Document Title:

PURIFIED HUMAN PANCREATIC ISLETS MASTER PRODUCTION BATCH RECORD (PRODUCT CODE PHPI-A-01) (CIT PROTOCOLS 03 – 07)

1.0 MASTER PRODUCTION BATCH RECORD APPROVAL

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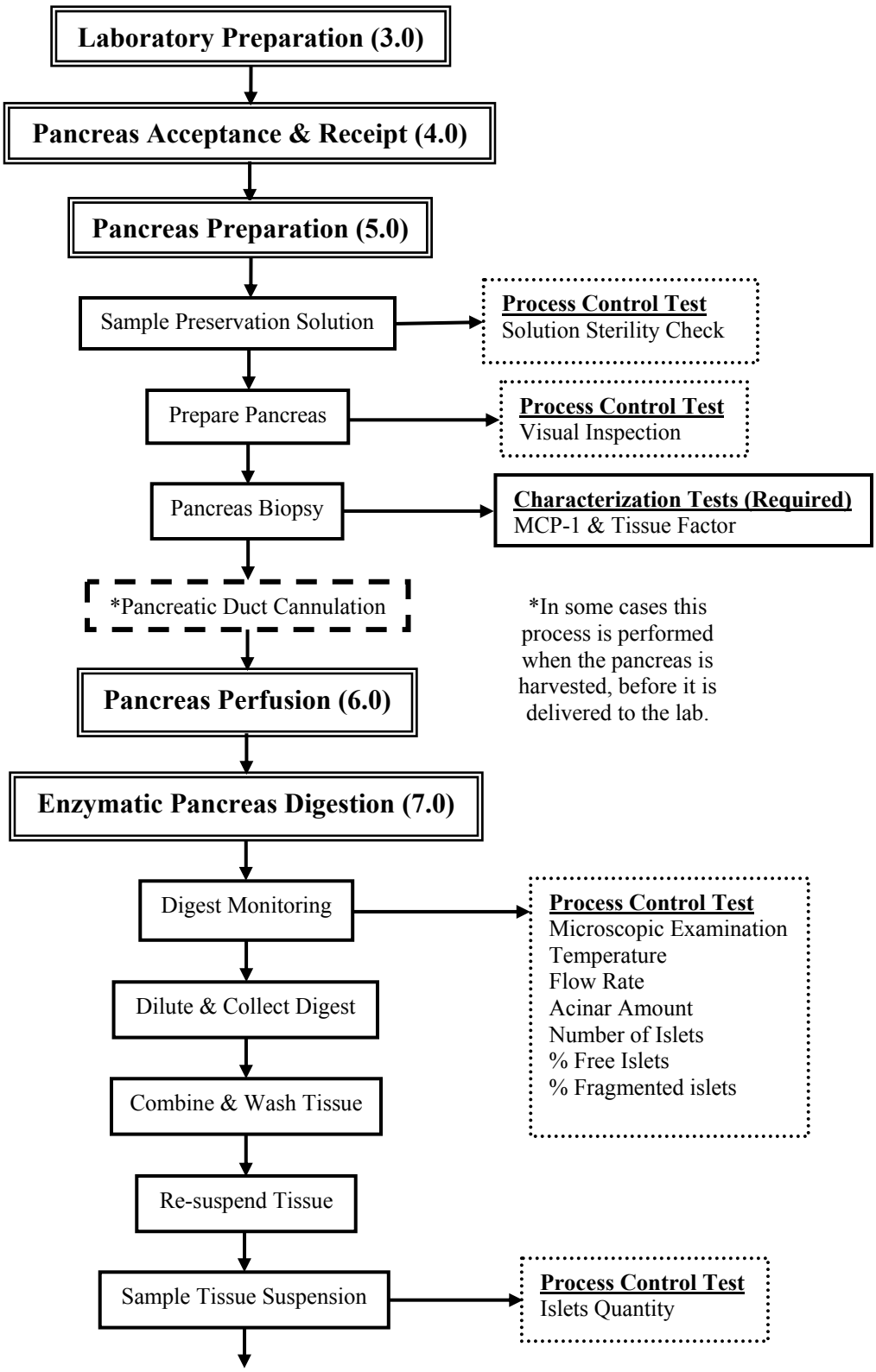
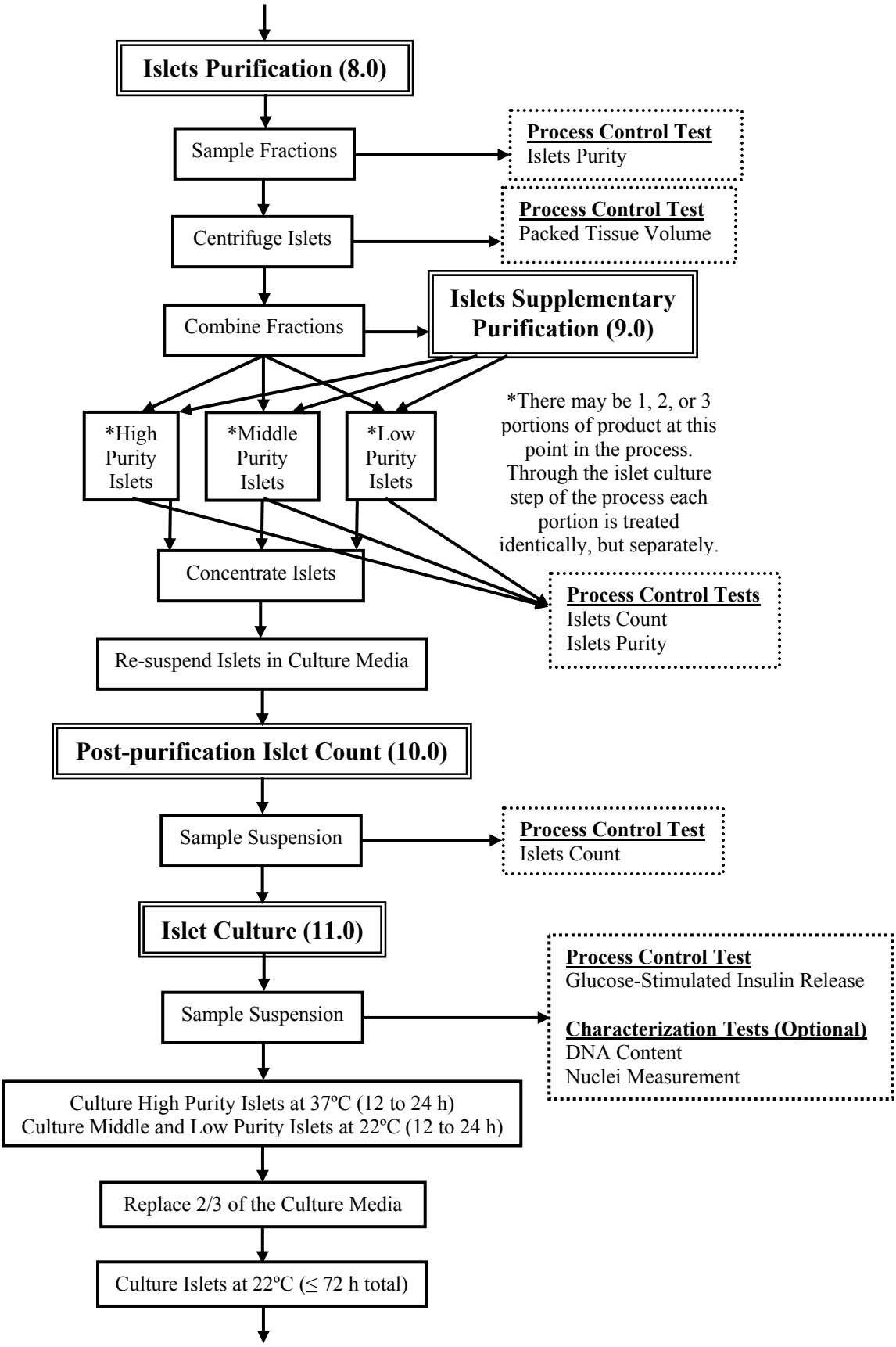
Changes to this Master Production Batch Record must be proposed to the Chief, Regulatory Affairs, DAIT, NIAID, NIH, and approved by all the original signatories, or their successors, before implementation.

2.0 FLOWCHART AND SAMPLING TABLE

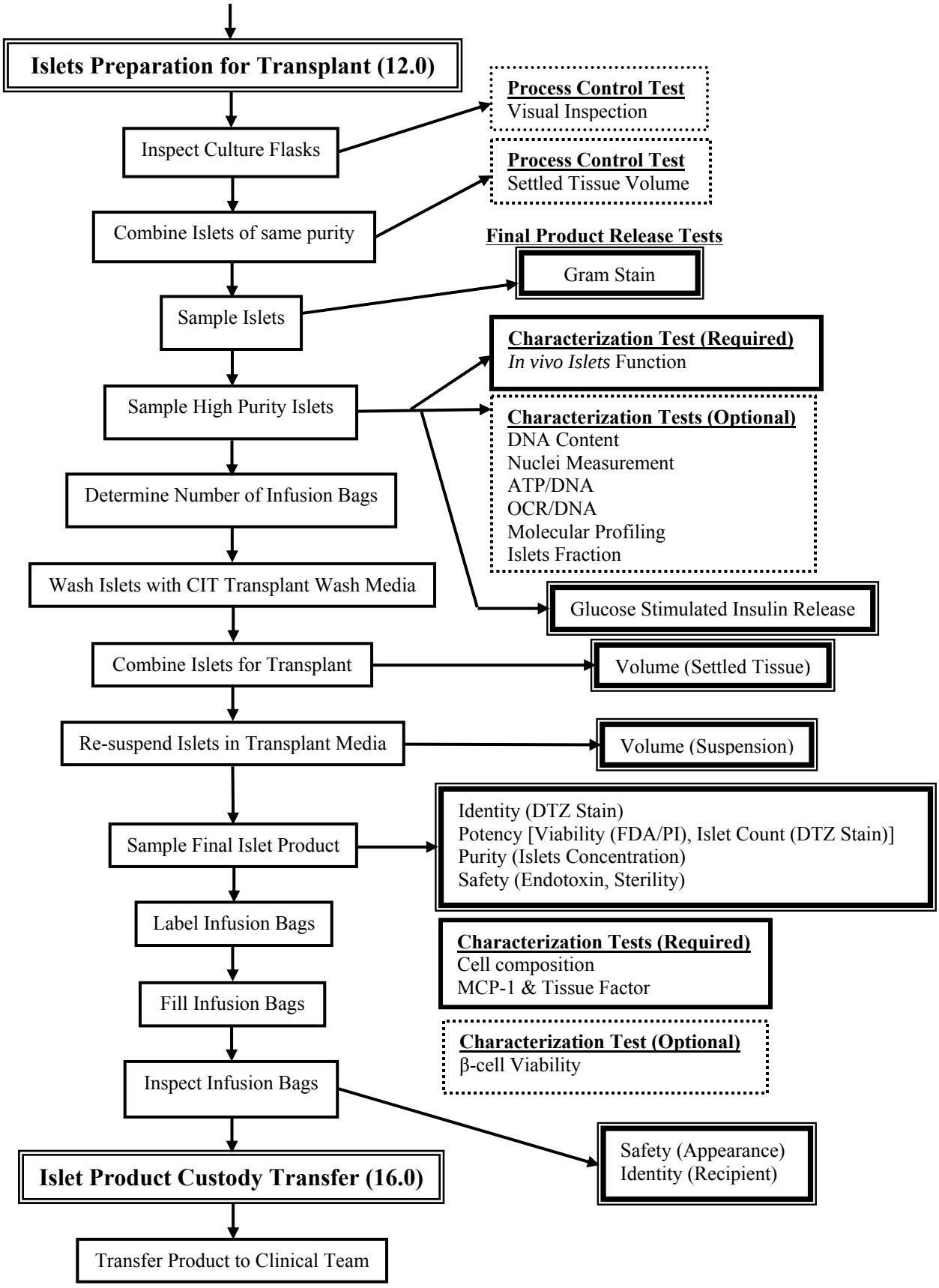
2.1 Production Process Flowchart (MPBR)



Islets Lot Number: _____



Islets Lot Number: _____



Islets Lot Number: _____

2.2 Samples and Tests

MPBR SECTION	SAMPLE TYPES & QUANTITIES PROCESS CONTROL TESTS	TESTS
5.1	Preservation Solution, ≥ 3 mL	Sterility (21 CFR 610.12) & Fungal Culture
7.1.3	Pancreas Digest, ≤ 1-2 mL periodically	Acinar Amount, # of Islets, % Free Islets, % Fragmented
7.5.1	Diluted Pancreas Digest, 2 X 100 µL	Islets Count
8.3.7	Purification Fractions, 0.5 mL/each of 12 fractions & 0.5 mL of W1 fraction, each COBE Run	Islets Purity (%)
8.4.3	Supplementary Purification Islets, 2 X 100 µL (Optional)	Islets Count
10.2	Purified Islets, 2 X 100 µL, High, Middle, Low Purity Levels	Islets Count
12.10	Cultured Islets, All Measured, High, Middle, Low Purity Levels	Settled Tissue Volume
12.13	Cultured Islets, 2 X 100 µL, High, Middle, Low Purity Levels	Post-culture Islets Count
	INTERIM & FINAL CERTIFICATES OF ANALYSIS	
11.1	Suspension, 400 IEQ, High Purity Islets	Glucose Stimulated Insulin Release
12.11.5	Supernatant above cultured islets, volume according to institution's procedure, High, Middle, Low Purity Levels	Gram Stain
12.13 & 12.14, or 12.17.1	Suspension, 2 X 100 µL/Each Final Product T-75 Flask	Islets Identity, Quantity, Concentration
12.17.2	Suspension, 100 IEQ/Each Final Product T-75 Flask	Viability
12.17.3	Supernatant above cultured islets, 1 mL/Each Final Product T-75 Flask	Endotoxin
12.18	Combined Islets, All Measured, High, Middle, Low Purity Levels	Settled Tissue Volume
	FINAL CERTIFICATE OF ANALYSIS ONLY	
12.14	Suspension, 400 IEQ, High Purity Islets (Post-culture Sample)	Glucose Stimulated Insulin Release
12.17.2	Volume according to institution's procedure of islets suspension in each T-75 Flask	Sterility (21 CFR 610.12) & Fungal Culture
	REQUIRED PRODUCT CHARACTERIZATION TESTS FOR INFORMATION ONLY	
5.6	Superficial biopsy of approximately 3 mm X 3 mm X 3 mm	MCP-1 and Tissue Factor
12.14	Suspension, 4,000 IEQ, High Purity Islets	*In vivo* (Nude Mouse) Islets Function
12.17.2	Suspension, 1,000 IEQ/Each Final Product T-75 Flask	Cell Composition
12.17.2	Suspension, 500 to 1,000 IEQ/Each Final Product T-75 Flask	MCP-1 and Tissue Factor
12.17.2	Suspension, 4 X 500 IEQ from T-75 Flask #1 in 1.8 mL cryovials	NIDDK Repository
	OPTIONAL PRODUCT CHARACTERIZATION TESTS FOR INFORMATION ONLY	
11.1	Suspension, 3 X 100 IEQ, High Purity Islets	Pre-culture DNA Content
11.1	Suspension, 3 X 100 IEQ, High Purity Islets	Nuclei Measurement
12.14	Suspension, 3 X 100 IEQ, High Purity Islets	Post-culture DNA Content
12.14	Suspension, 3 X 100 IEQ, High Purity Islets	Nuclei Measurement
12.14	Suspension, 500 IEQ, High Purity Islets	ATP/DNA
12.14	Suspension, 5,000 IEQ, High Purity Islets	OCR/DNA
12.14	Suspension, 5,000 IEQ, High Purity Islets	Molecular Profiling
12.14	Suspension, 500 IEQ, High Purity Islets	Islets Fraction
12.17.2	Suspension, 2,000 IEQ/Each Final Product T-75 Flask	β-cell Viability

Note: Materials used in this process may transmit infectious agents. Therefore, each person participating in this process must be trained in, and follow, the institution's procedures for handling potentially infectious agents. All waste materials from this process that may have contacted the pancreas or the islets must be discarded as Biohazardous Waste.

