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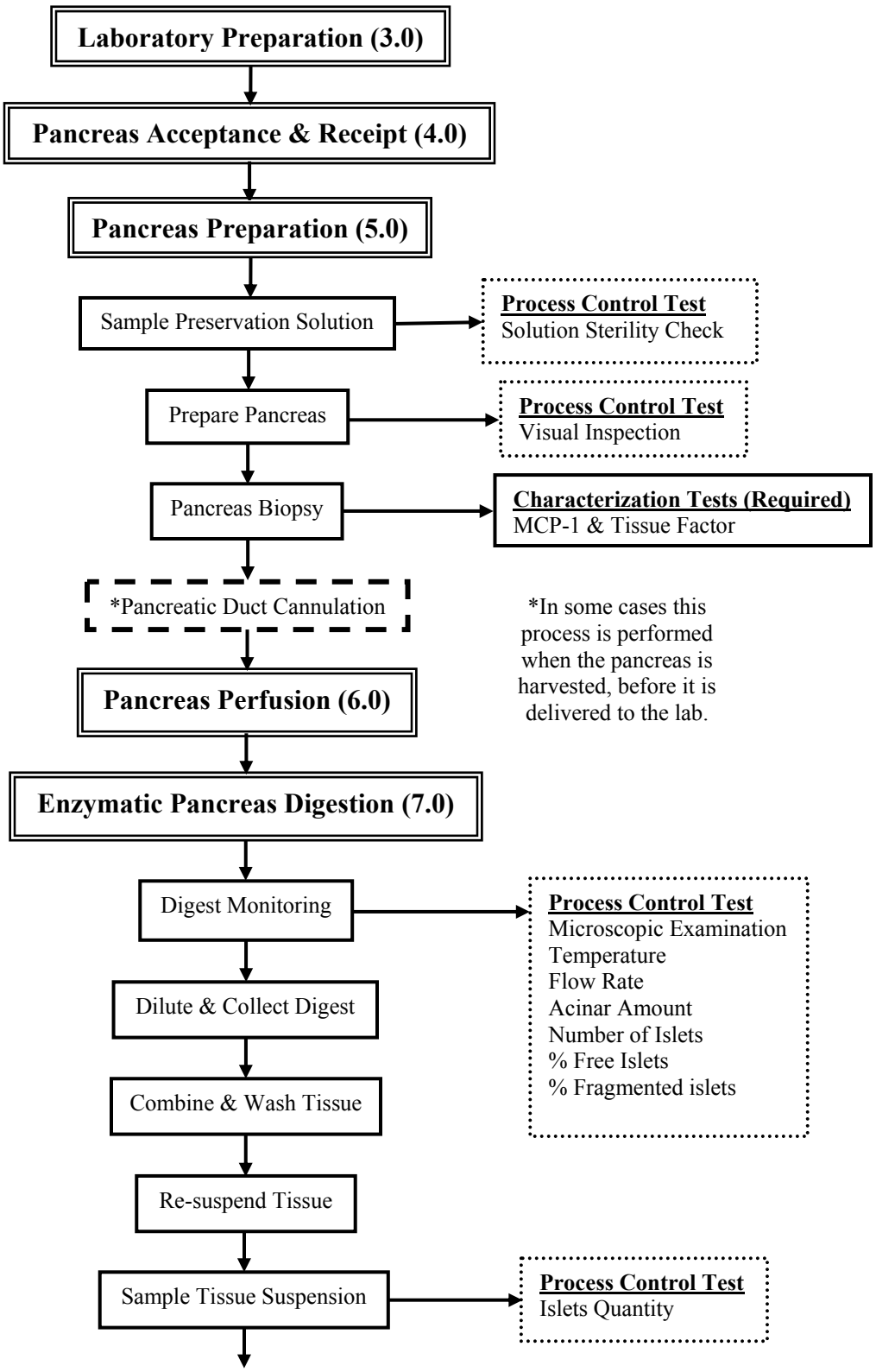
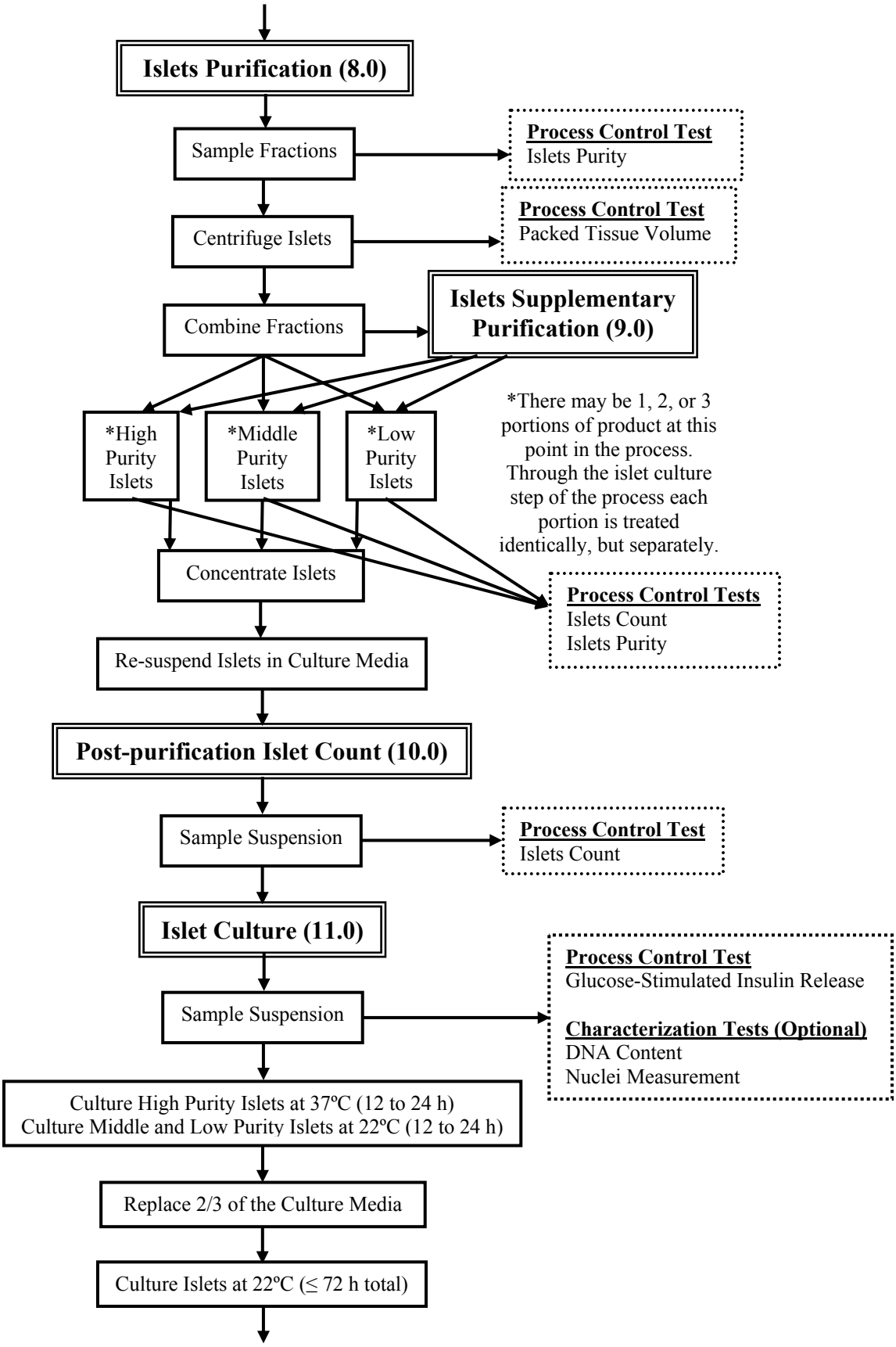
Changes to this Master Production Batch Record must be proposed to the Chief, Regulatory Affairs, DAIT, NIAID, NIH, and approved by all the original signatories, or their successors, before implementation.

