CIT-07: ISLET TRANSPLANTATION IN TYPE 1 DIABETES

LABORATORY MANUAL FOR CIT-07 STUDY-SPECIFIC CENTRAL ASSESSMENTS

VERSION 8.0
APRIL 25, 2013

CONFIDENTIAL

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APPENDICES

1. CIT-07 DCC Protocol Coordinator Information

DCC Protocol Coordinator

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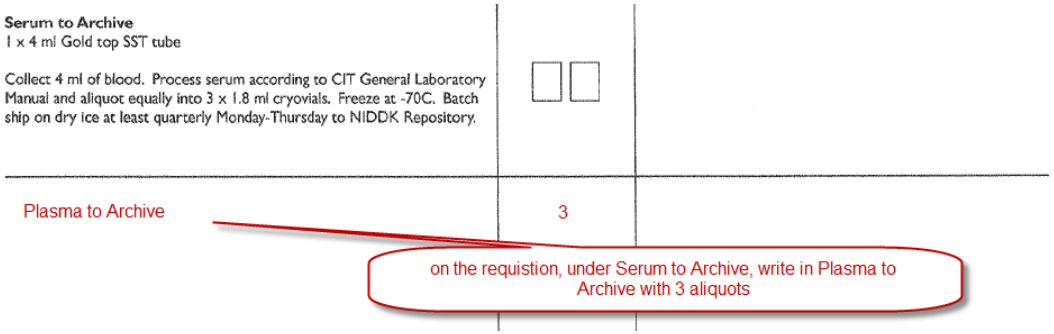
2. CIT-07 Central Laboratory Specimen Schedule

Central Laboratory Assessments					
Assessment	**Laboratory**	**Visit / Time-point**	**Volume**	**Collection Container**	**Shipping Instructions**
Hemoglobin A1c (HbA1c)	University of Washington	Visits 10, 13, 14, 15, Y1, 16, 17, 18, 19, Y2	2 mL Blood	(1) 2-mL Lavender top EDTA Vacutainer	Ship on cold pack within 24 hours of collection. Ship Monday-Thursday only.
Fasting serum glucose and c-peptide / serum creatinine	University of Washington	Visits 8, 9, 10†, 11, 12, 13*, 14*, 15, Y1, 17,19, Y2	2 mL Blood	(1) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
MMTT: Stimulated serum glucose and c-peptide	University of Washington	Visits 10†, 13, 14, 15, Y1, 17, 19, Y2	4 mL Total Blood • 2 mL at 60 minutes (only if checking for graft failure) • 2 mL at 90 minutes (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.
Insulin Modified FSIGT	University of Washington	Visits 10†, 15, 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.
Atherogenic Profile	University of Washington	Visit Y1, 19	8.5 mL Blood	(1) 8.5 mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Alloantibodies	University of Pennsylvania	Visits 03$^{\infty}$, 10, 13, 14, 15, Y1, 17, 19	2 mL Blood	(1) 3-mL Red-top Vacutainer	Ship on dry ice in batches at least quarterly. Ship Monday – Thursday only.

Assessment	Laboratory	Visit / Time-point	Volume	Collection Container	Shipping Instructions
Autoantibodies	Barbara Davis Center	Visits 10, 13, 14, 15, Y1, 17, 19	2 mL Blood	(1) 3-mL Red-top OR (1) 3.5-mL Gold SST	Ship on dry ice in batches at least quarterly .Ship Mon – Wed only. If collected on Thurs or Fri, freeze serum at -20°C or -70°C until Mon, Tues, or Wed. in dry ice.
Serum to Archive	NIDDK Repository	Visits 10, 13, 14, 15, Y1	4 mL Blood	(1) 4-mL Gold SST	Ship in batches at least quarterly.
Plasma to Archive (see below)	NIDDK Repository	Visits 10, 13, 14, 15, Y1	10 mL Blood	(1) 10-mL Na Heparin Vacutainer	Ship in batches at least quarterly.
GFR	University of Minnesota	Visits 08, 10, 15, Y1, 19	10 mL Blood 2 mL each at 120, 150, 180, 210 and 240 minutes	(5) 2-mL Na Heparin Tube	Ship in batches weekly on dry ice. Ship Mon – Thurs.
Albumin/Creatinine Ratio	University of Minnesota	Visits 08, 10, 15, Y1, 17, 19	5 mL Urine	Sterile Urine Container	Ship in batches weekly, frozen on dry ice. Ship on Monday – Thursday.

