, and the date and time the blood is drawn.

- Draw 30 mL of blood (4 CPT tubes) in the morning. (The site in Norway must arrange sampling time according to the regular flight schedule for Alranda Stockholm).
- Ship immediately at ambient temperature. The samples should arrive at Rudbeck Lab within 4-6 hours.

Packing and Shipping Instructions

- Follow packing instructions outlined in Section 5 of this manual.
- Shipment with courier JETPACK or DHL
- Delivery Monday through Wednesday. The laboratory is not staffed on weekends or holidays.

Elisabeth Wijkström
Clinical immunology
Rudbeck laboratory (C5), University Hospital
751 85 Uppsala, Sweden

Tel: +46-18-6114063

- On the day of shipment 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 Elisabeth.wijkstrom@klinimm.uu.se and anna.andersson@klinimm.uu.se.
- 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +46-18-553149.

The following is a list of equipment and reagents required at Rudbeck Laboratory:

- Centrifuge
- Conical Tubes, 15 mL, sterile
- Hematology Mixer, Model M26125 (Fisher #12-814-2)
- Nalgene Cryo Freezing Container filled with 2-propanol (Nalgene #5100-001, Fisher#15350-50)
- Pasteur Pipettes (Fisher# 13-678-20)
- Pipette Aids (Fisher #13-681-19)
- Vials, cryogenic, 1.8mL, sterile (Fisher#12-565-171N)
- Biological Safety Cabinet
- Alcohol Swabs, Gloves, Sharps Disposal System
- Guava Personal Cytometer (Guava Technologies #0100-1430)
- BD Vacutainer™ CPT™ Preparation Tubes with Sodium Citrate
- Phosphate Buffered Saline (PBS) sterile, Ca^{++}, Mg^{++} free
- Freezing Medium A (HuAB serum, Tissue culture tested, heat inactivated, filtered)
- Freezing Medium B (20% DMSO, HuAB serum, Tissue culture tested, heat inactivated, filtered)
- Trypan Blue

Specimen Processing

All work needs to be performed under the biological safety cabinet observing biosafety regulations and using sterile technique.

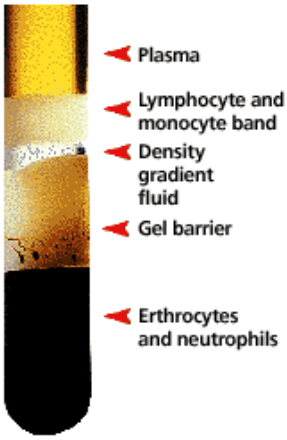
Take precautions while handling the glass tubes as they have the potential for breakage. Handle all biological samples and blood collections “sharps” in accordance with the policies and procedures of your facility.

PLASMA COLLECTION:

1. Before centrifugation, remix the blood by gently inverting the CPT tube 8 to 10 times.

2. Use a temperature controlled centrifuge and set the temperature to 18-25 °C. Centrifuge the tube that contains blood at 2800 RPM for 30 minutes at 20°C. Keep the brake OFF.

3. After the tubes are centrifuged, you will see the buffy layer (lymphocyte and monocyte band) on top of the Gel Barrier. It looks like the picture to the right:



4. Using a serological pipette, carefully transfer the top half of the plasma layer into a sterile 50 mL sterile disposable centrifuge tube, DO NOT DISTURB the lymphocyte and monolayer band (PBMC layer). Combine plasma from each CPT tube into one 50 mL tube.

5. From the 50 mL tube, aliquot 250 µl plasma into 1.8 mL cryovials. Freeze the cryovials with plasma at -70 C.

The samples will be registered in the biobank, re-labeled and shipped from Uppsala to Fisher BioServices when all of the samples are collected for a subject.

- Follow packing instructions outlined in Section 5 of this manual.
- Follow shipping instructions outlined in Section 6 of this manual.
- Please complete an Expanded Service International Air Waybill (With Dry Ice) – Appendix 6 and the Commercial Invoice form (Appendix 8).