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Note: It is extremely important to protect the pancreas and the islets from contamination by adventitious microorganisms and pyrogenic agents. Reagents and equipment that may contact the pancreas or islets must be sterile, pyrogen-free, and single-use whenever possible. The institution's procedures for aseptic technique must be followed throughout the execution of this Production Batch Record. All "open" procedure steps must be performed in a clean and disinfected Certified Class II area or Biological Safety Cabinet (BSC).

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Note If, at any time during the execution of this Production Batch Record, you observe:

1) potential discrepancies in the identification of the pancreas or islets,
2) unusual appearance of any materials,
3) unusual, or improper performance of any equipment, or
4) inadvertent deviations from the process as defined in this Production Batch Record or the institution's established procedures;

you must notify the Laboratory Director, or designee, immediately.

The Laboratory Director, or designee, must investigate the observation, and write, sign and date a report giving the details of the observation and its resolution according to the institution's procedures. The occurrence of the event is documented in this Production Batch Record by writing "See Report #X" at the location in the Batch Record where the observation occurred. When allowed by the institution's procedures the report, or a copy, must be filed with this Batch Record. When not allowed, it must be traceable through the unique identification number ("Report #X") written in the Batch Record. The process for reporting a deviation to the CMCMC as defined in DAIT SOP 3200 must also be followed.

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3.0 LABORATORY PREPARATION

3.1 Identification of Institution, Personnel, Raw Materials and Purchased Reagents, Sterilized Items, Equipment and Disposable Items

3.1.1 Institution Manufacturing Purified Human Pancreatic Islets Product

Name of Institution: _____

3.1.2 Personnel

Attach to this Batch Record a list of the names of all personnel directly involved in the execution of this Batch Record and their signatures and initials, or have them sign and initial the table below.

PRINTED NAME	SIGNATURE	INITIALS

3.1.3 Raw Materials and Purchased Reagents

Below is a list of the raw materials and purchased reagents used in this procedure, including their catalog numbers and suppliers, where specific Catalog Numbers and Suppliers are required. Record in the table the Catalog Number and Supplier, where not already specified, and the lot number and expiration date of each material used.

RAW MATERIAL AND PURCHASED REAGENTS	CATALOG NUMBER	SUPPLIER	LOT NUMBER	EXPIRATION DATE
1. CMRL 1066, Supplemented, CIT Modifications				
2. CMRL 1066 Transplant Media, contains Hepes and without Sodium Bicarbonate				
3. Hanks' Balanced Salt Solution (HBSS), 1X				
4. Heparin Sodium Injection USP, Preservative Free		_____ Units/mL		
5. HEPES Buffer, 1 M				
6. Gradient Stock Solution				
7. Phase I Solution				
8. Cold Storage/Purification Stock Solution				
9. Albumin Human USP, 25% Solution				
10. Hydrochloric Acid NF, 1 N				
11. Insulin-like Growth Factor-1 (IGF-1), 1.0 mg/vial	CM001	Cell Sciences		
12. Insulin Human Injection USP, Recombinant				

RAW MATERIALS AND PURCHASED REAGENTS (Continued)

RAW MATERIAL AND PURCHASED REAGENTS	CATALOG NUMBER	SUPPLIER	LOT NUMBER	EXPIRATION DATE
13a. Collagenase NB 1 GMP Grade	N0002937	SERVA/Nordmark		
13b. Neutral Protease NB GMP Grade	N0002936	SERVA/Nordmark		
14a. Collagenase NB 1 Premium Grade	17455	SERVA/Nordmark		
14b. Neutral Protease NB	30301	SERVA/Nordmark		
15a. CIzyme Collagenase HA	001-1000	VitaCyte LLC		
15b. CIzyme Thermolysin	002-1000	VitaCyte LLC		
16. Liberase MTF C/T GMP Grade	05339880001	Roche Diagnostics		
17. OptiPrep				
18. Trimming Solution				
19. Human Pancreas, Deceased Donor	See Section 4.2 and SOP 3108			
20. PentaStarch, 10% Solution				
21. Povidone Iodine USP, 10%				
22. Pulmozyme (dornase alpha), 2.5 mL/vial, 1 mg/mL	NDC No. 50242-100-40	Genentech		
23. RPMI 1640 with L-Glutamine				
24. Sterile Water for Injection USP				
25. Viaspan (UW Solution)				
26. Biocoll Separating Solution, Density 1.100	L6155	Biochrome AG/ Cedarlane		
27. Stock Polysucrose Solution, sterile	99-662-CVS	Mediatech		
28. Islet Gradient 1.037, sterile	99-690-CIS	Mediatech		
29. Islet Gradient 1.096, sterile	99-691-CIS	Mediatech		
30. Islet Gradient 1.108, sterile	99-692-CIS	Mediatech		
31. Calcium Chloride USP (Dihydrate) ($CaCl_2$ 2 H_2O)				
32. Calcium Chloride Injection USP				
33. Cefazolin Sodium USP				
34. Infusion Bag				

Verified by: _____ **Date:** _____

3.1.4 Sterilized Items

Attach a list of all items used in this process that have been sterilized, the sterilizer load numbers and dates, and verify that the sterilizations were performed within the time period validated by the institution.

Verified by: _____ **Date:** _____

3.1.5 Equipment

Attach a list of all equipment used in the manufacturing process, including identification numbers, serial numbers, etc.

Verified by: _____ **Date:** _____

3.1.6 Disposable Items

Attach a list of all disposable items used in this process, the supplier of each, the lot number, and the expiration date.

Verified by: _____ **Date:** _____

3.2 Biological Safety Cabinet and Laboratory Preparation

Prepare the laboratory, including the Biological Safety Cabinet (BSC), for islet isolation according to the institution's procedure(s) and record the preparation on the appropriate form(s) or logbook(s). Submit copies of the form(s) or logbook page(s) with this Batch Record.

Verified by: _____ **Date:** _____

3.3 Dilution Media Preparation

3.3.1 Equilibrate RPMI 1640 for digest dilution to room temperature prior to use for approximately 1 to 2 hours.

3.3.2 Prepare four 1L containers ahead of time and store at 2ºC to 8ºC before use:

REQUIRED	USED
1st Container	
400 mL of RPMI 1640	mL
200 mL of Albumin Human USP, 25% Solution	mL
200 Units of insulin (final concentration: 0.2 Units/mL)	Units
10,000 Units of heparin (final concentration: 10 Units/mL)	Units
2nd Container	
400 mL of RPMI 1640	mL
200 mL of Albumin Human USP, 25% Solution	mL
200 Units of insulin (final concentration: 0.2 Units/mL)	Units
10,000 Units of heparin (final concentration: 10 Units/mL)	Units

3rd Container	
500 mL of RPMI 1640	mL
100 mL of Albumin Human USP, 25% Solution	mL
200 Units of insulin (final concentration: 0.2 Units/mL)	Units
10,000 Units of heparin (final concentration: 10 Units/mL)	Units
4th Container	
500 mL of RPMI 1640	mL
100 mL of Albumin Human USP, 25% Solution	mL
200 Units of insulin (final concentration: 0.2 Units/mL)	Units
10,000 Units of heparin (final concentration: 10 Units/mL)	Units

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

3.3.3 Fill as many additional containers as needed with enough Albumin Human USP, 25% Solution each to provide a final concentration of 1.5% Albumin.

Number of additional containers: _____

Volume of each additional container: _____ mL

Volume collected in each additional container: _____ mL

Volume of Albumin Human USP, 25% Solution in each additional container _____ mL

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

4.0 PANCREAS ACCEPTANCE AND RECEIPT

4.1 Time of pancreas receipt in the lab: _____ (Record all times using the 24-hour clock)

Received by: _____ **Date:** _____

4.2 Pancreas Donor Qualification Record (NA = Not Available)

REQUIREMENTS A qualified donor must have "Yes" responses to all of the Inclusion Criteria (A), and "No" responses to all of the Exclusion Criteria (B & C).	Yes	No	NA
Container Label must specify Human Pancreas, and a UNOS or DDD number must be present.			
The Organ Procurement Organization (OPO) must be identified.			
A. Inclusion Criteria (The donor or pancreas must meet these criteria.)			
1. Pancreas Preservation in (i) UW, (ii) PF/UW, (iii) HTK, or (iv) PF/HTK Solution(s)			
2. Maximum 12 hour cold ischemia time			
3. Donor age 15-65 years			
4. Cause and circumstances of death acceptable to the transplant team			
B. Exclusion Criteria (Is there evidence of the following conditions?)			
1. History or biochemical evidence of Diabetes mellitus Type 1 or 2 (Transplant teams may consider donor HbA1C > 6.1% in the absence of transfusions in the week prior to death as an indication for exclusion, with discretion for donors who have received transfusions.)			
2. Pancreas from non-heart-beating cardiac death donors.			
3. Malignancies, other than resected basal squamous cell carcinoma or intracranial tumor as the cause of death			
4. Suspected or confirmed sepsis			
5. Evidence of clinical or active viral Hepatitis [A, B (HBcAg), C]. HBsAb+ is acceptable, if there is a history of vaccination.			
6. Acquired Immunodeficiency Syndrome (AIDS)			
7. HIV seropositivity (HIV-I or HIV-II), or HIV status unknown*			
8. HTLV-I or HTLV-II (Optional)			
9. Syphilis (RPR or VDRL positive)*			
10. Active viral encephalitis or encephalitis of unknown origin			
11. TSE or Creutzfeldt-Jacob Disease			
12. Suspected Rabies Diagnosis			
13. Treated or Active Tuberculosis			
14. Individuals who have received pit-hGH (pituitary growth hormone)			
15. Any medical condition that, in the opinion of the transplant team, precludes a reasonable possibility of a favorable outcome of the islet transplant procedure			
16. Clinical history and/or laboratory testing suggestive of West Nile Virus, Vaccinia, or SARS			
C. Exclusion Criteria – Behavioral Profiles (Is there evidence of the following conditions?)			
17. High-risk sexual behavior within 5 years prior to time of death: men who have had sex with men, individuals who have engaged in prostitution, and individuals whose sexual partners have engaged in high-risk sexual behavior			
18. Non-medical intravenous, intramuscular, or subcutaneous drug use within the past five years			
19. Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates			
20. Findings on history or physical examination consistent with an increased risk of HIV exposure			
21. Current inmates of correctional systems and individuals who have been incarcerated for more than 72 consecutive hours during the previous 12 months			

*Test results for Exclusion Criteria B. 7 and 9 are required by FDA regulation.

Islets Lot Number: _____

Is donor qualified as pancreas source? Yes No (Circle One)

Recorded by: _____ **Date:** _____

Review by: _____ **Date:** _____

4.3 Examine the container in which the pancreas arrived and its label. Is the container clean, intact and labeled with the UNOS or DDD number that has been accepted and are a proper name and donor records present?

Yes No (Circle One)

Is the product packaged properly?

Yes No (Circle One)

Comments: _____

_____

Examined by: _____ **Date:** _____

4.4 Record the following information from donor records provided by the OPO:

PANCREAS DONOR INFORMATION (NA = Not Available)

		ACCEPTABLE?		
	OBSERVED	**Yes**	**No**	**NA**
UNOS or DDD Number				
Name and Location of OPO				
OPO Unique Identifier (if applicable)				
Donor Consent for Islets Transplant Present				
Donor's Date of Birth				
Donor's Gender				
Donor's ABO				
Donor's Weight				
Donor's Height				
Donor's Body Mass Index				
Extent of Hemodilution (See Flowchart & Worksheet at the end of this document)				
Donor's CMV Status				

Recorded by: _____ **Date:** _____

5.0 PANCREAS PREPARATION

5.1 In-process Samples for Sterility Testing of Preservation Solution

Preservation Method: _____

Using sterile technique, open the pancreas container in a Class 100 area. Aseptically take at least a 3 mL sample of the preservation solution in which the pancreas was transported. Prepare and label the sample according to the institution's procedure and submit for sterility (21 CFR 610.12) and fungal culture testing to the appropriate laboratory. Attach a copy of the requisition form to the Production Batch Record.

Sample Collected by: _____ **Date:** _____

Record the test results, when available, in Section 17.1.

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Note: In some cases pancreas cleaning and cannulation are partially or completely performed immediately after the pancreas is procured and before it is delivered to the lab. In these cases, records of these activities will be made and filed with this Production Batch Record.

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5.2 Move the pancreas to a cold tray containing Trimming Solution plus 1 g/L Cefazolin Sodium USP and remove excess tissue.

Process Start time: _____

Performed by: _____ **Date:** _____

5.3 Examine the cleaned pancreas and record observations in the table below.

Check only one line in each category.

Fat	____ Clean	**Edema**	____ None
	____ Average		____ Interstitial Edema
	____ Patchy Infiltration		____ Slight Overall Swelling
	____ Heavily Infiltrated		____ Overly Distended
Flush	____ Well Flushed	**Texture**	____ Very Soft
	____ Poorly Flushed		____ Soft
			____ Firm (normal)
			____ Many Firm Areas (Fibrotic)
			____ Rigid Throughout
Blood	____ Blood on Capillaries	**Pancreas Condition**	____ Intact
	____ Blood in Intra-Parenchymal		____ Capsular Damage
	____ No Blood Present		____ Parenchymal Damage

Islets Lot Number: _____

Gross pathology observed? Yes No (Circle One)

Comments: _____

_____

_____

_____

_____

_____

Examined by: _____ **Date:** _____

5.4 Prepare the CIT Digestion Solution according to DAIT SOP 3106, B01, and file the record of preparation with this Batch Record.