* If blood is drawn locally at Months 7, 8, 10 and 11 (Visits 13a, 13b, 14a and 14b, respectively), sample should be sent from local lab to study site and then shipped to the central laboratory.
† Do not collect these samples at Day 75 for subjects with confirmed graft failure
∞Collect these samples beginning on Day -2 (pre-IS) for Visit 03.

Plasma to Archive Note– Specimen kits will not be modified to reflect the changes to archived samples. To collect Plasma to Archive samples, use one of the three PBMC/Plasma to Archive primary tubes that are currently in the kits. Draw only one green-top and discard the other two. To aliquot, take (3) 1.8 mL cryovials from bulk supply. Label each with an extra barcode label from the set inside the kit lid. In the STS (Process and Aliquot step), you will scan each of these aliquots as an extra sample and choose "Plasma to Archive" as the specimen type. On the kit requisition form, add (write in) a section for the Plasma to Archive samples. Ship the Plasma to Archive with the Serum to Archive to the NIDDK Repository.



3. CIT-07 Kit Components

Visit 03 **Day -2 through Day 0**	**Kit #3**	**TAT, C3a and c-peptide** (5) 2-mL EDTA Vacutainer (Lavender top) (5) 1.8-mL cryovial Vial	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial
Visit 08 **Day 28**	**Kit #4**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL cryovial	**GFR** (5) 2-mL Na Heparin Vacutainer Tubes (5) 1.8-mL cryovial
Visit 09 **Day 56**	**Kit #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials	
Visit 10 **Day 75**	**Kit #6**	***Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials ***MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovial **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL cryovial ***FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL cryovials	**GFR** (5) 2-mL Na Heparin Tubes (5) 1.8-mL cryovials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial ~~**RNA to Archive (NIDDK)**~~ ~~(3) 3-mLTempus RNA Tube~~ **(discard)** **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL cryovials ~~**PBMC and**~~ **Plasma to Archive** (3) 10-mL Na Heparin Tubes *Use (1) 10-mL Na Heparin Tube for Plasma and discard the other 2* *(3) 1.8-mL cryovial from bulk supply*
Visit 11 **Day 120**	**Kit #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials	

Visit 12 **Day 150**	**Kit #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials	
Visit 13 **Day 180**	**Kit #7**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial	**Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial ~~**RNA to Archive (NIDDK)**~~ ~~(3) 3-mLTempus RNA Tube~~ **(discard)** **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL cryovials ~~**PBMC and**~~ **Plasma to Archive** (3) 10-ml Na Heparin Tubes *Use (1) 10-mL Na Heparin Tube for Plasma and discard the other 2* *(3) 1.8-mL cryovial from bulk supply*
Visit 13a, 13b **Days 210, 240**	**Kit #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials	
Visit 14 **Day 270**	**Kit #7**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial	**Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial ~~**RNA to Archive (NIDDK)**~~ ~~(3) 3-mLTempus RNA Tube~~ **(discard)** **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL cryovials ~~**PBMC and**~~ **Plasma to Archive** (3) 10-mL Na Heparin Tubes *Use (1) 10-mL Na Heparin Tube for Plasma and discard the other 2* *(3) 1.8-mL cryovial from bulk supply*
Visit 14a, 14b **Days 300, 330**	**Kit #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials	