  Fisher BioServices Corporation
  Attention: Sandra Ke or Heather Higgins
  20301 Century Blvd, Bldg 6, Suite 400
  Germantown, MD USA 20874
  Office: +1-240-686-4702

- On the day of the shipment 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +1-301-515-4049.

8.10

8.11 RNA and Plasma to Archive (The Rudbeck Laboratory)

Specimen Collection and Processing

RNA and plasma to archive will be collected, processed, and shipped to the Rudbeck Laboratory. Specimens to be collected at the baseline visit, day 3, day 14, day 75, and 1year following the initial islet transplantation.

- Draw blood into a 10 mL EDTA vacutainer tubes are centrifuged at 2000-3000 RPM or 1100-1300 RCF(g) for 10 minutes.
- The total supernatant (i.e. EDTA plasma), are divided into 0.5 mL aliquots in 1.8 mL cryovials (up to 10 total).
- 0.5 mL aliquots of remaining cell pellet (i.e. red and white blood cells are transferred to 1.8 mL cryovials (up to 5 total).
- 1.2 mL of RNAlater™ (Ambion Inc) are thereafter added to each cryovial followed by mixing.
- The tubes are stored at – 70° C until shipping.

Packing and Shipping Instructions

- Follow packing instructions outlined in section 5 of this manual.

- Follow shipping instructions outlined in section 6 of this manual.
- The samples should be shipped, without preceding thawing, on dry ice to the address below using overnight delivery Monday through Wednesday. The laboratory is not staffed on weekends or holidays.

Elisabeth Wijkström
Clinical immunology
Rudbeck laboratory (C5), University Hospital
751 85 Uppsala, Sweden
Tel: +46-18-6114063

- On the day of shipment 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 Elisabeth.wijkstrom@klinimm.uu.se and anna.andersson@klinimm.uu.se .
- 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 9) and fax a copy of the form including the airbill tracking number to the laboratory at +46-18-553149.

9. DE-IDENTIFICATION OF ARCHIVED SAMPLES

Specimens that are collected for the purpose of archiving will be de-identified prior to shipping to the repository in the United States. The following procedures will be in place to ensure these specimens are de-identified appropriately:

1. Archived specimens will be collected at visit 01 (screen), visit 10 (day 75), visit 11 (month 6) and visit 12 (month 12), for a total of 4 collection time points.
2. The specimens will be labeled according to procedures outlined in section 4 of this manual, and then shipped to the Rudbeck Laboratory (Uppsala, Sweden) where they will be stored until the series of specimens are collected (all 4 collections).
3. Once the Rudbeck Laboratory has obtained the series of samples for a participant, the laboratory personnel will remove all study labels from the samples, re-label the specimens (i.e. removing all identifiers), and batch ship the specimens to the NIDDK Repository.
4. The specimens will be re-labeled according to the following criteria:
    a. A randomly chosen letter will replace the participant identification number. The same letter will be assigned to each sample in the participant's series of specimens.
    b. The collection time points will be alphabetically labeled, in order for the repository to be informed as to the order of collection, and the timing in the study the collection was obtained. Visit 01 (A), visit 10 (B), visit 11 (C), and visit 12 (D).

Example:

Study Labels:	Participant ID:	CIT01A0101
Re-Labeled:	De-Identified	J-A, J-B, J-C, J-D

Note: The Rudbeck Laboratory will destroy all specimens from participants who screen, but do not randomize in the study. These specimens (visit 01- screen) will not be re-labeled or shipped to the United States.