2.0 FLOWCHART AND SAMPLING TABLE

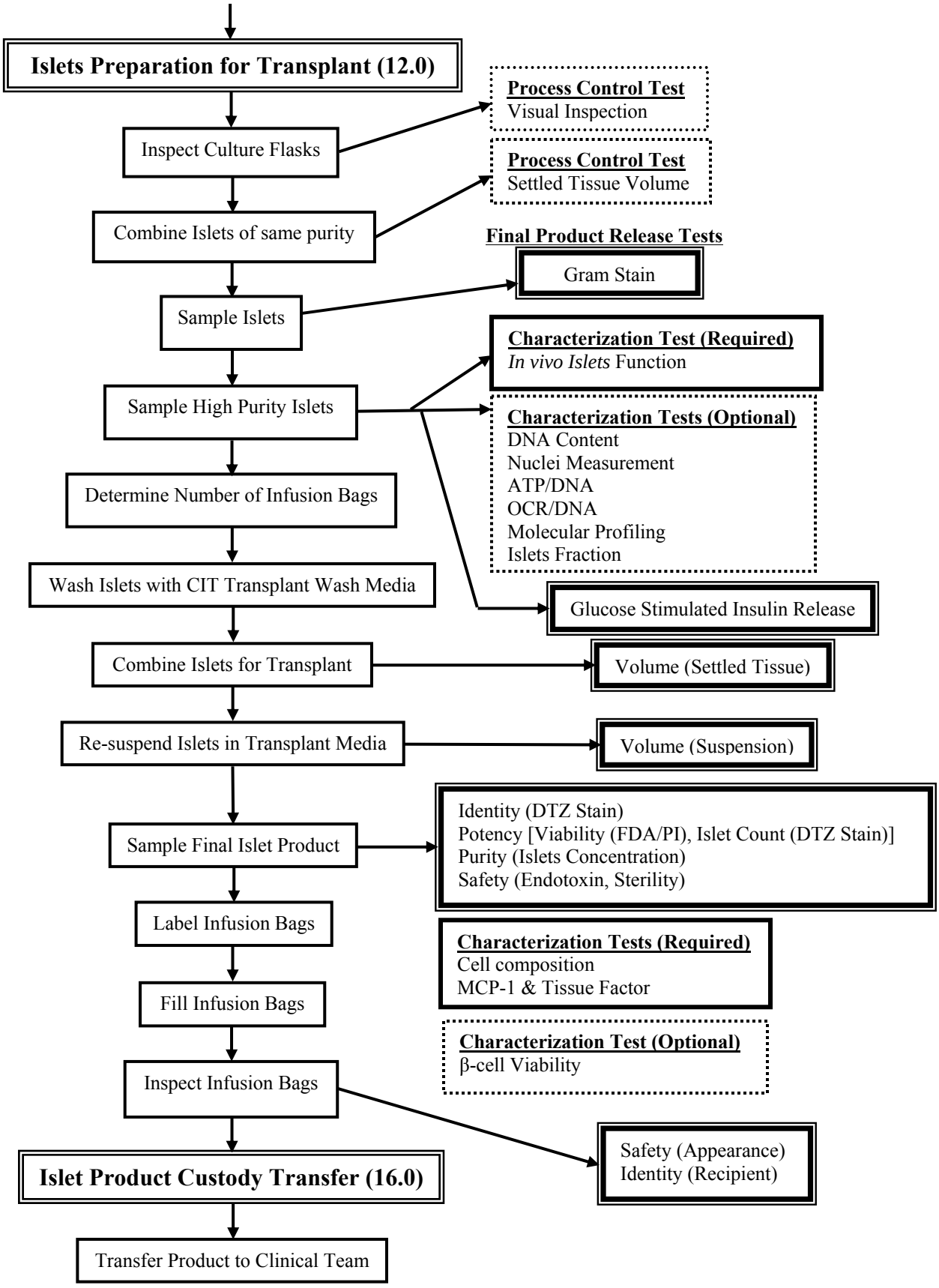
2.1 Production Process Flowchart (MPBR)