Performed by: _____ **Date:** _____

5.5 Optional Pancreas Surface Decontamination

If desired, place the pancreas in 250 mL of HBSS or preservation solution containing 1 mg/mL Cefazolin Sodium USP, or in 250 mL of 10% Povidone Iodine USP solution. Rinse the pancreas with 400 mL of plain HBSS 1X, transfer it to a new container of 400 mL of plain HBSS 1X, and rinse again. Remove the original pan and instruments from the BSC, and replace with clean, sterile pan and instruments.

Pancreas surface decontamination method: _____

Documented by: _____ **Date:** _____

5.6 Pancreas Biopsy

Collect a superficial biopsy of approximately 3 mm X 3 mm X 3 mm from the area within 1 cm of the main duct of the donor pancreas for required product characterization MCP-1 and tissue factor testing. Prepare and ship the sample according to instructions in the CIT Islets Lab Binder. Report the results in PBR Section 17.3.

Performed by: _____ **Date:** _____

5.7 Pancreas Weight

After excess tissue is trimmed from the pancreas, weigh the pancreas.

Initial Trimmed Pancreas Weight: _____ g

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

5.8 CIT Enzyme Solution Preparation

Prepare the CIT Enzyme Solution described in the appropriate procedure reference below. Cross out the two references not used.

5.8.1 Prepare the CIT Enzyme Solution – SERVA Enzymes according to DAIT SOP 3106, B11.

5.8.2 Prepare the CIT Enzyme Solution – Vitacyte Enzymes and VitaCyte/SERVA Enzymes Combination according to DAIT SOP 3106, B13.

5.8.3 Prepare the CIT Enzyme Solution – Roche Enzymes according to DAIT SOP 3106, B14.

File the record of CIT Enzyme Solution preparation with this Batch Record.

Recorded by: _____ **Date:** _____

5.9 CIT Enzyme Solution **(Specify Units of each enzyme)**

Collagenase Activity actually used: _____

Neutral Protease Activity actually used: _____

Thermolysin Activity actually used: _____

Cross out the line above not used.

CIT Enzyme Solution volume prepared: _____ mL

Verified by: _____ **Date:** _____

5.10 Pancreas Cannulation

The pancreas will be perfused in a controlled manner, using separate cannulae for the head and tail. After the pancreas is cleaned of excess tissue, cut the pancreas to separate the head and tail, and cannulate the main pancreatic ducts with 16 to 22 gauge cannulae, one at the head and one at the tail. You may use a small cannula as a thread down the duct from the head of the pancreas to facilitate the identification of the duct for the cannulation process.

Performed by: _____ **Date:** _____

5.11 After the two portions of pancreas are cannulated, continue to remove excess tissue if necessary. Place this additional trimmed tissue in a tared container.

Comments on pancreas receipt and preparation for perfusion: _____

_____

_____

Written by: _____ **Date:** _____

6.0 PANCREAS PERFUSION

6.1 Assemble perfusion equipment according to the institution's procedure.

Performed by: _____ **Date:** _____

6.2 Perfuse the pancreas with the CIT Enzyme Solution.

- If indicated by the institution's procedures, prime the perfusion circuit by pumping HBSS, 1X, through it. Confirm the absence of leaks or loose connections, and drain the perfusion circuit.
- Add CIT Enzyme Solution (Section 5.5) at 4ºC to 8ºC to the chamber and refill the perfusion circuit with it. Remove all air bubbles.
- Connect the perfusion tubing to the cannula and perfuse the pancreas for 4 to 10 minutes at 60 to 80 mm Hg, followed by 4 to 6 minutes (8 minutes maximum in case of poor distension) at 160 to 180 mm Hg at 4ºC to 14ºC. Note the Desired Pressure in the table below depending on when the pressure is increased.
- Record the Perfusion Start Time (enzyme solution enters the pancreas) in the table below.
- Monitor temperature and pressure during pancreas perfusion and record in the table below.
- Optionally monitor the flow rate and record it in the table below.
- Stop perfusion after 10 minutes (12 minutes in the case of poor distension). If perfusion time exceeds 12 minutes, attach to this record a justification for the additional time.

Pancreas Perfusion Pressures & Temperatures

			Start Time:				
			Head		Tail		
Desired Temp. (°C)	Desired Pressure (mm Hg)	Time (min)	Observed Pressure (mm Hg)	Observed Flow Rate (mL/min)*	Observed Pressure (mm Hg)	Observed Flow Rate (mL/min)*	Observed Temp. (°C)
4 – 14	60 – 80	2					
4 – 14	60 – 80	4					
4 – 14		6					
4 – 14		8					
4 – 14		10					
4 – 14							
4 – 14							
4 – 14	160 – 180	Finish Perfusion					
Perfusion completion			Finish time:		Finish time:		
Total Perfusion Time (Minutes)							
Enzyme Solution remaining after perfusion (Section 7.2)			g or mL (Circle One)				
Distention Quality (Circle One)			Excellent Good Partial		Excellent Good Partial		
Comments on pancreas distention (If partial distention, describe)							
Perfusion Method:			Automated		Manual		(Circle One)
Data recorded by:					Date:		

Continue to clean the pancreas during and after perfusion. Save all removed non-pancreatic tissue in the container from Section 5.11.
*Optional

Post-perfusion trim finish time: _____

Performed by: _____ **Date:** _____

6.3 Final Trimmed Pancreas Weight

After perfusion and trimming are complete, weigh the additional tissue removed after the Initial Trimmed Pancreas Weight was determined (Section 5.7, above). Record this weight in row B of the table below, and calculate the Final Trimmed Pancreas Weight.

A. Initial Trimmed Pancreas Weight (from Section 5.7)	g
B. Additional Trimmed Tissue Weight	g
C. Final Trimmed Pancreas Weight (A – B =C)	g
D. Undigested Tissue Weight (from Section 7.3)	g
E. Digested Pancreas Tissue Weight (C – D= E)	g

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Determine the volume of CIT Enzyme Solution to be added to the Ricordi Digestion Chamber using the preparation table in the appropriate Attachment (B11, B13, B14) to SOP 3106.

Performed by: _____ **Date:** _____

6.4 Assemble the pancreas digestion equipment according to the institution's procedure. Use the 600 mL Ricordi Digestion Chamber (Biorep Technologies, Inc., Model No. 600-MUL-03 with screen WM-533, or Model No. 600-mDUR-03, with screen WM-533).

Performed by: _____ **Date:** _____

6.5 Pancreas Preparation for Digestion

Cut the pancreas into 5 to 15 similar sized pieces of 1 to 2.5 inches length and place the pieces in a Ricordi digestion chamber. Place 6 to 10 marbles into the digestion chamber and add CIT Enzyme Solution up to the point where the screen is to be placed. Place a 533 µm woven stainless steel screen on top of the chamber and close it. Ensure that the digestion chamber is sealed properly to prevent leaking.

Performed by: _____ **Date:** _____

6.6 Pancreas Processing Times

Record information about the pancreas processing times in the table below. Calculate the Pancreas Preparation Time (Process Start Time, Section 5.2, to Perfusion Start Time, Section 6.2), and the Cold Ischemia Time (Cross Clamp Time, from donor records, to Perfusion Start Time, from Section 6.2) and record these in the table below.

	Date	Time
A. Cross Clamp (Donor Records)		
B. Process Start (Section 5.2)		
C. Perfusion Start (Section 6.2)		
	D. Pancreas Preparation Time (D = C – B)	Hours _____ Minutes _____
	E. Cold Ischemia Time* (E = C – A)	Hours _____ Minutes _____

*Cold Ischemia Time must be 12 hours or less. If the Cold Ischemia Time is more than 12 hours, immediately notify the site principal investigator.

Recorded by: _____ **Date:** _____

Calculate by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If the site principal investigator is notified of excessive Cold Ischemia Time, complete the following:

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

7.0 ENZYMATIC PANCREAS DIGESTION

7.1 Pancreas Digestion

7.1.1 Add any remaining residual CIT Enzyme Solution to the recirculation flask for introduction into the digestion circuit.

Add 0 to 5 mL of Pulmozyme (2.5 mL/ampoule, 1 mg/mL) to the Ricordi Digestion Chamber

Volume of Pulmozyme (1 mg/mL) added: _____ mL

Performed by: _____ **Date:** _____

7.1.2 Start pumping the solution at a rate of 230 ± 20 mL/min to fill the system. Record this as the Digestion Start Time in the table in Section 7.2. Add as much CIT Digestion Solution to the recirculation flask as needed to fill the system and to completely eliminate air from the circuit.

Immediately begin recording the temperature inside the chamber, and the flow rate in the table in Section 7.2.

Rock the chamber gently for the first 5 minutes and then decrease the flow rate to 110 ± 20 mL/min. Start shaking the chamber after 5 minutes. It takes approximately 3 - 5 minutes for the chamber to reach a target temperature of 32 to 38ºC.

Verified by: _____ **Date:** _____

7.1.3 When tissue is observed in the circulating digest, take a 1 – 2 mL sample of the digest from the sampling port with a syringe. Place the digest sample in a 35 mm dish and add dithizone (DTZ) stain solution. Observe the digest under a microscope. Repeat this sampling (taking the same sample volume each time) and examination every 1-2 minutes during the digestion. Record the digestion chamber temperature, the flow rate and your observations on the stained sample in the table below. Maintain temperature between 32ºC and 38ºC, based on digest quality, considering the following factors that help in determining when to stop digestion and start dilution:

Factors	Ideal Ranges for Switching from Digestion to Dilution*
Amount of Tissue	3 to 6
Number of Islets	> 45 islets
% Free Islets	> 50%
% Fragmented (Over-digested) Islets	< 10%

*See definitions in Note, below.

Verified by: _____ **Date:** _____

Note: **Criteria for evaluating the digest and determining the end of digestion**

- **Estimate the amount of tissue by centering the tissue in the dish, viewing the mass with a microscope at 40X power, and estimating the amount of the visual field covered (6 = tissue covers entire visual field, 3 = tissue covers about 1/2 of the visual field, 0 = no tissue).**
- **Estimate the number of islets (a rough visual count, 10 – 20, 30 – 50, 80 – 90 islets, etc.).**
- **Estimate the % free islets (free islets versus the total number of islets, 25%, 50%, 90%, etc.). Free islets have less than 25% of the border attached to acinar tissue.**
- **Estimate the % fragmented islets (number of fragmented islets versus the total number of islets, 10%, 15%, 50%, etc.). Fragmented islets are those with a ragged border due to damage by overexposure to the enzyme (Over-digested).**

7.1.4 When the decision to stop digestion is made, start dilution and collection of islets. Record the Dilution Start Time (= Digestion Stop Time) at the end of the table in Section 7.2 and calculate the Total Digestion Time.

Decided by: _____ **Date:** _____

Verified by: _____ **Date:** _____

7.2 Dilution and Collection of Islets

- Adjust the flow rate to 230 ± 20 mL/min, and continue shaking the digestion chamber.
- Add fresh RPMI 1640 at room temperature to the intake container as needed.
- Adjust the temperature of the chamber to ≤ 30 ºC during dilution and collection.
    - If a large number of imbedded islets are observed in the digest, the chamber temperature may be maintained between 30°C and 38°C during dilution.
- Collect the digest into the 1L containers prepared in 3.3.2.
- Gently swirl each container periodically as it fills. When it reaches a volume of 1L, immediately decant the solution into 250 mL conical tubes for centrifugation at 170 X g and 2ºC to 8Cº for 3 to 4 minutes.
- Periodically take 1 to 2 mL samples of the diluted digest from the sample port with a syringe. Stain with Dithizone (DTZ) solution and observe the stained sample under a microscope. Record your observations in the table below.
- When no islets are observed in the stained samples and little tissue remains in the chamber, discontinue the addition of media to the system, collect the media remaining in the system, and stop the circulation pump.
- Record the Dilution Stop Time at the end of the table below, and calculate and record the Total Dilution Time.

Verified by: _____ **Date:** _____

Pancreas Digestion Record

Digestion Start Time: _____

Time (min)	Desired Temp. (° C)	Observed Temp. (° C)	Desired Flow Rate (mL/min)	Observed Flow Rate (mL/min)	Acinar Amount (0 – 6)	# of Islets (Range)	% Free Islets	% Fragmented Islets
0			210 – 250					
1			210 – 250					
2			210 – 250					
3			210 – 250					
4			210 – 250					
5	32 – 38		90 – 130					
6	32 – 38		90 – 130					
7	32 – 38		90 – 130					
8	32 – 38		90 – 130					
	≤ 30		210 – 250					
	≤ 30		210 – 250					
	≤ 30		210 – 250					
	≤ 30		210 – 250					
	≤ 30		210 – 250					
	≤ 30		210 – 250					

Record Desired Temperatures and Desired Flow Rates in vacant cells based on Digestion Stop Time.

Dilution Start Time = Digestion Stop Time: _____ Digestion Time: _____ minutes

Dilution Stop Time: _____ Dilution Time: _____ minutes

Comments: _____

_____

Recorded by: _____ **Date:** _____

Islets Lot Number: _____

7.3 Remove the undigested pancreas material from the digestion chamber, weigh it, record the weight below, and in the table in Section 6.3. Calculate the weight of digested tissue in the table in Section 6.3.

Examine the undigested pancreas material remaining in the digestion chamber, and estimate the percentages of pancreatic tissue and connective tissue (should equal 100%). Record these estimates below.

Weight of undigested tissue remaining in chamber (record also in Section 6.3): _____ g
Calculate the Digested Pancreas Weight in Section 6.3 table, above.

Estimate of undigested pancreatic tissue: _____%

Estimate of undigested connective tissue: _____%

Performed by: _____ Date: _____

7.4 Tissue Recovery and Washing

7.4.1 Prior to the end of digestion prepare CIT Purification Solution and CIT Wash Solution according to DAIT SOP 3106, B02, and B12, respectively. Attach the record of preparation to this Production Batch Record and keep both solutions at 2ºC to 8ºC until used.

7.4.2 As tissue is collected during dilution, transfer it to 250 mL conical tubes for the first four liters and centrifuge at 170 X g and 2ºC to 8ºC for 3 to 4 minutes, to pellet the tissue.

7.4.3 Decant all of the supernatant and transfer pellets to a 1 L container containing 900 mL of CIT Wash Solution (keep cold).