APPENDIX 1: KIT COMPONENTS

VISIT 01	**KIT #1**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL Cryogenic Vials **FSIGT** (24) 3.5-mL Gold SST Tubes (48) 1.8-mL Cryogenic Vials **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Alloantibody (Rudbeck)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial	**Serum to Archive (NIDDK)** (1) 4-mL Gold SST Tube (3) 1.8-mL Cryogenic Vials **PBMC and Plasma to Archive (NIDDK)** (4) 8-mL CPT™ Tube (1) 15 mL Conical Tube (1) 50 mL Sterile Disposable Tube (16) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube
VISIT 02	**KIT #2**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Alloantibody (Rudbeck)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial	**Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials **RNA &Plasma to Archive (Rudbeck)** (1) 10-mL EDTA Vacutainer Tube (15) 1.8-mL Cryogenic Vials
VISIT 02 **Waitlist**	**Kit #2a**	**HbA1C **(repeat q6mo)**** **(1) 2-mL EDTA Vacutainer Tube**	
VISIT 02 **Waitlist**	**Kit #2b**	**Alloantibody (U. Penn)**(repeat q6mo)**** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Alloantibody (Rudbeck) **(repeat q6mo)**** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial	
VISITS 03 - 04 (all in 1 kit)	**KIT #3**	**TAT, C3a, C-peptide** (9) 4-mL EDTA Vacutainer Tube (18) 1.8-mL Cryogenic Tube	
VISITS 05 & 07	**KIT #4**	**RNA and Plasma to Archive (Rudbeck)** (1) 10-mL EDTA Vacutainer Tube (15) 1.8-mL Cryogenic Vials	
VISIT 09	**KIT #5**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube	

VISIT 10 **(DAY 75)**	**KIT #6**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube ***MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL Cryogenic Vials ***FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial	**Serum to Archive (NIDDK)** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **PBMC and Plasma to Archive (NIDDK)** (4) 8-mL CPT™ Tube (1) 15 mL Conical Tube (1) 50 mL Sterile Disposable Tube (16) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mL Tempus RNA Tube **RNA & Plasma to Archive (Rudbeck)** (1) 10-mL EDTA Vacutainer Tube (15) 1.8 mL Cryogenic Vials *Do not collect these samples at Day 75 for subjects with confirmed graft failure.
VISIT 11	**KIT #7**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL Cryogenic Vials **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials	**Serum to Archive (NIDDK)** (1) 4-mL Gold SST Tube (3) 1.8-mL Cryogenic Vials **PBMC and Plasma to Archive (NIDDK)** (4) 8-mL CPT™ Tube (1) 15 mL Conical Tube (1) 50 mL Sterile Disposable Tube (16) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mL Tempus RNA Tube
VISIT 12	**KIT #8**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL Cryogenic Vials **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials	**Serum to Archive (NIDDK)** (1) 4-mL Gold SST Tube (3) 1.8-mL Cryogenic Vials **PBMC and Plasma to Archive (NIDDK)** (4) 8-mL CPT™ Tube (1) 15 mL Conical Tube (1) 50 mL Sterile Disposable Tube (16) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mL Tempus RNA Tube

VISIT Y1 **(1 year post first transplant)**	**KIT #9**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL Cryogenic Vials **FSIGT** (24) 3.5-mL Gold SST Tubes (58) 1.8-mL Cryogenic Vials **Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial	**Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vials **RNA & Plasma to Archive (Rudbeck)** (1) 10-mL EDTA Vacutainer Tube (15) 1.8 mL Cryogenic Vials

Reduced Follow-up (1 year post-last transplant)	**Kit #50**	**Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**90 min c-peptide post-MMTT & serum creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials
Reduced Follow-up (Monthly and Quarterly)	**Kit #50X**	**Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial	
Suspected Graft Failure	**Kit #50z**	**MMTT** (6) 3.5-mL Gold SST (12) 1.8-mL cryovials	**Alloantibody (U. Penn)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody (Barbara Davis Ctr)** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial

APPENDIX 2: SUPPLY ORDER FORM: KIT SUPPLY

Protocol CIT-01: Please complete form and fax to University of Iowa @ +**1-319-353-3960**

Site Name: _____ Site Number: _____

Order Date: _____ Due Date @ Site: _____

Requested By: _____ Requestor's phone: _____

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

KIT(s) #	**QUANTITY**
Kit(s) # _____	_____
Kit(s) # _____	_____
Kit(s) # _____	_____
Kit(s) # _____ _	_____
Kit(s) # _____	_____

You will receive an initial supply of kits for 10 participants upon notice of your site activation. The initial supply of kits will include (1) Kit #1 and (1) Kit# 2, per subject.

