

**CIT 05: B-Lymphocyte Immunotherapy in
Islet Transplantation: Toward Calcineurin
Inhibitor Free Immunosuppression
AND
CIT 07 PENN SUBJECTS**

Version 2.0
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1. CIT 05 ADDITIONAL STUDY CONTACT INFORMATION

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2. CIT 05 PENN CENTRAL LABORATORY SPECIMEN SCHEDULE

Please follow the CIT-07 Site Specific Laboratory Manual for collection of central labs. The only exception will be the collection of Rituximab and HACA Evaluations.

On Visits 03, 08, 13, and 14 collect the Rituximab and HACA levels in a 5 mL Red/Black speckled top. This will be sent to the Covance Laboratory. It will be batched and shipped quarterly on dry ice. The address is on page 3. The instructions for collecting and handling is detailed in the next this section.

3. RITUXIMAB AND HACA EVALUATION INSTRUCTIONS

INSTRUCTIONS FOR COLLECTING AND STORING SPECIMENS FOR RITUXAN Serum HACA AND/OR Serum PK ASSAYS

Rituxan HACA and Pharmacokinetics (PK) Assays – Serum

Collection Tube Requirements (*NOT* provided by Covance Labs Inc.):

1. 5.0 mL “Red/Black speckled-top” vacutainer tube (Serum Separator Tube with clot activator and gel for serum separation). OR, also acceptable is a “Gold-top” plastic SST vacutainer tube with clot activator and gel (See www.bd.com/vacutainer for reference).

Sample Collection Requirements: These items are provided in your package

1. Non-sterile **2-2.0 mL round bottomed, polypropylene Specimen tube** (12mm x 48mm) with screw-top polystyrene cap (Corning #2028 or VWR 66008-284).
2. Specimen Label to fit the above referenced tube.

Serum Collection Instructions:

1. Draw 3-5 mL of blood (yielding ~1.5 mL of serum) into a red/gray top vacutainer tube using standard venipuncture techniques. If the vacutainer tube has clot activator, invert tube 5 times *gently* to mix.
2. Label the vacutainer tube with the patient’s identification (patient number), date and time of blood draw (dd-MMM-yy format for the date (i.e. 01-JAN-03) and 24:00 hour clock format for the time).
3. Take a plastic bag and label the bag with the barcode from the correlating Fisher kit.
4. Allow the blood clot upright at room temperature for **30** minutes. . (If using plain red top vacutainer with no clot activator, allow the blood clot upright at room temperature for **60** minutes)
5. Centrifuge the sample to isolate the serum (supernatant) from the red blood cells at 1000 x g (approx. 2000 rpm) for at least 10 minutes.
6. Completely fill out the Covance Laboratories, Inc. provided sample label (YELLOW for HACA, WHITE for PK) with the corresponding patient

identification, date and time of blood draw (dd-MMM-yy format for the date (i.e. 01-JAN-03) and 24:00 hour clock format for the time) and affix it to one of the room temperature polypropylene sample transport tubes provided.

7. Draw off the supernatant and pipette a 1.0-1.5 ml aliquot of the serum into the already labeled polypropylene sample tube.
8. Freeze and store the samples upright at or below -20°C as soon as possible.
9. Fill out the Specimen Shipping Manifest on the provided color-coded hard copies. The provided hardcopy of the Sample Shipping Manifest only needs to be filled out if you are unable to fill out the electronic version.
10. Scan the plastic bag with the barcode into the STS system and follow the STS steps. You will need to also fill out the hard copy manifest and put it in the shipment to Covance.
11. When shipping, it is very important for you to print out a paper copy to send with the shipment to Covance and to keep a copy for records.
12. Please be sure to use 25-30 pounds of Dry Ice when shipping the samples to Covance Laboratories, Inc. A completed PK and/or HACA **Rituxan Specimen Shipping Manifest** MUST accompany the samples being shipped. (NOTE: **Ice Packs will not sufficiently freeze the samples during shipment.**)

SHIP TO:

Specimen Management
c/o 6281 Sample Coordinator
Covance Immunochemistry Services
3635 Concorde Parkway Ste 100
Chantilly, VA 20151-1130
Telephone: 1-703-245-2200 ext 2850 or 1-703-409-9796
Fax: 1-703-245-2291
Email: kurt.reber@covance.com

4. CIT 05 PENN KIT COMPONENTS

Please follow the CIT 07 Site Specific Laboratory Manual for the kit components. Please note that the collection tubes for the Rituximab and HACA levels will be provided by Penn.