NOTE: Be sure the flask is kept level during recombination to avoid tissue aggregation and hypoxic conditions.

7.4.4 If residual tissue remains, wash it with 3 to 5 mL of CIT Wash Solution.

7.4.5 After dilution is completed and all the tissue has been recombined into the CIT Wash Solution, mix the flask thoroughly by gentle swirling and transfer the contents into as many 250 mL sterile conical tubes as required. Centrifuge each tube at 170 X g and 2ºC to 8ºC for 3 to 4 minutes.

7.4.6 Wash the recombined tissue with CIT Wash Solution until the extracellular debris and DNA strings have been minimized. As the washing progresses, reduce the number of conical tubes to two, then one by combining tissue.

NOTE: If, during collection, DNA stings are observed after centrifugation with loose pellet formation, transfer the suspension portion of those tubes containing the majority of cells into one separate 250 mL conical tube, and keep it lying flat on the bench for 5 minutes after adding up to 200 mL of CIT Wash Solution and 200 µL (1 µg/mL) of Pulmozyme. After re-centrifugation, when the DNA strings have disappeared, recombine with other pellets.

7.4.7 After the washing is complete, centrifuge the final tube at 170 X g and 2ºC to 8ºC for 3 to 4 minutes and visually estimate the total packed tissue volume in the final 250 mL container. Aspirate the supernatant down to the pellet.

Total Packed Tissue Volume: _____ mL

Islets Lot Number: _____

7.4.8 Re-suspended the islets to 100 to 250 g or mL, depending on the amount of tissue, with CIT Purification Solution. Ensure there are no clumps (dissolve if necessary). Record the volume or weight.

Total Suspension Volume or Weight: _____ mL or _____ g

Verified by: _____ **Date:** _____

7.5 Pre-purification Islets Count

7.5.1 Re-suspend tissue evenly. Take two 100 µL samples and count each sample once.

7.5.2 Perform pre-purification count according to the institution's procedure and record the data in the table below and attach spreadsheet, if used, to Production Batch Record.

Pre-purification Islets Counts & Calculations

Sample Volume			µL	
Total Volume			mL	
Dilution Factor				
Diameter (µm), Factor	Counts		IPN (Avg.)	IEQ
50 – 100, 0.167				
101 – 150, 0.648				
151 – 200, 1.685				
201 – 250, 3.500				
251 – 300, 6.315				
301 – 350, 10.352				
> 350, 15.833				
		Sample Total		
		Suspension Total		
% Trapped				
% Fragmented				
Technicians' Initials				

Comments: _____

_____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

7.5.3 The maximum tissue volume for purification is 25 mL per COBE run. If the tissue volume is < 25 mL, centrifuge the islets suspension and re-suspend the tissue in 100 mL of CIT Purification Solution. If the tissue volume is > 25 mL, using the Packed Tissue Volume from Section 7.4.8, calculate the number of COBE runs required to process ≤ 25 mL of packed tissue per run. Divide the tissue evenly into separate sterile 250 mL conical tubes and fill each to the 100 mL mark with additional CIT Purification Solution. During purification of the first tube, the additional conical tubes should be kept in the cold room or refrigerator for subsequent COBE runs (keep tube lying flat and mix occasionally to avoid tissue aggregation) until ready to be loaded into the COBE.

Number of conical tubes and COBE runs: _____

Volume of tissue distributed into each tube: _____ mL

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

7.5.4 When ready to load the first COBE run, add 20 mL of Albumin Human USP, 25% Solution to the tissue and mix well. Continue to Section 8.2.11.

For subsequent COBE runs, centrifuge the conical tube at 170 X g and 2ºC to 8°C for 3 – 4 minutes. Remove the supernatant, add 20 mL of Albumin Human USP, 25% Solution to the tissue and mix well to re-suspend. Bring the tissue suspension to 120 mL in a 250 mL tube or beaker with CIT Purification Solution. Continue to Section 8.2.11.

8.0 ISLETS PURIFICATION

8.1 COBE 2991 Preparation

Set up the COBE according to the Operational Manual and the institution's procedures. The COBE must be refrigerated or placed in a cold room.

- Prepare High (1.10 g/mL) and Low (1.06 g/mL) CIT Purification Density Gradients according to SOP 3106, B10, and file the records of their preparation with this Production Batch Record.
- Label 13 X 250 mL conical tubes with the COBE run number, and "W1" and fraction numbers 1 through 12 (See tables in Section 8.3). Label a 14th 250 mL conical tube with the COBE run number and "Bag."
- Fill tubes 1 through 12 with 225 mL of CMRL 1066, Supplemented, and store at 2ºC to 8°C.

Verified by: _____ **Date:** _____

8.2 COBE 2991 Procedure – Gradient and Tissue Loading

8.2.1 Assemble the COBE bag onto COBE cell processor according to institution's procedure. Place clamps near the main line on all colored tubing except one line to be used for loading the COBE bag.

8.2.2 Place gradient-maker on magnetic stir plate and aseptically connect one end of size 16 tubing to gradient-maker and the other end to green tubing of the COBE bag.

8.2.3 Place a sterile stir bar into the left chamber (next to outlet) and turn on the stir plate.

8.2.4 Run tubing through pump and set pump to 60 mL/min.

8.2.5 Sanitize the exterior of all solution bottles before placing in the hood.

8.2.6 Pour 120 mL of the High Density Gradient (1.10 g/mL) into the left chamber of the gradient maker.

8.2.7 Start to pump High Density Gradient (1.10 g/mL) into COBE bag. Once this gradient reaches the bag, start the COBE at 1800 – 2000 rpm.

8.2.8 Once the entire 120 mL of High Density Gradient (1.10 g/mL) is loaded, remove excess air from the COBE bag by pressing Superout while unclamping the red tubing. Press the Hold button once the Bottom Gradient has reached the T (junction of red/green tube). Re-clamp the red tubing line and press the Stop/Reset button.

8.2.9 Wait for the final centrifugation of the digest tissue and then begin loading the continuous density gradient into the COBE bag (Section 7.5.4).

- Pour 125 mL High Density Gradient (1.10 g/mL) in the left chamber (nearest the outlet) of the gradient maker. Open and close the port between the two chambers just enough to fill the opening.
- Pour 125 mL Low Density Gradient (1.06 g/mL) in the right chamber of gradient maker (away from outlet)
- Start the COBE and ensure that the centrifuge speed is between 1800 and 2000 rpm.

  Centrifuge Speed: _____ rpm

  Recorded by: _____ Date: _____
- Open the port between the chambers, set pump to 20 mL/min and load gradient up to the T of the COBE bag tubing. Stop the pump when the gradient has reached the T-connection.

NOTE: Observe the gradient maker to ensure that gradients are mixing during the continuous gradient loading.

8.2.10 Load the continuous gradient by unclamping the green tubing and starting the pump. Load the entire 250 mL of continuous gradient at 20 mL/minute.

8.2.11 When all of the gradient has been loaded, stop the pump just as the last portion of the gradient enters the tubing attached to the gradient maker.

NOTE: COBE must remain spinning during the rest of the purification process. If abnormal signs appear from rotating seal (e.g. leak, unusual noise, burnt smell, etc.), replace COBE bag and make new density gradients.

8.2.12 Aseptically remove the tubing from gradient maker port and move it to the beaker with tissue. Reverse the pump to purge the air.

8.2.13 Load the tissue with the pump at a setting of 20 mL/min. Gently swirl the beaker to keep the tissue well-suspended during the loading.

8.2.14 To ensure tissue does not back-up on the gradient (a heavy tissue line observed on the gradient), periodically turn the pump off allowing tissue to enter the gradient and then turn the pump back on again. Repeat as necessary every 1 to 2 minutes.

NOTE: As an alternate, turn the pump off for 30 seconds, followed by loading tissue for 45 seconds.

8.2.15 As soon as the tissue is loaded, add 30 mL of additional CIT Purification Solution to the 250 mL beaker to rinse. Load this rinse onto the COBE.

8.2.16 After the last portion of the rinse has entered the COBE bag, stop the pump.

8.2.17 Vent the system by carefully unclamping the red tubing. Re-clamp the tubing when liquid (capping solution) is approximately one inch above the ceramic seal. This is the start of centrifugation time.

NOTE: Air left in the ceramic rotating seal can cause seal failure which may lead to leaking, seal occlusion and possible system shutdown due to overpressure during Superout.

8.2.18 Clamp the green line and allow the COBE to spin for 3 minutes. Record data on Purification Data Log for each COBE run, below.

Verified by: _____ **Date:** _____

8.3 COBE 2991 Procedure – Tissue Collection

8.3.1 During the 3 minute spin disconnect tubing from the pump. Prepare for collection of tissue fractions.

8.3.2 Verify that the Superout Rate is set at 100 mL/min.

8.3.3 After 3 minute spin slowly remove the blue clamp on the green line and quickly press the Superout button.

8.3.4 Collect the first 150 mL of effluent into the conical tube labeled "W" and 12 X 25 mL fractions into the numbered conical tubes each pre-filled with 225 mL CMRL 1066, Supplemented, CIT Modifications, as described on the Purification Data Log for each respective COBE run.

8.3.5 Once the fractions are collected, stop the COBE and aseptically collect the contents of the COBE bag into a 250 mL conical tube labeled "bag." Discard the COBE bag and tubing.

8.3.6 Dilute the COBE bag contents up to 200 mL with CMRL 1066, Supplemented, CIT Modifications. Take a 200 µL sample and place it into 35 mm dish. Stain the sample with dithizone according to the institution's procedure and examine it for the presence of islets. If a significant number of free islets are present keep the diluted COBE bag contents at 2ºC to 8ºC for further processing as instructed in Section 8.4.1. If there are not a significant number of free islets, discard the COBE bag contents.

8.3.7 To evaluate each COBE fraction quickly, gently but thoroughly mix each fraction from Section 8.3.4, then quickly transfer a 0.5 mL sample to one well of a 12-well microtiter plate and 0.5 mL of the W fraction to a 35 mm dish.

8.3.8 Stain each sample with dithizone according to the institution's procedure and observe for islets. Record Islets Purity (%) and disposition of each fraction on the Purification Data Log for each COBE run.

8.3.9 Centrifuge the 250 mL tubes for 3 minutes at 140 X g and 2ºC to 8ºC. Record Packed Tissue Volumes of each COBE fraction on the Purification Data Log for each respective COBE run. Discard supernatant.

8.3.10 Combine the islets fractions by transferring the pellets with 10 mL pipets into four labeled 250 mL conical tubes containing 100 mL of CMRL 1066, Supplemented, to obtain the following purity levels after recombination:

- High Purity (≥ 70%) (H),
- Middle Purity (40% to 69%) (M),
- Low Purity (30% to 39%) (L), and
- Supplementary Purification Islets (< 30%) (S).

Discard fractions (D) that contain little or no tissue. For the other four categories of islets purity, keep the conical tubes flat on the bench at room temperature until the tissue of all COBE runs has been combined into the respective conical tubes.

NOTE: Depending on the analysis and disposition of each fraction, there may be up to one 250 mL conical tube for each Purity Level (High, Middle, Low Purity Islets), and one 250 mL conical tube for the Supplementary Purification Islets, if there are any.

8.3.11 Repeat steps 8.2.1 to 8.3.10 for each COBE purification run. Combine fractions of similar purity into the 250 mL conical tubes prepared in Section 8.3.10.

NOTE: Scoring Guidelines for purified layers in Purification Data Logs:

- Packed Tissue Volume: estimate of the tissue volume in the individual conical tubes after they have centrifuged for 3 minutes at 140 X g and 2ºC to 8ºC.
- % Purity: estimate relative amount (%) of islets to total tissue.
- H M L S D: This is the disposition of each fraction as defined in the column header.

Repeat this purification process for each of the tubes.

Purification Data Log, COBE Run #1:

Layer	Medium	Amount
Capping Layer	CIT Purification Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Purification Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		Centrifuge Stop Time	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, S: Supplementary, D: Discard (Circle One)
W	0	150 mL				H M L S D
1	225	25				H M L S D
2	225	25				H M L S D
3	225	25				H M L S D
4	225	25				H M L S D
5	225	25				H M L S D
6	225	25				H M L S D
7	225	25				H M L S D
8	225	25				H M L S D
9	225	25				H M L S D
10	225	25				H M L S D
11	225	25				H M L S D
12	225	25				H M L S D
Bag	0	95				S D

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

Purification Data Log, COBE Run #2

Layer	Medium	Amount
Capping Layer	CIT Purification Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Purification Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		Centrifuge Stop Time	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, S: Supplementary, D: Discard (Circle One)
W	0	150				H M L S D
1	225	25				H M L S D
2	225	25				H M L S D
3	225	25				H M L S D
4	225	25				H M L S D
5	225	25				H M L S D
6	225	25				H M L S D
7	225	25				H M L S D
8	225	25				H M L S D
9	225	25				H M L S D
10	225	25				H M L S D
11	225	25				H M L S D
12	225	25				H M L S D
Bag	0	95				S D

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

Purification Data Log, COBE Run #3

Layer	Medium	Amount
Capping Layer	CIT Purification Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Purification Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		Centrifuge Stop Time	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, S: Supplementary, D: Discard (Circle One)
W	0	150				H M L S D
1	225	25				H M L S D
2	225	25				H M L S D
3	225	25				H M L S D
4	225	25				H M L S D
5	225	25				H M L S D
6	225	25				H M L S D
7	225	25				H M L S D
8	225	25				H M L S D
9	225	25				H M L S D
10	225	25				H M L S D
11	225	25				H M L S D
12	225	25				H M L S D
Bag	0	95				S D

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

Purification Data Log, COBE Run #4

Layer	Medium	Amount
Capping Layer	CIT Purification Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Purification Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		Centrifuge Stop Time	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, S: Supplementary, D: Discard (Circle One)
W	0	150				H M L S D
1	225	25				H M L S D
2	225	25				H M L S D
3	225	25				H M L S D
4	225	25				H M L S D
5	225	25				H M L S D
6	225	25				H M L S D
7	225	25				H M L S D
8	225	25				H M L S D
9	225	25				H M L S D
10	225	25				H M L S D
11	225	25				H M L S D
12	225	25				H M L S D
Bag	0	95				S D

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

Purification Data Log, COBE Run #5

Layer	Medium	Amount
Capping Layer	CIT Purification Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Purification Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		Centrifuge Stop Time	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, S: Supplementary, D: Discard (Circle One)
W	0	150				H M L S D
1	225	25				H M L S D
2	225	25				H M L S D
3	225	25				H M L S D
4	225	25				H M L S D
5	225	25				H M L S D
6	225	25				H M L S D
7	225	25				H M L S D
8	225	25				H M L S D
9	225	25				H M L S D
10	225	25				H M L S D
11	225	25				H M L S D
12	225	25				H M L S D
Bag	0	95				S D

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

Note: If the initial purification process, above, did not yield a sufficient number of sufficiently pure islets for transplant, and there is a substantial quantity of tissue containing impure islets in the Middle and/or Low Purity Islets 250 mL conical tubes, and/or in the Supplementary Purification 250 mL conical tube, follow the procedure in Section 8.4, below.