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

If you have any questions or concerns please contact Traci Ripperda at +1-319-384-4180

Comments:

APPENDIX 3: SUPPLY ORDER FORM: BULK SUPPLY (COLLECTION CONTAINERS/MATERIALS)
Protocol CIT-__ __ : Please complete form and fax to University of Iowa @+1-319-335-6535

Site Name: _____ Site Number: _____

Order Date: _____ Due Date @ Site: _____

Requested By: _____ Requestor's phone: _____

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

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 # 02-657-32	10-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 Tubes	_____/100 Tubes
Fisher # 02-683-99B	3.5-mL Gold Top SST Vacutainer Tubes	_____/100 Tubes
Fisher # 02-688-81	8-mL Cell Preparation Tubes (CPT ™)	_____/20 Tubes
Fisher # 02-688-8	2-mL Green-top Sodium Heparin Tubes	_____/100 Tubes
Fisher # NC9215055	3-mL Tempus Blood RNA Tubes	_____/50 Tubes
T311-2P	1.8-mL Cryogenic Vials	_____/100Vials
Fisher # 12-565-162N	4-mL Cyrogenic Vial	_____/400 Tubes
Fisher # 05-539-9	50-mL Sterile Disposable Centrifuge Tubes	_____/20 Tubes
Fisher # 05-538-51	15-mL Sterile Conical Tubes	_____/100 Tubes
Fisher #13-678-20	Pasteur Pipettes	
Fisher # 13-681-19	Pipette Aids	____/ 960 tips
Fisher # 13-711-61	Sterile Urine Container	____/ 1 each
Fisher # 23-111-253	Multi-Stix	__/100 test strips
REAGENTS		
AM7020 Ambion	RNAlater Solution	_____/100 mL

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-521-1000 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 Rudbeck Laboratory	airbills
FedEx Airbills to NIDDK Repository	airbills