5. CIT-05/07 BLOOD VOLUME TABLE

CIT05 - MAXIMUM RESEARCH BLOOD VOLUME TABLE																			
TIME POINTS/VISITS																			
TIMING OF STUDY PARTICIPATION	Days				Weeks					Months									
	SCRN	BL	BL	TX	0	3	1	2	3	4	2	2.5 (Day 75)	4	5	6, 7, 8	7a	9, 10, 11	12	12
VISIT	1	2	2 (1)	3	4	5	6	7	8	9	10	11	12	13**	13**	14***	15	15 (1)	1 yr post initial tx
BLOOD VOLUMES																			
LOCAL LABORATORY ASSESSMENTS																			
CBC (WBC + Diff & Plat)	4	4		24		4	4	4	4	4	4	4	4	4	4		4	4	
Chemistry (P18 + Mg or P20)	4	4		16		4	4	4	4	4	4	4	4	4	4		4	4	
Lipids	4	4									4			4	4		4	4	
Thyroid Function	4	4															4		
PSA	4	4																	4
Serology	4	4																	
EBV IgG	8																		
CMV IgG, CMV IgM		4																	4
Coagulation (PT, PTT, INR)	4	4		24															
Blood Type & HLA	4																		
Crossmatch				10															
RA	2	2									2				2		2	2	
Fasting and 2 post-prandial (1-3 hrs) c-pep					2	2					2				2		2	2	
Glucose-Potential Arginine											92						92		
Glucose Counter-Regulation (1)		140	140											140	140		140	140	
Rituximab PK				4					4					4		4			
Sirolimus drug levels (trough)					4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CENTRAL LABORATORY AND METABOLIC ASSESSMENTS																			
EBV and CMV by PCR		8							8	8	8	8	8	24		24	8		8
GFR (5 timed specimens/timept; 2 ml each)	10	10							10		10						10		10
HbA1c	4	4							4		4			4		4	4		4
Fasting glucose & c-pep / serum creatinine	2	2			2	2	2	2	2	2	2	2	2	6		6	2		2
60, 90 min c-pep, gluc (MMTT)	4																		
90 min c-pep, gluc (MMTT)											2			2		2	2		2
Insulin modified FSIGT (c-pep, insulin, gluc)		48									48						48		48
CENTRAL MECHANISTIC ASSAYS																			
Alloantibody		2									2			2		2	2		2
Autoantibody		2									2			2		2	2		2
TAT, c-peptide & C3a		2		8															
PENN MECHANISTIC ASSAYS																			
General B and T cell flow cytometry		4	4		4				4				4	4	12		12		12
HiD B cell tube			8		8				8				8	8			8		24
CD19+ separation for B cell spectratyping		12												12		12	12		12
Clone tracking		4	4		4	4	4	4		4	4	4	4	12		12			12
T cell ELISpot and Cytokine Profile	18		18							18				18		18			18
HLA Antibody Typing		4									4			4		4	4		4
High resolution class II typing		4																	
B Cell ELISpot			10													10	10		10
CENTRAL ARCHIVED SAMPLES																			
Serum		4									4			4		4	4		4
PBMC / Plasma			30								30			30		30	30		30
RNA			9								9			9		9	9		9
TOTALS (mls)	80.0	280.0	184.0	86.0	24.0	20.0	18.0	30.0	40.0	48.0	198.0	38.0	42.0	262.0	144.0	132.0	368.0	144.0	182.0
BL - WK 6 TOTAL (mls)	682.0																		
YEAR TOTAL (mls)	2320.0																		

**13, 13a, and 13b

***14, 14a, and 14b

q 3mos

q 6mos

1. Will occur up to one month following initial Glucose Counter Regulation

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.