8.4 Supplementary Purification Fractions and COBE Bag Contents Processing

8.4.1 If, upon examination of the COBE bag contents, a significant number of islets is present (See Section 8.3.6), centrifuge the 250 mL conical tube containing the diluted COBE bag contents at 140 X gravity and 2ºC to 8ºC for three minutes, and transfer the packed tissue to the Supplementary Purification Islets 250 mL conical tube.

8.4.2 List all fractions combined for Supplementary Purification:

COBE Run #	Fractions and/or COBE Bags Combined for Supplementary Purification
1	
2	
3	
4	
5	

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

8.4.3 Bring the volume of the Supplementary Purification Islets 250 mL conical tube to 100 to 250 mL with CMRL 1066, Supplemented, CIT Modifications, and take one or two 100 µL samples for counting, if desired.

8.4.4 Dilute the Supplementary Purification Islets to 250 mL with CMRL 1066, Supplemented, CIT Modifications. Lay the tube on its side at 2ºC to 8ºC if counts are performed.

Verified by: _____ **Date:** _____

8.4.5 If desired, count islets according to the institution's procedure in the Supplementary Purification Islets sample and record counts in the table below and attach any spreadsheets used. Indicate in the Comments space if the tissue will be re-purified. Supplementary Purification may be indicated if there are a significant number of islets (greater than 50,000 IEQ). If Supplementary Purification is to be performed, record which of the two procedures will be used on the Comments lines below the Counts table, and proceed to Section 9.0. If Supplementary Purification is not to be performed, record the disposition of the Supplementary Purification Islets on the Comments lines below the Counts table.

Optional Pre-supplementary Purification Islets Counts & Calculations

Sample Volume				µL
Total Volume				mL
Dilution Factor				
Diameter, Factor	Counts		IPN (Avg.)	IEQ
50 – 100, 0.167				
101 – 150, 0.648				
151 – 200, 1.685				
201 – 250, 3.500				
251 – 300, 6.315				
301 – 350, 10.352				
> 350, 15.833				
		Sample Total		
		Suspension Total		
% Trapped				
% Fragmented				
Technicians' Initials				

Comments: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Decided by: _____ **Date:** _____

Islets Lot Number: _____

8.5 Tissue Preparation for Re-purification

If the decision in Section 8.4, is to perform a Supplementary Purification of the islets, centrifuge the 250 mL conical tube containing all the Supplementary Purification Islets at 140 X gravity and 2ºC to 8ºC for three minutes. Remove and discard the supernatant.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

9.0 ISLETS SUPPLEMENTARY PURIFICATION

If islets tissue insufficiently purified by the procedure described in Section 8.0 is present, this tissue may be re-purified by one of the three procedures defined in SOP 3109. Cross out all three references, if no Supplementary Purification is performed. Cross out the two references not used, if Supplementary Purification is performed.

9.1 SOP 3109, B01, Supplementary Purification, OptiPrep Procedure & Record

9.2 SOP 3109, B02, Supplementary Purification, Continuous Biocoll Procedure & Record

9.3 SOP 3109, B03, Supplementary Purification, Discontinuous Polysucrose Procedure & Record

File the Supplementary Purification record with this Production Batch Record.

Recorded by: _____ **Date:** _____

Approved by: _____ **Date:** _____

10.0 POST-PURIFICATION ISLETS COUNT

10.1 After all islets are combined into the three Purity Levels, wash each Purity Level once with CIT Culture Media prepared according to DAIT SOP 3106, B04. Allow the tissue in the conical tubes to settle for 3 to 5 minutes. After the tissue in each purity level has settled, remove the supernatant and re-suspend the final tissue in 50 to 250 mL of CIT Culture Media in T-75 flasks labeled for each Purity Level with Lot Number and isolation date.

Verified by: _____ **Date:** _____

10.2 Gently mix each Purity Level and take two 100 µL samples of each for Post-purification Islet Count. Enter the count data in the table below, attach a spreadsheet, if used, and calculate the Total Islet Number (IPN) and Total IEQ. The contents of these T-75 flasks are now ready to proceed to Islet Culture, Section 11.

Sampled by: _____ **Date:** _____

Post-purification Islets Counts

	High Purity				Middle Purity				Low Purity			
Sample Volume			µL				µL				µL	
Total Volume			mL				mL				mL	
Dilution Factor												
Diameter, Factor	Counts		Avg.	IEQ	Counts		Avg.	IEQ	Counts		Avg.	IEQ
50 – 100, 0.167												
101 – 150, 0.648												
151 – 200, 1.685												
201 – 250, 3.500												
251 – 300, 6.315												
301 – 350, 10.352												
> 350, 15.833												
Total												
% Trapped												
% Fragmented												
% Purity												
Islet Quality Grade*												
Technicians' Initials												

Islets Lot Number: _____

Post-purification Islets Calculations

	High Purity	Middle Purity	Low Purity	Total
Post-purification IPN				
Post Purification IEQ				
Pre-purification IEQ (Section 7.5.2)				
IEQ Recovery (%) (from Pre-purification IEQ)				
Total IEQ/g of Final Trimmed Pancreas (Section 6.3)				
Comments				

*See Note, below, for Islets Quality Grade guidelines

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Note: **Islets Quality Grade**

Grade the quality of the islets based on these parameters and criteria:

Parameter	0 Points	1 Point	2 Points
Shape (3D)	flat/planar	in between	spherical
Border (2D)	irregular	in between	well-rounded
Integrity	fragmented	in between	solid/compact
Single Cells	many	a few	almost none
Diameter	all < 100 μm	a few > 200 μm	> 10% > 200 μm

Add up the points for each sample to obtain the following grades:

- 9 to 10 points = A
- 7 to 8 points = B
- 4 to 6 points = C
- 2 to 3 points = D
- 0 to 1 point = F

11.0 ISLET CULTURE

11.1 For product characterization tests samples, gently re-suspend the contents of the High Purity (≥ 70%) Islets culture flask. Based on the count results in Section 10, take a sample containing ≥ 400 IEQ for a Pre-culture Glucose Stimulated Insulin Release Test according to the institution's procedure. This islets sample is cultured in a culture dish simultaneously with, but separately from, the bulk islets product. Report Result in Section 14.4 and on the Certificates of Analysis.

Also, take samples of the High Purity Islets suspension for the Pre-culture DNA Content, and Nuclei Measurement product characterization tests according to the table, below. Report the results of these tests in Section 20.

CHARACTERIZATION TEST	IEQ	IEQ/mL	SAMPLE REMOVED (mL)
Example –Low Yield	400	1,000	0.40 mL
Example – High Yield	400	5,000	0.08 mL
Interim Certificate of Analysis			
REQUIRED PRE-CULTURE GLUCOSE STIMULATED INSULIN RELEASE	400		
Optional Product Characterization, For Information Only			
PRE-CULTURE DNA CONTENT	3 X 100		
PRE-CULTURE NUCLEI MEASUREMENT	3 X 100		
Sampled by:			Date:
Verified by:			Date:

11.2 Calculate the number of T-175 culture flasks needed for a target of 10,000 to 30,000 IEQ/Flask using the equation (Round decimals up to the next higher whole number of flasks):

$$\frac{\text{IEQ in Purity Level}}{\text{(20,000 to 30,000 IEQ/Flask) X Purity (in decimal form)}} = \text{\# of T-175 Culture Flasks}$$

Purity Level	IEQ in Level	Purity	Target IEQ/Flask	Number of T-175 Culture Flasks
Example – High Purity	352,423	0.95	27,500	13.48988, rounded up to 14
Example – Middle Purity	53,817	0.50	25,000	4.30536 rounded up to 5
High Purity				
Middle Purity				
Low Purity				
Calculated by:				Date:
Verified by:				Date:

11.3 Obtain the calculated number of sterile T-175 flasks, inspect each for cracks, and label them.

Performed by: _____ **Date:** _____

11.4 Transfer the target quantity of islets (Section 11.2, above, 10,000 to 30,000 IEQ) to each T-175 culture flask and bring the volume to 30 mL with CIT Culture Media

Fraction	Number of T-175 Culture Flasks	Media Needed (30 mL/flask)	CIT Culture Media Volume (Section 10.2)	CIT Culture Media Added or Removed
Example 1 – High Purity	14	420 mL	100 mL	+ 320 mL
Example 2 – Middle Purity	5	150 mL	120 mL	+ 30 mL
Example 3 – Low Purity	2	60 mL	100 mL	– 40 mL
High Purity				
Middle Purity				
Low Purity				

Calculated by:	Date:
Verified by:	Date:
Performed by:	Date:

11.5 Add 15 mL of CIT Culture Media to the culture dish containing the sample for Glucose Stimulated Insulin Release Assay (Section 11.1) and culture its contents with the High Purity Islets.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

11.6 Place all the flasks of High Purity Islets in an incubator at 37ºC, 95% air, and 5% carbon dioxide, and record the date and time as the High Purity Islets 1st Culture Start Date & Time here and in Section 12.5 table, below, using the 24-hour clock format.

High Purity Islets' 1st Culture Start Date & Time: _____

Performed by: _____ **Date:** _____

The islets' 1st Culture Stop Date &Time must be between 12 and 24 hours after the High Purity Islets' 1st Culture Start Date & Time. Calculate these dates and times and record them here and in Section 12.5 table, below.

Date and time of minimum 1st Culture Stop Date & Time: _____

Date and time of maximum 1st Culture Stop Date & Time: _____

The islets' 2nd Culture Stop Date & Time must be between 36 and 72 hours after the High Purity Islets' 1st Culture Start Date & Time. Calculate these dates and times and record them here and in the Section 12.5 table, below.

Date and time of minimum 2nd Culture Stop Date & Time: _____

Date and time of maximum 2nd Culture Stop Date &Time: _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Notify the Site Principal Investigator, or designee, of the calculated minimum and maximum 2nd Culture Stop Dates and Times.

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____

11.7 Place all the flasks of Middle and Low Purity Islets in an incubator at 22ºC, 95% air, and 5% carbon dioxide with the T-neck in the up position and record the date and time as the Middle and Low Purity Islets 1st Culture Start Time here and in Section 12.5 table, below.

Date and time Middle and Low Purity Islets 1st Culture Start Date & Time: _____

Performed by: _____ **Date:** _____

11.8 Media Change, 1st Culture Stop Date & Time

11.8.1 After 12 to 24 hours remove all the flasks from the incubator(s) and record the date(s) and time(s) that each purity level of islets product is removed from the incubator(s) in the table in Section 12.5 as the 1st Culture Stop Date & Time.

Performed by: _____ **Date:** _____

11.8.2 Inspect the contents of each flask for gross appearance, cloudiness, stranding or clumping. Using a microscope, examine the morphology of the islets, including the extent of fragmentation and the numbers of single cells; and the fluid in each flask for microorganisms. Signs of contamination (cloudiness, microorganisms upon microscopic examination) or unusual islets morphology, including extensive fragmentation or large numbers of single cells, must be reported to the Site Principal Investigator, or designee, immediately, and investigated according to the institution's procedures. Record observations and dispositions of flasks below.

_____

_____

_____

_____

Inspected by: _____ **Date:** _____

Islets Lot Number: _____

If the Site Principal Investigator, or designee, is notified of any unusual islets morphology or evidence of microbial contamination, complete the following:

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____

11.8.3 Equilibrate the CIT Culture Media at room temperature. Place each flask in the BSC, tilt each at a 45º angle, and allow the islets to settle for 2 to 3 minutes. Aseptically remove 20 mL of supernatant media from each flask, and place all the removed supernatant from each purity level in as many containers as necessary for that purity level.

Add 20 mL of fresh CIT Culture Media to each flask, and replace the cap on each flask.

Verified by: _____ **Date:** _____

11.8.4 Transfer the supernatants to 250 mL conical tubes and centrifuge at 140 X g for 3 minutes. Remove supernatant and transfer tissue (if present) to a separate T-175 culture flask for each purity level.

	High Purity Supernatant	Middle Purity Supernatant	Low Purity Supernatant
Tissue Observed and recovered?	**Yes No**	**Yes No**	**Yes No**

Checked by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If no tissue is observed, discard the supernatant as biohazardous waste.

Performed by: _____ **Date:** _____

11.9 Place all the T-175 culture flasks (High, Middle, and Low Purity Levels) into an incubator at 22ºC, 95% air, and 5% carbon dioxide with the T-neck in the up position, and record the date(s) and time(s) that each purity level of islet product is placed in the incubator(s) in the table in Section 12.5 as the 2nd Culture Start Dates & Times.

Verified by: _____ **Date:** _____

12.0 ISLET PREPARATION FOR TRANSPLANT

12.1 Record the date and time scheduled for transplant of this lot of islets.

Scheduled Islet Transplant Date: _____

Scheduled Islet Transplant Time: _____

Recorded by: _____ **Date:** _____

12.2 Physician's Order for Transplant

Verify that the physician's signed order for transplant (if used by the institution) is present, and the order, or a copy, is attached to this batch record.

Yes No (Circle One)

Physician's Name: _____

Verified by: _____ **Date:** _____

12.3 Recipient & Donor Information

From the source documents record the information about the prospective recipient in the table below. Attach a copy of the Request for Islet Transplant form to this Production Batch Record.

	Islets Recipient Information	**Donor Information**
Hospital Name		UNOS or DDD #
Recipient Medical Record Number		
Recipient Study ID #		
Date of Birth		
Gender		
ABO		
CMV Status		
Allergies (Cipro, Penicillin, etc.)		
Current Weight (kg)		

Recorded by: _____ **Date:** _____

Compare this information with the Donor information in Section 4.4.

Blood Type Compatible? Yes No (Circle One)

CMV Status Reviewed? Yes No (Circle One)

Allergies Reviewed? Yes No (Circle One)

Information Reviewed with Clinician? Yes No (Circle One)

Compared by: _____ **Date:** _____
Lab Manager or designee

Reviewed by: _____ **Date:** _____

12.4 Before the scheduled transplant time:

12.4.1 Prepare the laboratory, including the Biological Safety Cabinet (BSC), for islet preparation according to the institution's procedure(s) and record the preparation on the appropriate form(s) or logbook(s). Submit copies of the form(s) or logbook page(s) with this Batch Record.