APPENDIX 5: FSIGT WORKSHEET

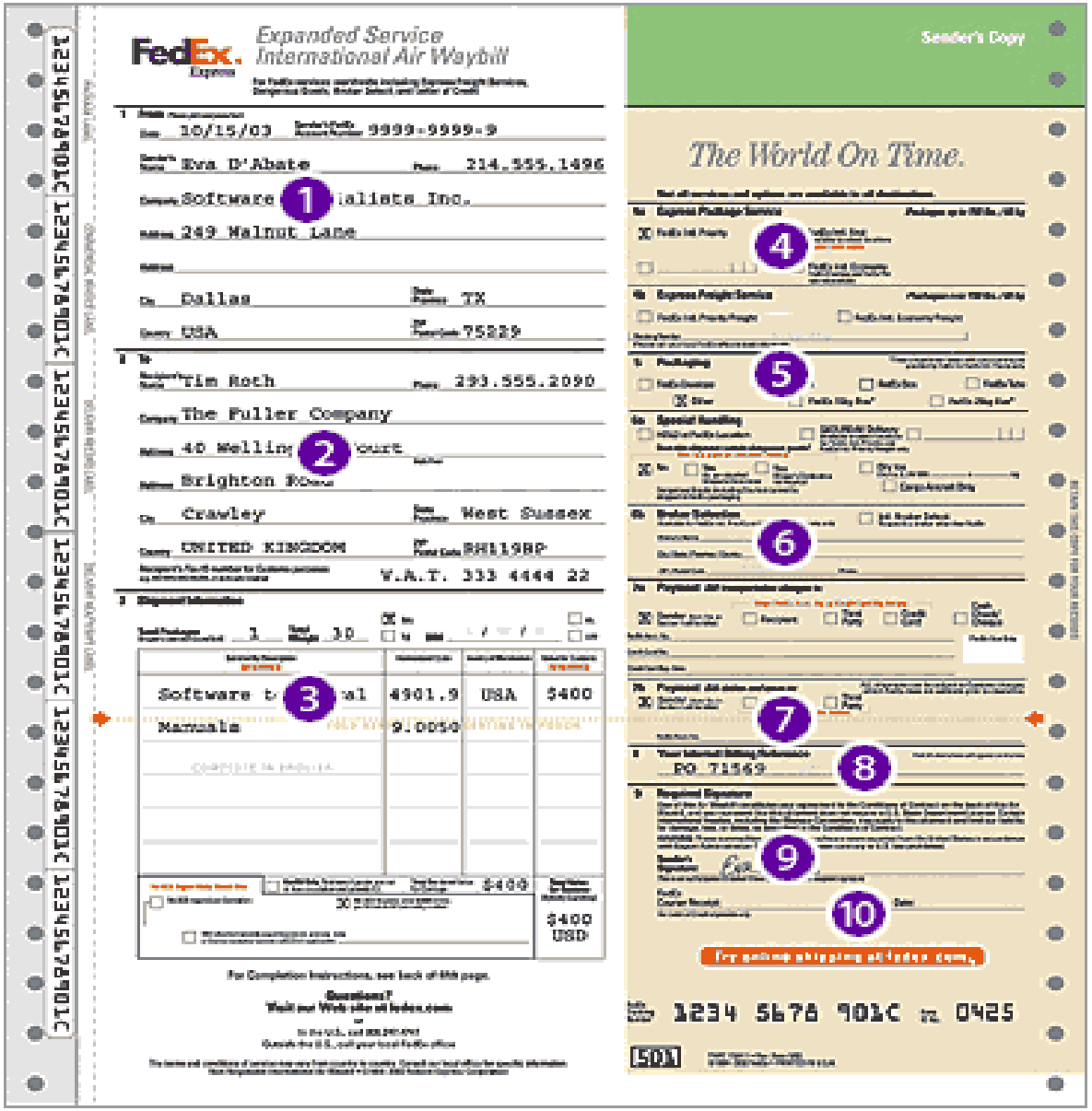
STUDY#:_____

SUBJECT ID: _____ DATE: _____

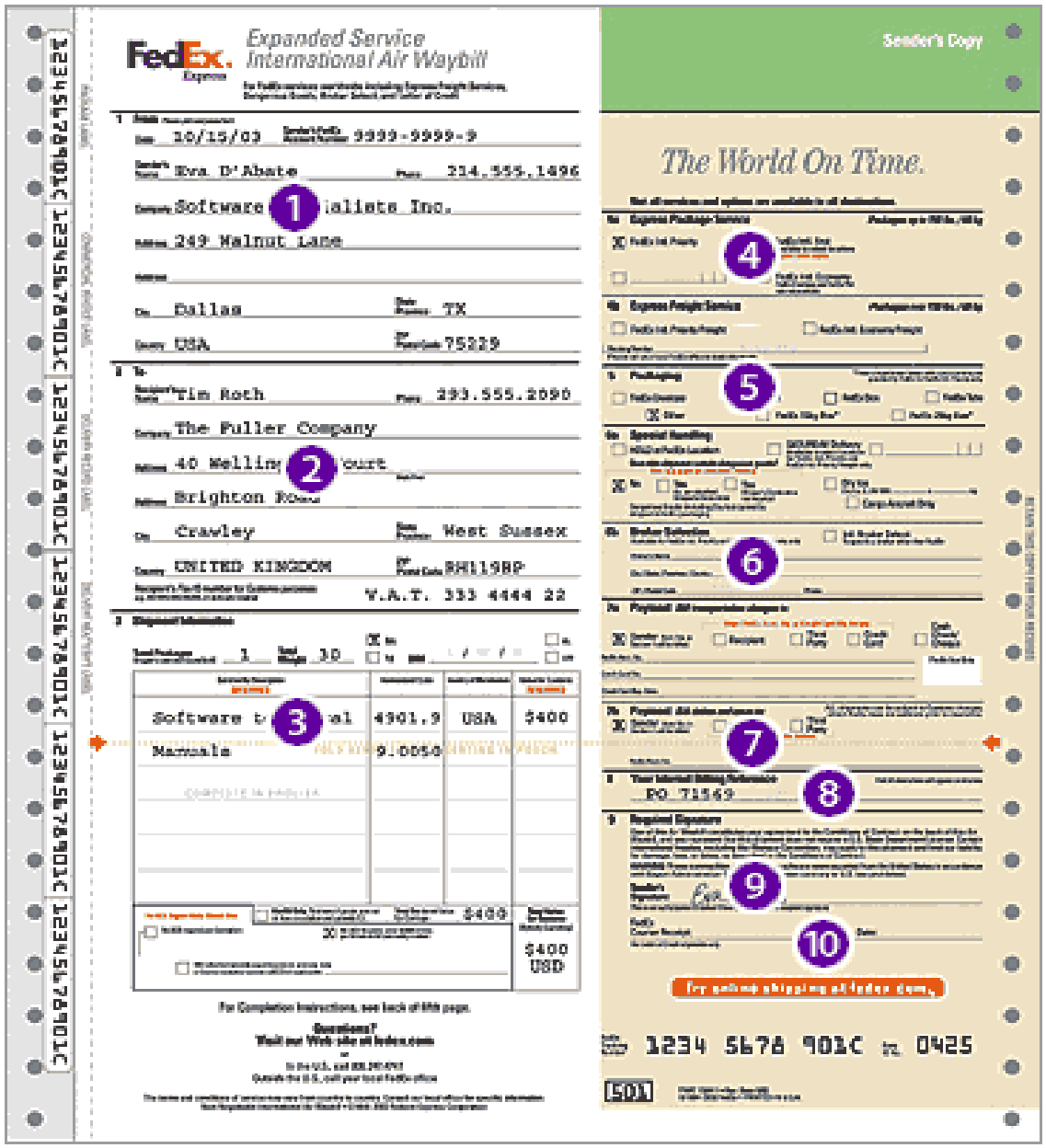
FSIGT PROTOCOL				
Relative Time (minutes)	**Exact Time (hours)**	**Sample #**	**2 ml SST**	**Notes/BG**
-30				IVs placed
-10		1	Yes	
-5		2	Yes	
-1		3	Yes	
0				**Dextrose (begin pushing dextrose @ -30sec, push over 1 min)**
1		4	Yes	
2		5	Yes	
3		6	Yes	
4		7	Yes	
5		8	Yes	
7		9	Yes	
10		10	Yes	
12		11	Yes	
14		12	Yes	
16		13	Yes	
18		14	Yes	
20 (draw sample at 19min45sec)		15	Yes	**After 20 min sample drawn (begin pushing insulin , 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 after 20 minute sample drawn)

APPENDIX 6: EXPANDED SERVICE INTERNATIONAL AIR WAY BILL COMPLETION INSTRUCTIONS (**WITH DRY ICE**)