Visit 15 **Day 365**	**Kit #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Albumin/Creatinine Ratio** (1) Urine Specimen Container **(**1) 4.0-mL cryovials **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL cryovials **GFR** (5) 2-mL Na Heparin Tubes (5) 1.8-mL cryovials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial ~~**RNA to Archive (NIDDK)**~~ ~~(3) 3-mLTempus RNA Tube~~ **(discard)** **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL Cryogenic Vials ~~**PBMC and**~~ **Plasma to Archive** (3) 10-mL Na Heparin Tubes *Use (1) 10-mL Na Heparin Tube for Plasma and discard the other 2* *(3) 1.8-mL cryovial from bulk supply*
Visit Y1 **365 days post initial transplant**	**Kit #8**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL cryovial **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL cryovials **GFR** (5) 2-mL Na Heparin Tubes (5) 1.8-mL cryovials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial ~~**RNA to Archive (NIDDK)**~~ ~~(3) 3-mLTempus RNA Tube~~ **(discard)** **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL cryovials ~~**PBMC and**~~ **Plasma to Archive** (3) 10-mL Na Heparin Tubes *Use (1) 10-mL Na Heparin Tube for Plasma and discard the other 2* *(3) 1.8-mL cryovial from bulk supply* **Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials
Visit 16 **Month 15**	**Kit #12**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube	

Visit 17 Month 18	**Kit #9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL cryovial **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial
Visit 18 Month 21	**Kit #12**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube	
Visit 19 Month 24	**Kit #10**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL cryovial **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**GFR** (5) 2-mL Na Heparin Tubes (5) 1.8-mL cryovials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials
Visit Y2	**Kit #11**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials
Reduced Follow-Up (Year 1 and 2post-initial transplant)	**Kit #50**	**90 min c-peptide post MMT, Serum Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial	**HBA1C** (1) 2-mL EDTA Vacutainer Tube

Reduced Follow-Up (monthly and quarterly)	**Kit #50x**	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial	
Suspected Graft Failure	**Kit #50z**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL cryovials **MMTT (stimulated glucose and c-peptide)** (2) 3.5-mL Gold SST (4) 1.8-mL cryovials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL cryovial

***** Do not collect these samples at Day 75 for subjects with confirmed graft failure.
SEE APPENDIX 1 FOR KIT SUPPLY ORDER FORM

4. Kit Usage

Kit #6 (Visit10 and Visit 15), kit # 7 (visit 13 and visit 14), kit #8 (visit Y1), kit #9 (visit 17), kit #10 (visit 19) and kit #11 (Y2) 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.

5. CIT-07 Blood Volume Table

CIT07 - MAXIMUM RESEARCH BLOOD VOLUME TABLE																				
TIME POINTS/VISITS																				
	Days				**Weeks**				**Months**											
TIMING OF STUDY PARTICIPATION	**SCRN**	**BL**	**TX 0**	**3**	**1**	**2**	**3**	**4**	**2**	**2.5** (Day 75)	**4**	**5**	**6, 7, 8**	**9, 10, 11**	**12**	1 yr post initial tx	**15**	**18**	**21**	**24**	2 yrs post initial tx
VISIT	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13***	**14****	**15**	**Y1**	**16**	**17**	**18**	**19**	**19**
BLOOD VOLUMES																					
LOCAL LABORATORY ASSESSMENTS																					
CBC (WBC + Diff & Plat)	5	5	5		5	5	5	5	5	5	5	5	5	5	5		5	5	5	5	
Chemistry	4	4	4		4	4	4	4	4	4	4	4	4	4	4		4	4	4	4	
Lipids	4	4								4			4	4	4			4		4	
Thyroid Function	4	4																			
Serology	7	7														7					
EBV IgG	2																				
CMV IgG, CMV IgM		4														4					
Coagulation (PT, PTT, INR)	5	5	5																		
Blood Type & HLA		11																			
Crossmatch		10																			
PRA		10																			
Fasting and 2 post-prandial (1-3 hrs) c-pep				9	9																
Sirolimus drug levels (trough)			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
Tacrolimus drug level (trough)				4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
EBV by PCR		4																			
CMV by PCR		4								4			4								
CENTRAL LABORATORY AND METABOLIC ASSESSMENTS																					
GFR (5 timed specimens/timept; 2 ml each)	10	10						10		10					10	10				10	
HbA1c	2	2								2			2	2	2	2	2	2	2	2	2
Fasting glucose & c-pep / serum creatinine	2	2						2	2	2	2	2	6	6	2	2					
60, 90 min c-pep, gluc (MMTT) / serum creat	4																				
90 min c-pep, gluc (MMTT) / serum creat										2			2	2	2	2		2		2	2
Insulin modified FSIGT (c-pep, insulin, gluc)		48								48					48	48					
Atherogenic profile		8.5														8.5				8.5	
CENTRAL MECHANISTIC ASSAYS																					
Alloantibody	2	2								2			2	2	2	2		2		2	
Autoantibody		2								2			2	2	2	2		2		2	
TAT, c-peptide & C3a		2	8																		
CENTRAL ARCHIVED SAMPLES																					
Serum		4								4			4	4	4	4					
PBMC / Plasma		10								10			10	10	10	10					
TOTALS (mls)	**51.0**	**162.5**	**26.0**	**17.0**	**26.0**	**17.0**	**17.0**	**29.0**	**19.0**	**107.0**	**19.0**	**19.0**	**53.0**	**49.0**	**103.0**	**109.5**	**19.0**	**29.0**	**19.0**	**47.5**	**4.0**
BL - WK 6 TOTAL (mls)	**294.5**																				
YEAR 1 TOTAL (mls)	**891.0**																				
YEAR 2 TOTAL (mls)	**118.5**																				