Verified by: _____ **Date:** _____

12.4.2 In a BSC prepare CIT Transplant Wash Media and CIT Transplant Media according to DAIT SOP 3106, B05 and B06, respectively, and attach the record of preparation to this Production Batch Record. Equilibrate these media to room temperature before use.

Verified by: _____ **Date:** _____

12.5 End of Culture

Remove all the islets product flasks from the incubator(s) and record the dates and times in the table below as the 2nd Culture Stop Dates & Times.

		High Purity Islets	Middle Purity Islets	Low Purity Islets	Recorded by	Verified by
1st Culture Start Date &Time	Date					
	Time					
1st Culture Stop Date & Time	Date					
	Time					
1st Culture Time (Hours:Minutes)						
Minimum 1st Culture Stop Date & Time						
Maximum 1st Culture Stop Date & Time						
2nd Culture Start Date & Time	Date					
	Time					
2nd Culture Stop Date & Time	Date					
	Time					
2nd Culture Time (Hours:Minutes)						
Minimum 2nd Culture Stop Date & Time						
Maximum 2nd Culture Stop Date & Time						
Total Culture Time (Hours:Minutes)						

Is the 1st Culture Stop Date & Time within the minimum and maximum 1st Culture Stop Date & Time calculated in Section 11.6?

Yes No (Circle One)

Is the 2nd Culture Stop Date & Time within the minimum and maximum 2nd Culture Stop Date & Time calculated in Section 11.6?

Yes No (Circle One)

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If the answer to either question above is "No," immediately notify the Principal Investigator, or designee.

If the Site Principal Investigator, or designee, is notified of a culture time deviation, complete the following:

Name of Person notified: _____

Notified by: _____ **Date & Time Notified:** _____

12.6 Inspect the contents of each flask for gross appearance, cloudiness, stranding or clumping. Using a microscope, examine the morphology of the islets, including the extent of fragmentation and the numbers of single cells; and the fluid in each flask for microorganisms. Signs of contamination (cloudiness, microorganisms upon microscopic examination) or unusual islets morphology, including extensive fragmentation or large numbers of single cells, must be reported to the Site Principal Investigator, or designee, immediately, and investigated according to the institution's procedures. Record observations and dispositions of flasks below.

_____

_____

_____

_____

_____

_____

Inspected by: _____ **Date:** _____

If the Site Principal Investigator, or designee, is notified of any unusual islets morphology or evidence of microbial contamination, complete the following:

Name of Person notified: _____

Notified by: _____ **Date & Time Notified:** _____

12.7 Post-Culture Islet Recombination – High Purity Islets

12.7.1 Place all the High Purity Islets T-175 culture flasks at a 45º angle and allow the islets to settle to the bottom corner for 3 to 5 minutes.

12.7.2 After the supernatant is observed to be clear, carefully transfer the tissue in approximately 10 mL of media from each T-175 culture flask to a T-75 flask labeled "Islets – High Purity."

12.7.3 Rinse the interior surfaces of each T-175 culture flask with the 20 mL of media remaining and transfer these rinses to a new T-175 flask labeled "Supernatant – High Purity."

12.7.4 Allow the pooled islets in the "Islets – High Purity" T-75 flask to settle for approximately 3 to 5 minutes. Remove the supernatant from the top to leave 100 mL (=100 g) of suspension in the T-75 flask. Place the supernatant into the "Supernatant – High Purity" T-175 flask.

12.7.5 Examine the "Supernatant – High Purity" T-175 flask under a microscope to determine if islets are present. If islets are present, transfer the supernatant to a 250 mL conical tube and centrifuge at 140 X g for 2 to 3 minutes at 2ºC to 8ºC. Transfer the tissue to the "Islets – High Purity" T-75 flask.

Verified by: _____ **Date:** _____

12.8 Post-Culture Islet Recombination – Middle Purity Islets

12.8.1 Place all the Middle Purity Islets T-175 culture flasks at a 45º angle and allow the islets to settle to the bottom corner for 3 to 5 minutes.

12.8.2 After the supernatant is observed to be clear, carefully transfer the tissue in approximately 10 mL of media from each T-175 culture flask to a T-75 flask labeled "Islets – Middle Purity."

12.8.3 Rinse the interior surfaces of each T-175 culture flask with the 20 mL of media remaining and transfer these rinses to a new T-175 flask labeled "Supernatant – Middle Purity."

12.8.4 Allow the pooled islets in the "Islets – Middle Purity" T-75 flask to settle for approximately 3 – 5 minutes. Remove the supernatant from the top to leave 100 mL (=100 g) of suspension in the T-75 flask. Place the supernatant into the "Supernatant – Middle Purity" T-175 flask.

12.8.5 Examine the "Supernatant – Middle Purity" T-175 flask under a microscope to determine if islets are present. If islets are present, transfer the supernatant to a 250 mL conical tube and centrifuge at 140 X g for 2 to 3 minutes at 2ºC to 8ºC. Transfer the tissue to the "Islets – Middle Purity" T-75 flask.

Verified by: _____ **Date:** _____

12.9 Post-Culture Islet Recombination – Low Purity Islets

12.9.1 Place all the Low Purity Islets T-175 culture flasks at a 45º angle and allow the islets to settle to the bottom corner for 3 to 5 minutes.

12.9.2 After the supernatant is observed to be clear, carefully transfer the tissue in approximately 10 mL of media from each T-175 culture flask to a T-75 flask labeled "Islets – Low Purity."

12.9.3 Rinse the interior surfaces of each T-175 culture flask with the 20 mL of media remaining and transfer these rinses to a T-175 flask labeled "Supernatant – Low Purity."

12.9.4 Allow the pooled islets in the "Islets – Low Purity" T-175 flask to settle for approximately 3 to 5 minutes. Remove the supernatant from the top to leave 100 mL (=100 g) of suspension in the T-75 flask. Place the supernatant into the "Supernatant – Low Purity" T-175 flask.

12.9.5 Examine the "Supernatant – Low Purity" T-175 flask under a microscope to determine if islets are present. If islets are present, transfer the supernatant to a 250 mL conical tube and centrifuge at 140 X g for 2 to 3 minutes at 2ºC to 8ºC. Transfer the tissue to the "Islets – Low Purity" T-75 flask.

Verified by: _____ **Date:** _____

12.10 Estimate the Settled Tissue Volume in the final product T-75 flasks

- Allow the tissue to settle in the corner of each T-75 flask for 3 to 5 minutes.
- Gently aspirate all the tissue into a sterile 10 mL glass pipet.
- Allow the tissue to settle in the pipet while holding it vertically for 3 to 5 minutes.
- Estimate the settled tissue volume from the pipet and record result in the table below.

Record the Settled Tissue Volumes in the table in Section 12.12, below.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.11 Wash Tissue in Preparation for Loading into Transplant Bags

12.11.1 Allow the tissue in each T-75 flask (High, Middle and Low Purity) to settle for 3 to 5 minutes.

12.11.2 Transfer each supernatant to 250 mL conical tubes and centrifuge at 140 X g for 3 to 5 minutes.

12.11.3 Wash the settled tissue in each T-75 with approximately 100 mL CIT Transplant Wash Media.

12.11.4 Remove the supernatant from each 250 mL conical tube and return any tissue to the appropriate T-75 flask.

12.11.5 Bring the volume in each T-75 flask (High, Middle, and Low Purity) to 50 to 250 mL with CIT Transplant Media after the second wash. Take a sample of each **supernatant** for a Gram Stain according to the institution's procedure and send it to the appropriate lab. Report the results in Section 12.12.

Purity Level	High	Middle	Low
Suspension Volume (mL)			
Sample Volume (mL)			
Remaining Suspension Volume (mL)			

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.12 The Final Product Composition Plan

This plan is based on the Settled Tissue Volume and the Gram Stain results recorded in the table, below. Determine and record which flasks will be combined, if any, so that:

- If there is ≤ 7.5 mL Total Settled Tissue Volume, all tissue may be combined into one Final Product T-75 flask.
- There is ≤ 7.5 mL of Settled Tissue Volume in **any one** Final Product T-75 flask.
- There is ≤ 15 mL of total Settled Tissue Volume in **all** Final Product T-75 flasks.

Purity Level	Settled Tissue Volume (mL) (Section 12.10)	Gram Stain Results (Section 12.11.5)*	Disposition Identify which flasks will be combined or not combined for transplant, and which will be used for research or discarded.
High			
Middle			
Low			
Total			

*These Gram Stain results are reported on the Certificates of Analysis.

Determined by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If a positive Gram Stain result is reported for any purity level, immediately notify the Site Principal Investigator, or designee.

If the Site Principal Investigator, or designee, is notified of a positive Gram Stain result, complete the following:

Name of Person notified: _____

Notified by: _____ **Date & Time Notified:** _____

12.13 Take two 100 µL samples of each purity level and perform counts and calculations. Attach spreadsheet(s) if used.

Post-culture Islets Counts

	High Purity Islets				Middle Purity Islets				Low Purity Islets			
Sample Volume			µL				µL				µL	
Total Volume*			mL				mL				mL	
Dilution Factor												
Diameter, Factor	Counts		Avg.	IEQ	Counts		Avg.	IEQ	Counts		Avg.	IEQ
50 – 100, 0.167												
101 – 150, 0.648												
151 – 200, 1.685												
201 – 250, 3.500												
251 – 300, 6.315												
301 – 350, 10.352												
> 350, 15.833												
Total												
% Trapped												
% Fragmented												
Purity (%)												
Islet Quality Grade*												
Technicians' Initials												

*Remaining Suspension Volume recorded in Section 12.11.5, above.

Post-culture Islets Calculations

	High Purity Islets	Middle Purity Islets	Low Purity Islets	Total
Post-culture IPN				
Post-culture IEQ				
Pre-purification IEQ (Section 7.5.2)				
IEQ Recovery (%) (from Pre-purification IEQ)				
Post-purification IEQ (Section 10.2)				
IEQ Recovery (%) (from Post-purification IEQ)				
IEQ/g of Final Trimmed Pancreas (Section 6.3)				
Comments				

*See Islet Quality Grade Note at the end of Section 10.2, for guidelines

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Total Post-purification Islets Count: _____ IEQ

Total Post-culture Islets Count: _____ IEQ

Percent Change: _____%

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If the Post-culture Islets Count is > 30% less than the Post-purification Islets Count, Section 10.2, notify the Site Principal Investigator, or designee, immediately.

If the Site Principal Investigator, or designee, is notified of > 30% decrease in IEQ, complete the following:

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____

12.14 Post-culture Sampling of High Purity Islets Suspension

Based on the Post-culture count, Section 12.13, take samples of the High Purity Islets suspension according to the table below and record test results in Section 17.2, the Certificates of Analysis and Section 20.0, as required.

From the High Purity Islets Total IEQ and suspension volume (Section 12.13, above) calculate the High Purity Islets concentration:

Total IEQ _____ / Suspension Volume _____ mL = _____ IEQ/mL

SAMPLE QUANTITY	REQUIRED FOR CERTIFICATE OF ANALYSIS, FOR INFORMATION ONLY	SAMPLE VOLUME (mL)	SAMPLE IEQ
Suspension, 400 IEQ	Post-culture Glucose Stimulated Insulin Release Index		
	REQUIRED PRODUCT CHARACTERIZATION, FOR INFORMATION ONLY		
Suspension, 4,000 IEQ	*In vivo* (Nude Mouse) Islets Function		
	OPTIONAL PRODUCT CHARACTERIZATION, FOR INFORMATION ONLY		
Suspension, 3 X 100 IEQ	Post-culture DNA Content*		
Suspension, 3 X 100 IEQ	Nuclei Measurement*		
Suspension, 500 IEQ	ATP/DNA		
Suspension, 5,000 IEQ	OCR/DNA*		
Suspension, 5,000 IEQ	Molecular Profiling*		
Suspension, 500 IEQ	Islets Fraction*		
	Total Removed from High Purity Islets Suspension Volume & IEQ		
	High Purity Islets Suspension Volume & IEQ Before Sampling (Section 12.13)		
	Remaining High Purity Islets Volume & IEQ		

*Note: Follow instructions in the CIT Lab Binder for preparation and shipment of samples.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.15 Combine the Islets Suspensions (cross out, initial and date unused sub-sections below)

12.15.1 If, according to the plan in Section 12.12, there will be **one infusion bag**, combine all islets into one T-75 flask rinsing the emptied flasks with CIT Transplant Media. Combine by settling and removing supernatant as in Section 12.11, above, as necessary. Adjust the volume in the single T-75 flask after combination to 100 mL with CIT Transplant Media.

Final Volume in one T-75 flask: _____ mL

Verified by: _____ **Date:** _____

12.15.2 If, according to the plan in Section 12.12, there will be **two infusion bags**, combine the islets into two T-75 flasks according to the plan, rinsing the emptied flasks with CIT Transplant Media. Combine by settling and removing supernatant as in Section 12.11, above, as necessary. Adjust the volume in each T-75 flask after combination to 100 mL with CIT Transplant Media.

Final Volume in T-75 flask #1: _____ mL

Final Volume in T-75 flask #2: _____ mL

Verified by: _____ **Date:** _____

12.15.3 If, according to the plan in Section 12.12, there will be **three infusion bags**, combine the islets into three T-75 flasks according to the plan. Combine by settling and removing supernatant as in Section 12.11, above, as necessary. Adjust the volume in each T-75 flask after combination to 100 mL with CIT Transplant Media.

Final Volume in T-75 flask #1: _____ mL

Final Volume in T-75 flask #2: _____ mL

Final Volume in T-75 flask #3: _____ mL

Verified by: _____ **Date:** _____

12.16 Label sample containers for the release and characterization testing samples according to the institution's procedures.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.17 Sampling and Testing of Final Product T-75 Flasks

12.17.1 If Islets Purity Levels are combined according to the plan in Section 12.12, take two 100 µL samples of each final Product T-75 Flask and perform counts and calculations. Attach spreadsheet(s) if used. If no Islets Purity Levels are combined, use the IEQ values from Section 12.13 for Middle and Low Purity Islets and from Section 12.14 for High Purity Islets.