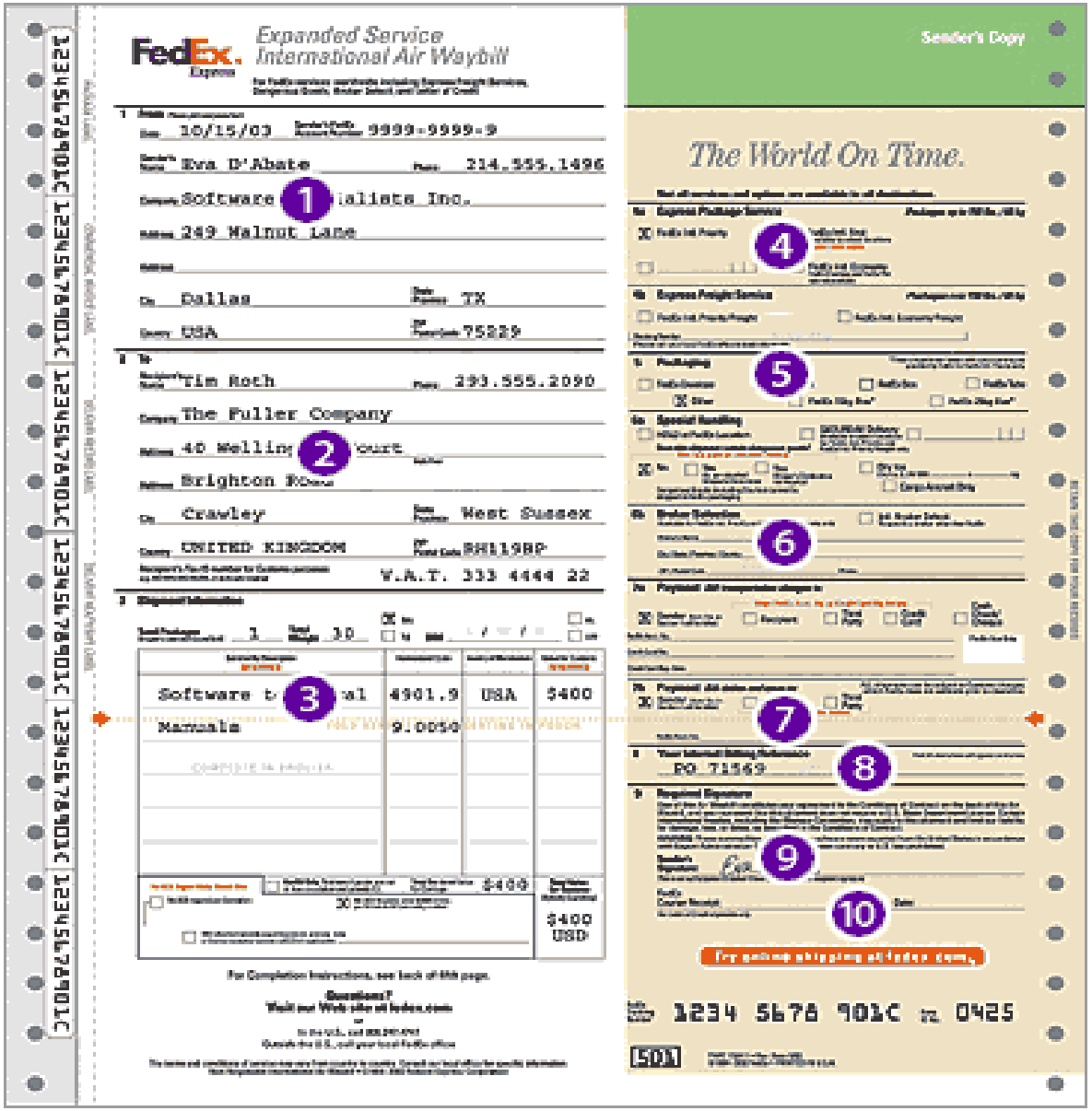
1. **Sender Information**
   Enter your shipping information.
   This includes the address you are shipping from, your name, your phone number, the FedEx account number should already be pre-printed on the airbill. *If the FedEx account number is not pre-printed on the airbill you may enter this information,* ***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 Diagnostic Specimens – (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