* 13, 13a, and 13b
** 14, 14a, and 14b

Appendix 1: Kit Supply Order Form

Please complete form and fax to University of Iowa @ **+1-319-335-6580**

Protocol #: _____

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 through Kit# 5, per subject.

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

Appendix 2: University of Miami Substudy

1. Additional Study Specimen Schedule

For Miami CIT-07 subjects:
Follow the CIT-07 Site Specific Laboratory Manual for collection of central labs, except for the following tests: fasting serum glucose and c-peptide/serum creatinine, and MMTT. The details of these assessments are below.

For the MMTT at Visit 10, 13, 14, 15, Y1, 17, and 19, please combine the MMTT package of 2* tubes (60 minute and 90 minute) and aliquots from kits #6, #7, #8, #9, or #10 and the MMTT package of tubes (9) and aliquots from kit #3Y to complete all of the time points.

Central Laboratory Assessments					
Assessment	Laboratory	Visit / Time-point	Volume	Collection Container	Shipping Instructions
Fasting serum glucose and c-peptide / serum creatinine	University of Washington	V04,05,06,07, 08a,08b,08c,09, 10a,10b	2 mL Blood	(1) 3.5-mLGold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
MMTT: Stimulated serum Glucose and c-peptide (Boost Extra, 15,30,60*90*,120,150,180, 210,240,270,300 min)	University of Washington	V10,13,14,15,Y1,17,19	22 mL Total Blood 2 mL at 15,30,60, 90,120,150,180,210, 240,270, and 300 minutes each	(11) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.

*These tubes and cryovials are included in the parent kit (kits #6, 7, 8, 9,10 or 11).

2. Additional Study Kit Components

For Miami CIT-07 subjects:
Follow the CIT-07 Site-Specific Laboratory Manual for kit components and the kits listed below.

VISIT 04 – 07 Days 3,7,14,21 **VISIT 8a-8c** Days 35, 42, 49 **VISIT 10 a** Day 90 **VISIT 10 b** Day 105	**KIT #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials
VISIT 10 Day 75 **VISIT 13** Day 180 **VISIT 14** Day 270 **VISIT 15** Day 365 **VISIT Y1** 365 days post-initial transplant **VISIT 17** Month 18 **VISIT 19** Month 24	**KIT #3Y**	**MMTT (stimulated glucose and c-peptide)** (9) 3.5-mL Gold SST (18) 1.8-mL Cryogenic Vials

See Appendix 1 for kit supply order form.