Final Product Islets (Post-combination) Counts & Calculations

	Final Product T-75 Flask #1				Final Product T-75 Flask #2				Final Product T-75 Flask #3			
Sample Volume				µL				µL				µL
Total Volume (Section 12.15)				mL				mL				mL
Dilution Factor												
Diameter (µm), Factor	Counts		Avg.	IEQ	Counts		Avg.	IEQ	Counts		Avg.	IEQ
50 – 100, 0.167												
101 – 150, 0.648												
151 – 200, 1.685												
201 – 250, 3.500												
251 – 300, 6.315												
301 – 350, 10.352												
> 350, 15.833												
Sample Totals												
Purity Level Totals												
% Trapped												
% Fragmented												
Purity (%)												
Islet Quality Grade*												
Technicians' Initials												

*See Islets Quality Grade Note at the end of Section 10.2 for guidelines

Total Final Product Islets Quantity: _____ IEQ

Total IEQ/g of Final Trimmed Pancreas (Section 6.3): _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.17.2 Sample the **suspension(s)** in the Final Product T-75 flask(s) before filling the infusion bags, and send the samples to the appropriate laboratory for the tests indicated in the table below. Report the test results in Sections 14.0 and 20.0, and on the Certificates of Analysis, as indicated.

Islets Lot Number: _____

If Islets Purity Levels were not combined, use the IEQ values in Section 12.13 for Middle and Low Purity Islets, the IEQ value in Section 12.14 for High Purity Islets, and the Suspension Volumes in Section 12.15, to calculate the Islets concentrations (IEQ/mL) in the suspensions.

If Islets Purity Levels were combined, use the IEQ values and the Suspension Volumes in Section 12.17.1, to calculate the Islets concentrations (IEQ/mL) in the suspensions.

		T-75 #1	T-75 #2	T-75 #3	
IEQ in flask (Section 12.13, 12.14, or 12.17.1)					
Volume in Flask (mL) (Section 12.15, or 12.17.1)					
Islets Concentration (IEQ/mL)					
Sample Type & Quantity		**Sample Removed (mL)**			
Required for Certificates of Analysis	**Tests**	T-75 #1	T-75 #2	T-75 #3	**Testing Lab**
100 IEQ/Each T-75 Flask	Viability				
500 IEQ/Each T-75 Flask (Combine with Supernatant Volume taken is Section 12.17.3)	Sterility (21 CFR 610.12), & Fungal Culture				
Required Product Characterization, For Information Only					
1,000 IEQ/Each T-75 Flask	Cell Composition				University of Miami*
500 to 1,000 IEQ/Each T-75 Flask	MCP-1 & Tissue Factor				Uppsala University Hospital, Sweden*
4 X 500 IEQ from T-75 flask #1 in 1.8 mL cryovials	Repository				NIDDK Repository*
Optional Product Characterization, For Information Only					
2,000 IEQ/Each T-75 Flask	β-cell Viability				
Suspension Volume Removed from each T-75 Flask					
Suspension Volume in each T-75 Flask before sampling (Section 12.15, or 12.17.1)					
Suspension Volume in each T-75 Flask after sampling					
IEQ in each T-75 Flask after sampling					

*Follow instructions in the CIT Islets Lab Binder for preparation and shipment of samples for Cell Composition analysis, for MCP-1 and Tissue Factor analyses, and for the NIDDK Repository.

Remaining IEQ in each T-75 Flask = Suspension Volume in each T-75 Flask after sampling X Islets Concentration (IEQ/mL) in each T-75 Flask

Is the islets suspension the source of all these samples? Yes No (Circle One)

Sampled by: _____ **Date:** _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

12.17.3 Remove 1 mL of supernatant from each T-75 flask for Endotoxins testing, and the volume required by the institution's procedures from each T-75 flask for Sterility testing. Report the Endotoxins results in Section 14, below, and on the Certificates of Analysis, and the Sterility results in Section 17.1.2, below, and on the Certificate of Analysis.

	T-75 Flask #1	T-75 Flask #2	T-75 Flask #3
Remaining Suspension Volume (Section 12.17.2) (mL)			
Endotoxins Sample Volume (mL)			
Sterility test sample volume according to institution's procedure of islets suspension from each T-75 Flask (Combined with 500 IEQ taken in Section 12.17.2 for testing) (mL)			
Remaining Suspension Volume (mL)			

Note: The Remaining Suspension Volume in each T-75 Flask is used to calculate the Endotoxins/kg in Section 14.5, below.

Sampled by: _____ **Date:** _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.18 After sampling, Section 12.17.2, above, estimate the Tissue Volume in the final product containers
- Allow the tissue to settle in the corner of each T-75 flask for 3 to 5 minutes.
- Gently aspirate all the tissue into a sterile 10 mL glass pipet.
- Allow the tissue to settle in the pipet while holding it vertically for 3 to 5 minutes.
- Estimate the settled tissue volume from the pipet and record result in the table below.

T-75 FLASK	#1	#2	#3
SETTLED TISSUE VOLUME (ML)			

Report these results on the Interim and Final Certificates of Analysis.

Verified by: _____ **Date:** _____

12.19 Set up the labeled product bag(s), 150 mL rinse bag(s), 60 mL syringe(s) in the BSC as follows:
- Connect the tubing from the 150 mL rinse bag to the Ricordi Infusion bag.
- Clamp off the line connecting the bags with a hemostat at both ends.
- Place a syringe in ring stand and remove its plunger.
- Connect the syringe to the Luer lock port of the Ricordi Infusion bag.
- Repeat this setup for the 2nd and 3rd bag systems, if the final tissue volume warrants multiple bags.

Performed by: _____ **Date:** _____

12.20 Calculation of Heparin Quantity Addition

****

Heparin is not a part of the product. It is added to the product at the discretion of the recipient's physician.

****

Optionally, to the final product add 70 Units of heparin per kg of recipient body weight.

Recipient Body Weight (Section 12.3): _____ kg

Heparin Concentration: _____ units/mL

Divide the heparin equally among the infusion bags.

_____ kg X 70 U/kg / _____ # of bags = _____ Units of Heparin to add to each product bag

_____ Units of Heparin to add to each product bag / _____ U/mL = _____ mL of Heparin to add to each product bag

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.21 Label with the following information one Purified Human Pancreatic Islets product infusion bag for each T-75 flask remaining, after combining in Section 12.12, that will be transplanted:

- "Human Islets," "Human Islets Product," or similar
- Islets Lot Number
- Donor Identification (UNOS or DDD) Number
- Donor Blood Type
- Total IEQ in Bag
- "Bag X of Y"
- Recipient Name (This is redacted from the label copy sent to the sponsor for review)
- Recipient Medical Record Number (This is redacted from the label copy sent to the sponsor for review)
- Recipient Study ID #
- Recipient Blood Type
- "Sterility testing has not been completed."
- "Biohazard: Human Tissue"
- "New drug. Limited by law to investigational use only"
- Suspension Volume
- Name of the Manufacturing Institution
- FDA Registration Number, if available
- "BB-IND 9336"
- Storage Temperature (15°C to 30°C)
- "Contains Heparin, Units in this bag: _____"
- Use by Date: _____, Time: _____ (6 hours after filling)

Additional information may be added as required by the institution's procedures.

Make three identical labels for each bag. Place one on each bag, place one for each bag in the file with the Production Batch Record, and send one with each product bag with an instruction to affix it to the recipient's medical record chart.

Labeled by: _____ **Date:** _____

Checked by: _____ **Date:** _____

Islets Lot Number: _____

12.22 Filling Infusion and Rinse Bags #1

12.22.1 Add 100 mL of CIT Transplant Media to Infusion Bag #1. Unclamp tubing to drain the media from the infusion bag to the rinse bag. Remove all air from rinse bag and re-clamp tubing.

12.22.2 Transfer the tissue in 100 mL of CIT Transplant Media from the flask to Infusion Bag #1 through the syringe.

12.22.3 Record the time as Infusion Bag #1 Filling Start Time: _____

12.22.4 If heparin is to be added to the product, add the amount of heparin calculated in Section 12.21, to Infusion Bag #1 at this point.

Units of Heparin added to Infusion Bag #1: _____ units

Volume of Heparin added to Infusion Bag #1: _____ mL

Performed by: _____ **Date:** _____

12.22.5 Add 50 mL of CIT Transplant Media to the T-75 flask, rinse the surfaces of the flask with this media, and transfer this rinse media into the infusion bag.

12.22.6 Rinse the T-75 flask again with another 50 mL of CIT Transplant Media, and transfer this rinse media into the infusion bag. After transferring the entire final product to the infusion bag remove the air using a "burping" technique and clamp the port with a hemostat so that no air enters the bag.

12.22.7 Record the time as the Infusion Bag #1 Filling End Time: _____

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.23 Filling Infusion and Rinse Bags #2

12.23.1 Add 100 mL of CIT Transplant Media to Infusion Bag #2. Unclamp tubing to drain the media from the infusion bag to the rinse bag. Remove all air from rinse bag and re-clamp tubing.

12.23.2 Transfer the tissue in 100 mL of CIT Transplant Media from the flask to the Infusion Bag #2 through the syringe.

12.23.3 Record the time as Infusion Bag #2 Filling Start Time: _____

12.23.4 If heparin is to be added to the product, add the amount of heparin calculated in Section 12.21, to Infusion Bag #2 at this point.

Units of Heparin added to Infusion Bag #2: _____ units

Volume of Heparin added to Infusion Bag #2: _____ mL

Performed by: _____ **Date:** _____

12.23.5 Add 50 mL of CIT Transplant Media to the T-75 flask, rinse the surfaces of the flask with this media, and transfer this rinse media into the infusion bag.

12.23.6 Rinse the T-75 flask again with another 50 mL of CIT Transplant Media, and transfer this rinse media into the infusion bag. After transferring the entire final product to the infusion bag remove the air using a "burping" technique and clamp the port with a hemostat so that no air enters the bag.

12.23.7 Record the time as the Infusion Bag #2 Filling End Time: _____

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.24 Filling Infusion and Rinse Bags #3

12.24.1 Add 100 mL of CIT Transplant Media to Infusion Bag #3. Unclamp tubing to drain the media from the infusion bag to the rinse bag. Remove all air from rinse bag and re-clamp tubing.

12.24.2 Transfer the tissue in 100 mL of CIT Transplant Media from the flask to Infusion Bag #3 through the syringe.

12.24.3 Record the time as Infusion Bag #3 Filling Start Time: _____

12.24.4 If heparin is to be added to the product, add the amount of heparin calculated in Section 12.21, to Infusion Bag #3 at this point.

Units of Heparin added to Infusion Bag #3: _____ units

Volume of Heparin added to Final Product Bag #3: _____ mL

Performed by: _____ **Date:** _____

12.24.5 Add 50 mL of CIT Transplant Media to the T-75 flask, rinse the surfaces of the flask with this media, and transfer this rinse media into the infusion bag.

12.24.6 Rinse the T-75 flask again with another 50 mL of CIT Transplant Media, and transfer this rinse media into the infusion bag. After transferring the entire final product to the infusion bag remove the air using a "burping" technique and clamp the port with a hemostat so that no air enters the bag.

12.24.7 Record the time as Infusion Bag #3 Filling End Time: _____

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.25 Inspect each infusion bag to ensure that it is intact, there are no leaks, the label is legible, and the contents are a light yellow to amber liquid with visible islets in each bag. These observations are reported on the Interim Certificate of Analysis and the Certificate of Analysis.

Does each product infusion bag meet these criteria?

Bag #1:	Yes	No	(Circle One)
Bag #2:	Yes	No	(Circle One)
Bag #3:	Yes	No	(Circle One)

If any infusion bag does not meet these criteria, the Laboratory Director, or designee, must be notified immediately, and they must initiate an investigation according to the institution's procedures. The process for reporting a deviation to the CMCMC as defined in DAIT SOP 3200 must also be followed.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If the Laboratory Director, or designee, is notified of an infusion bag not meeting the criteria, complete the following:

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

12.26 Place the product infusion bags in a cooler with following:

- Absorbent material
- Room temperature pack
- Temperature monitor
- Infusion Set

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

13.0 CHECKLIST OF RECORDS FILED WITH THIS PRODUCTION BATCH RECORD

13.1 Required Solution and Media Preparation Records

MPBR SECTION	DAIT SOP 3106,	Solution and Media Preparation Records	PRESENT?	
			YES	NO
5.4	B01	CIT Digestion Solution		
5.8.1	B11	CIT Enzyme Solution – SERVA Enzymes		
5.8.2	B13	CIT Enzyme Solution – VitaCyte Enzymes or VitaCyte/SERVA Enzymes		
5.8.3	B14	CIT Enzyme Solution – Roche Enzymes		
7.4.1	B02	CIT Purification Solution		
7.4.1	B12	CIT Wash Solution		
8.1	B10	CIT Purification Density Gradients		
9.1	B10	CIT Purification Density Gradients (If OptiPrep Supplementary Purification, performed)		
10.1	B04	CIT Culture Media		
12.4.2	B05	CIT Transplant Wash Media		
12.4.2	B06	CIT Transplant Media		

Verified by: _____ **Date:** _____

13.2 Required Lists

MPBR SECTION	LISTS	PRESENT?	
		YES	NO
3.1.2	Personnel participating in this manufacturing process		
3.1.4	Sterilized Items		
3.1.5	Equipment		
3.1.6	Disposable Items		

Verified by: _____ **Date:** _____

13.3 Required Test Reports (Results not recorded in previous Sections of this Batch Record)

MPBR SECTION	TEST REPORTS	PRESENT?	
		YES	NO
12.11.6	Gram Stain		
12.18.2	Final Product Viability		
12.18.2	Final Product Endotoxins		
12.18.2	Pre-culture Sample Glucose Stimulated Insulin Release		

Verified by: _____ **Date:** _____

13.4. Supplementary Purification Records (if performed)

MPBR SECTION	DAIT SOP 3109,	SUPPLEMENTARY PURIFICATION RECORD	PRESENT?	
			YES	NO
9.1	B01	Supplementary Purification, OptiPrep Procedure		
9.2	B02	Supplementary Purification, Continuous Biocoll Procedure		
9.3	B03	Supplementary Purification, Discontinuous Polysucrose Procedure		

13.5 Additional Records

MPBR SECTION	ADDITIONAL RECORDS	PRESENT?	
		YES	NO
3.2, & 12.4.1	Laboratory and Biologic Safety Cabinet Preparation Records		
12.12	Physician's order for transplant, if used		
12.21	Product Infusion Bag Label(s)		
	All Deviation and Discrepancy Investigation Reports, if any		

Verified by: _____ **Date:** _____

14.0 Pre-transplant Test Results

14.1 From the tests conducted on the samples taken in Section 12.17.1, 12.17.2, 12.17.3, and 12.18, above, enter the results in the table below.