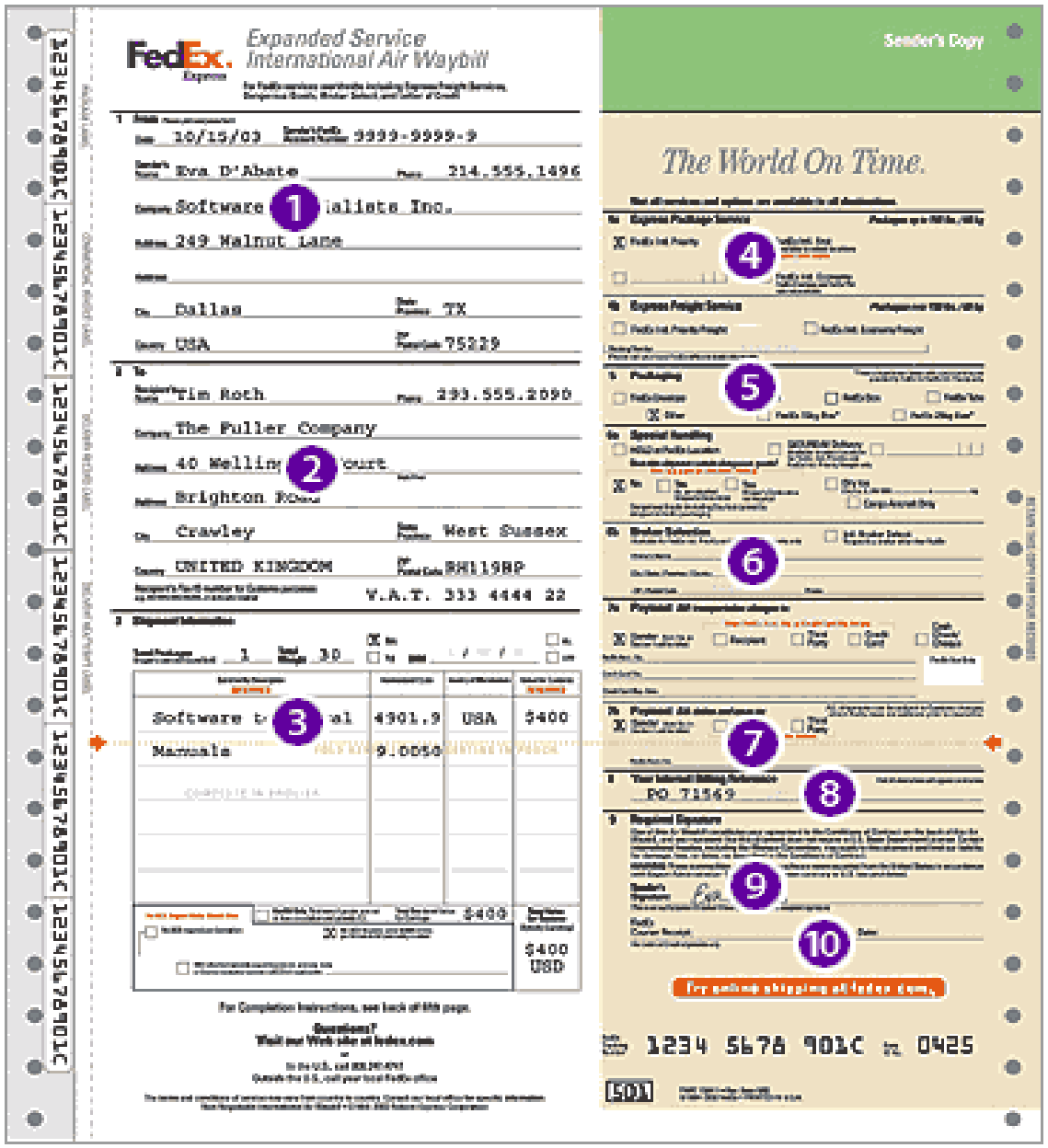
4. **Express Package Service**
   4a. Indicate FedEx International Priority®.
   4b. Leave Blank
5. **Packaging**
   Indicate the type of FedEx Express packaging you are using, or mark "other" if you are using your own packaging.
6. **Special Handling**
   Does the shipment contain dangerous goods?
   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, ***4261-1614-6*** .

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

APPENDIX 7: EXPANDED SERVICE INTERNATIONAL AIR WAY BILL COMPLETION INSTRUCTIONS (**WITHOUT DRY ICE**)



1. **Sender Information**
   Enter <u>your</u> 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 <u>recipients</u> 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 Diagnostic Specimens – (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, ***4261-1614-6*** .

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

APPENDIX 8: 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 MEA-SURE	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 9: 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 #: _____