FINAL PRODUCT INFUSION BAG	#1	#2	#3	TOTAL
Settled Tissue Volume (mL)* (Section 12.18)				
Suspension Volume (mL) in Infusion Bag* (Sections 12.22, 12.23, 12.24, above)				
Islets Identity (Yes/No)* (Section 12.17.1)				
Islets Equivalents (IEQ) in Infusion Bag (Section 12.17.2)				
Islets Quantity (IEQ/kg)* (Calculate in Section 14.2, below)				
Islets Concentration (IEQ/mL Tissue)* (Calculate in Section 14.3, below)				
Mean Glucose Stimulated Insulin Release Index (High Purity Islets, Pre-culture sample taken in Section 11.1, above) (Calculated in Section 14.4, below)*				
Viability (%)* (from Viability test report)				
Endotoxins Concentration (EU/mL) (from Endotoxins test report)				
Endotoxins (EU/kg Recipient Weight)* (Calculate in Section 14.5, below)				

***These results are also reported on the Interim and Final Certificates of Analysis.**

14.2 Calculate the Islets Quantity (IEQ/kg) in each T-75 Flask and their sum from the Islets Equivalents (IEQ) in each infusion bag and the Recipient Body Weight (kg), and record the results in the tables here and in Section 14.1, above:

$$\frac{\text{Islets Equivalents (IEQ)}}{\text{Recipient Body Weight (kg)}} = \text{Islets Quantity (IEQ/kg)}$$

Final Product T-75 Flasks	Islets Equivalents (IEQ) (Section 12.17.2)	Recipient body Weight (kg) (Section 12.3)	Islets Quantity (IEQ/kg)
1			
2			
3			
		Total	

Entered and calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

14.3 Calculate the Islets Concentration in each T-75 Flask and their sum from the Islets Equivalents and the Settled Tissue Volumes in Section 14.1, above, and record the results in the tables here and in Section 14.1, above:

$$\frac{\Sigma \text{ Islets Equivalents (IEQ)}}{\Sigma \text{ Settled Tissue Volume (mL)}} = \text{Islets Concentration (IEQ/mL Tissue)}$$

Final Product T-75 Flasks	Islets Equivalents (IEQ)	Settled Tissue Volume (mL)	Islets Concentration (IEQ/mL)
1			
2			
3			
Total			

To calculate the total IEQ/mL of tissue if there are more than one infusion bag, first add the IEQ and mL of tissue separately, then divide.

Entered and calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

14.4 Glucose Stimulated Insulin Release Test Results (Pre-culture Sample)

High Purity Islets	Index 1	Index 2	Index 3	Mean Index
Pre-culture Sample (PBR Section 11.1)				

Report the Mean Index in PBR Section 14.1, above, and on the Certificates of Analysis.

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

14.5 Calculate the Endotoxins Units per kg of recipient body weight in each T-75 Flask and the Total Endotoxins Units per kg of recipient body weight from the Endotoxins Concentration (EU/mL) in Section 14.1, the Remaining Suspension Volume (mL) in Section 12.17.3, and the Recipient Body Weight (kg) in Section 12.3, above, and record the results in the tables here and in Section 14.1 above:

$$\frac{\text{Endotoxins Concentration (EU/mL)} \times \text{Suspension Volume (mL)}}{\text{Recipient Body Weight (kg)}} = \text{EU/kg Recipient Weight}$$

Final Product T-75 Flasks	Endotoxins Concentration (EU/mL)	Suspension Volume (mL) (Section 12.17.3)	Recipient Body Weight (kg) (Section 12.3)	EU/kg
1				
2				
3				
			Total	

Entered and calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

15.0 PRE-TRANSPLANT BATCH RECORD REVIEW AND INTERIM APPROVAL

After the completion of all activities and records of this manufacturing process to this point, and before transplant of this batch of islets, a qualified technician, and the Laboratory Director, Operations Manager, or designee, must review the Production Batch Record to verify that it is complete and accurate to this point.

We have reviewed the Production Batch Record and verified that it is complete and accurate to this point.

_____ Date: _____
Qualified Technician

_____ Date: _____
Laboratory Director, Operations Manager, or designee

16.0 ISLET PRODUCT CUSTODY TRANSFER

16.1 If required by the institution's procedures, notify the clinical team that the islets are ready for transplant.

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

16.2 Custody Transfer Record

If required by the institution's procedures, complete and file the original or a copy of the institution's product custody transfer record with this production batch record.

Performed by: _____ **Date:** _____

16.3 Review the product bag label(s) with a clinical team member to assure that the intended recipient and the UNOS or DDD Number are correctly identified (See Section 12.3). Report this identity verification on the Interim and Final Certificates of Analysis.

UNOS or DDD Number Correct? Yes No (Circle One)

Recipient Identity Correct? Yes No (Circle One)

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

17.0 POST-TRANSPLANT TEST RESULTS & REPORTS

17.1 Sterility Test Results

17.1.1 Record the 24-hour and final test results of the 21 CFR 610.12 sterility test and fungal culture on the Preservation Solution (Section 5.1) in the table below, when available.

PRESERVATION SOLUTION	24-HOUR RESULT		FINAL RESULT	
	Sterility	Fungal Culture	Sterility	Fungal Culture
#1				

If there is a positive result, record the identity of the organism(s): _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

17.1.2 Record the Final Results of the sterility test (21 CFR 610.12) and fungal culture on the samples from the Final Product T-75 Flasks (taken at Section 12.17.2) in the table below. Report these results on the final Certificate of Analysis, when available.

FINAL PRODUCT T-75 FLASKS	24-HOUR RESULT		FINAL RESULT	
	Sterility	**Fungal Culture**	**Sterility**	**Fungal Culture**
#1				
#2				
#3				

If there is a positive result reported, record the identity of the organism(s): _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If any positive result is reported, immediately notify the attending physician.

Name of Physician Notified: _____

Notified by: _____ **Date:** _____ **Time:** _____

17.2 Glucose Stimulated Insulin Release Test Results (Post-culture Samples)

HIGH PURITY ISLETS	INDEX 1	INDEX 2	INDEX 3	MEAN INDEX
POST-CULTURE SAMPLE (PBR SECTION 12.14)				

Report the Mean Index on the Certificate of Analysis.

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

17.3 Required Test Reports (Results not recorded in previous Sections of this Batch Record)

MPBR SECTION	TEST REPORTS	PRESENT?	
		YES	NO
5.1	Preservation Solution Sterility		
12.14	Final Product Glucose Stimulated Insulin Release		
12.17.2	Final Product Sterility		

Verified by: _____ **Date:** _____

18.0 PRODUCT DISPOSITION

Was this product transplanted? Yes No (Circle one)

If this product was transplanted, record the Recipient Study ID #: _____

If this product, or any portion of it, was not transplanted, explain why not and state its final disposition.

_____

_____

_____

_____

Recorded by: _____ **Date:** _____

19.0 POST-TRANSPLANT BATCH RECORD REVIEW AND FINAL APPROVAL

After completion of Sections 16, 17, and 18, above, a qualified technician, and the Laboratory Director, Operations Manager, or designee review these Sections to verify that they are complete and accurate.

We have reviewed Sections 16, 17, and 18, above, and verified that they are complete and accurate.

_____ Date: _____

Qualified Technician

_____ Date: _____

Laboratory Director, Operations Manager or designee

A qualified representative of the institution's Quality Unit must review the entire Production Batch Record and verify that it is complete and accurate

I have reviewed this entire Batch Production Record and verified that it is complete and accurate.

_____ Date: _____

Quality Unit Representative

20.0 Product Characterization Test Results (For Information Only)

Record results of the following tests in the table below. File copies of the raw data with this PBR. "FPTF" means Final Product T-75 Flask.

SAMPLES FROM MPBR SECTION	REQUIRED PRODUCT CHARACTERIZATION	RESULT
5.7	Pancreas Biopsy MCP-1	
5.7	Pancreas Biopsy Tissue Factor	
12.14	*In Vivo* Islet Function (Nude Mouse Assay)	High Purity Islets: _____ (Hyperglycemia Reversed, or Not Reversed)
12.17.2	Cell Composition (Laser Scanning Cytometry & Immunofluorescence)	FPTF #1, β-cells: _____ % δ-cells: _____ % α-cells: _____ % PP-cells: _____ % FPTF #2, β-cells: _____ % δ-cells: _____ % α-cells: _____ % PP-cells: _____ % FPTF #3, β-cells: _____ % δ-cells: _____ % α-cells: _____ % PP-cells: _____ %
12.17.2	Final Product MCP-1	FPTF 1: _____ FPTF 2: _____ FPTF 3: _____
12.17.2	Final Product Tissue Factor	FPTF 1: _____ FPTF 2: _____ FPTF 3: _____

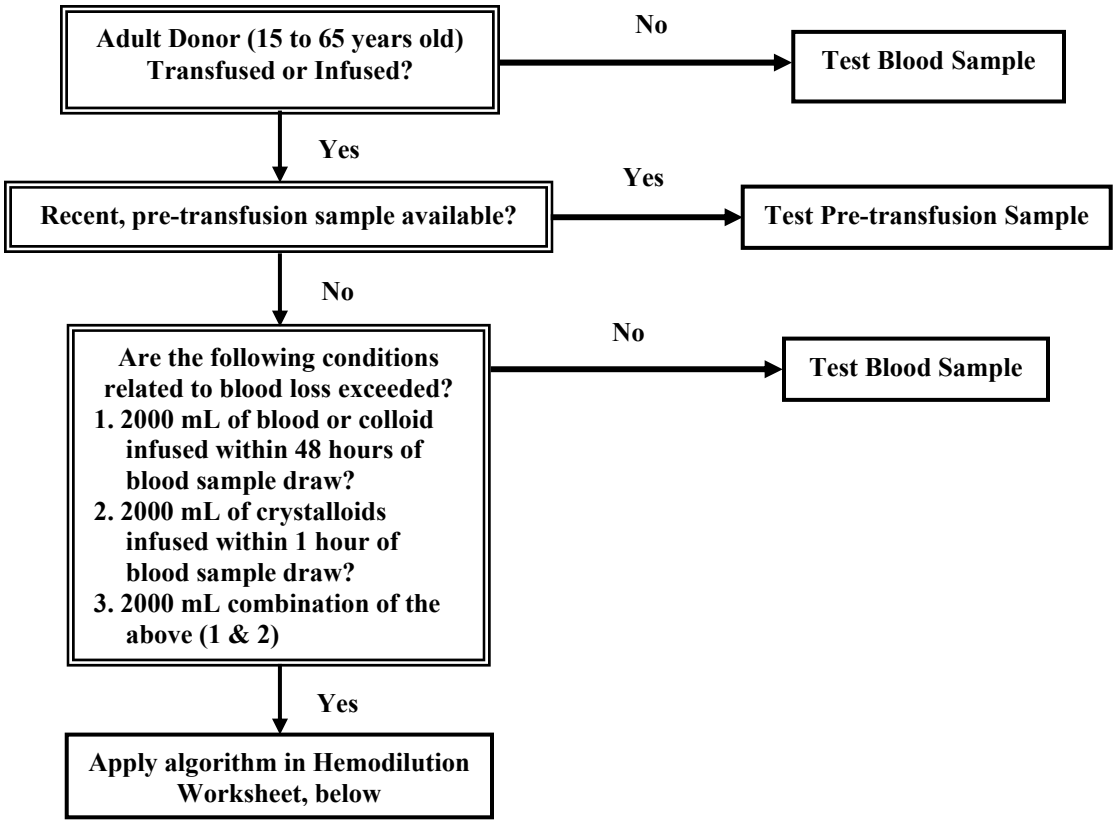
SAMPLES FROM MPBR SECTION	OPTIONAL PRODUCT CHARACTERIZATION	RESULT
11.1	Pre-culture DNA Content	High Purity Islets: _____ µg DNA
11.1	Pre-culture Nuclei Measurement	nuclei
12.14	Post-culture DNA Content	High Purity Islets: _____ µg DNA
12.14	Post-culture Nuclei Measurement	nuclei
12.14	ATP/DNA Ratio	
12.14	OCR/DNA	nmol O_2/min/mg DNA
12.14	Molecular Profiling	
12.14	Islet Fraction	
12.17.2	β-Cell Viability (Flow Cytometry)	FPTF #1: _____ % FPTF #2: _____ % FPTF #3: _____ %

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

HEMODILUTION FLOWCHART

DONOR SPECIMEN SUITABILITY FOR INFECTIOUS DISEASE TESTING FLOWCHART



Definitions:

1. Blood or blood component: any part of a single-donor unit of blood separated by physical or mechanical means.

2. Colloid: a protein or polysaccharide solution that can be used to increase or maintain osmotic (oncotic) pressure in the intravascular compartment such as albumin, dextran, hetastarch; or certain blood components, such as plasma or platelets.

3. Crystalloid: a balanced salt and/or glucose solution used for electrolyte replacement or to increase intravascular volume such as saline, Ringer's Lactate solution, or 5% dextrose in water.

HEMODILUTION WORKSHEET

Instructions: Use this worksheet when (1) no pre-transfusion sample is available and (2) the determination needs to be made if the post-transfusion sample is suitable for infectious disease testing due to transfusion or infusion.

Donor UNOS # _____ Date: _____

Date and Time of Sampling	a.m. p.m.
Donor Weight (kg)	kg
Plasma Volume (PV)	Donor weight (kg): _____/0.025 = _____ mL
Blood Volume (BV)	Donor weight (kg): _____/ 0.015 = _____ mL
A. Total Volume of Blood transfused/48 hours 1 unit packed red cells = 250 mL Date and Time of Transfusion	RBC's transfused/48 hrs: _____ mL Whole blood transfused / 48 hrs: _____ mL Reconstituted blood transfusion: _____ mL **Total of A: _____ mL**
B. Total Volume of colloid transfused/48 hours 1 unit FFP = 250 mL 1 unit platelet pheresis = 225 mL 1 platelet pool = 300 mL Date and Time of Transfusion	Dextran / 48 hrs: _____ mL Plasma / 48 hrs: _____ mL Platelets / 48 hrs: _____ mL Albumin / 48 hrs: _____ mL Hetastarch / 48 hrs: _____ mL Other (_____): _____ mL Other (_____): _____ mL **Total of B: _____ mL**
C. Total Volume of crystalloid transfused/1 hour	Saline: _____ mL Dextrose in Water: _____ mL Ringer's Lactate: _____ mL Other (_____): _____ mL Other (_____): _____ mL **Total of C: _____ mL**

Islets Lot Number: _____

HEMODILUTION WORKSHEET (CONTINUED)

D. Determination of Suitability	
B _____ mL + C _____ mL = _____mL A _____ mL + B _____ mL + C _____ mL = _____ mL	1. Is B + C > PV? (circle one) Yes No 2. Is A + B + C > BV? (circle one) Yes No *If the answers to both 1 and 2 are NO, then test sample.* *If the answer to either 1 or 2 is YES, then reject donor.*

Test blood sample? (circle one) Yes No

Donor Suitable? (circle one) Yes No

Recorded by :_____ Date: _____

Reviewed by :_____ Date: _____