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
7.1.3	Pancreas Digest, ≤ 1-2 mL periodically	Acinar Amount, # of Islets, % Free Islets, % Fragmented
7.5.1	Diluted Pancreas Digest, 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, 100 µL	Islets Count
9.1.3.6 or 9.2.21	Purification Fractions, 0.5 mL/each of 12 fractions & 0.5 mL of W1 fraction	Islets Purity (%)
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 CERTIFICATE OF ANALYSIS	
11.1	Suspension, 400 IEQ, High Purity Islets	Glucose Stimulated Insulin Release
	INTERIM & FINAL CERTIFICATES OF ANALYSIS	
12.11.6	Supernatant above cultured islets, volume according to institution's procedure, High, Middle, Low Purity Levels	Gram Stain
12.18.1	Combined Islets, All Measured, High, Middle, Low Purity Levels	Settled Tissue Volume
12.18.2	Suspension, 2 X 100 µL/Each Final Product T-75 Flask	Islets Identity, Quantity, Concentration
12.18.2	Suspension, 100 IEQ/Each Final Product T-75 Flask	Viability
12.18.2	Supernatant, 1 mL/Each Final Product T-75 Flask	Endotoxin
	FINAL CERTIFICATE OF ANALYSIS	
12.14	Suspension, 400 IEQ, High Purity Islets	Glucose Stimulated Insulin Release
12.18.2	Suspension, 3 mL/Each Final Product T-75 Flask	Sterility, 21 CFR 610.12
	REQUIRED PRODUCT CHARACTERIZATION TESTS FOR INFORMATION ONLY	
5.7	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
12.18.2	Suspension, 1,000 IEQ/Each Final Product T-75 Flask	Cell Composition
12.18.2	Suspension, 500 to 1,000 IEQ/Each Final Product T-75 Flask	MCP-1 and Tissue Factor
	OPTIONAL PRODUCT CHARACTERIZATION TESTS FOR INFORMATION ONLY	
11.1	Suspension, 3 X 100 IEQ, High Purity Islets	DNA Content
11.1	Suspension, 3 X 100 IEQ, High Purity Islets	Nuclei Measurement
12.14	Suspension, 3 X 100 IEQ, High Purity Islets	DNA Content
12.14	Suspension, 3 X 100 IEQ, High Purity Islets	Nuclei Measurement
12.14	Suspension, 500 IEQ, High Purity Islets	ATP/DNA Ratio
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
12.18.2	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		

RAW MATERIALS AND PURCHASED REAGENTS (Continued)

RAW MATERIAL AND PURCHASED REAGENTS	CATALOG NUMBER	SUPPLIER	LOT NUMBER	EXPIRATION DATE
12. Insulin Human Injection USP, Recombinant				
13a. Collagenase NB 1 GMP Grade	17452	SERVA/Nordmark		
13b. Neutral Protease NB GMP Grade	30303	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. OptiPrep	1114542	Nycomed		
17. Trimming Solution				
18. Human Pancreas, Deceased Donor	See Section 4.2 and SOP 3108			
19. PentaStarch, 10% Solution				
20. Povidone Iodine USP, 10%				
21. Pulmozyme (dornase alpha), 2.5 mL/vial, 1 mg/mL	NDC No. 50242-100-40	Genentech		
22. RPMI 1640 with L-Glutamine				
23. Sterile Water for Injection USP				
24. Viaspan (UW Solution)	1000-46-06	Duramed Pharmaceuticals		
25. Biocoll Separating Solution, Density 1.100	L6155	Biochrome AG/ Cedarlane		
26. Calcium Chloride USP (Dihydrate) ($CaCl_2$ 2 H_2O)				
27. Cefazolin Sodium USP				
28. Ricordi Infusion Bag	IB-01	Biorep Technologies, Inc.		

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 associated form. File the form(s), or copy, 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*			
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, 8, and 9 are required by FDA regulation.

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 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 Hanks 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 Cannulation and Biopsy

The pancreas will be perfused in a controlled manner, using separate cannulae for the head and tail. Cut the pancreas to separate the head and tail, and cannulate the main pancreatic duct with 16 to 22 gauge cannula, 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:** _____

5.7 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 the sample and ship it according to instructions in the Laboratory Manual. Report the results in Section 17.3.

Performed by: _____ **Date:** _____

5.8 Pancreas Weight

Taring the containers before each step, weigh the pancreas before perfusion and the cannulae, sutures and trimmed tissue after perfusion. Record the data in the table below, and calculate the Trimmed Pancreas Weight.

A. Cannulated Pancreas Weight (before Perfusion)	g
B. Weight of Cannulae, Sutures, and Trimmed Tissue	g
C. Trimmed Pancreas Weight (C = A – B)	g
D. Undigested Tissue Weight (Section 7.3)	g
E. Digested Tissue Weight (E = C – D)	g

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Comments on pancreas receipt and preparation: _____

_____

_____

_____

_____

_____

Verified by: _____ **Date:** _____

5.9 CIT Enzyme Solution Preparation **(Cross out lines not used.)**

5.9.1 Prepare the CIT Enzyme Solution – SERVA Enzymes according to DAIT SOP 3106, B11, and file the record of preparation with this Batch Record.

OR

5.9.2 Prepare the CIT Enzyme Solution – Vitacyte Enzymes according to DAIT SOP 3106, B13, and file the record of preparation with this Batch Record.

5.9.3 CIT Enzyme Solution (SERVA or VitaCyte Enzymes)

Collagenase Activity actually used: _____ (Specify Units)

Neutral Protease Activity actually used: _____ Units

Thermolysin Activity actually used: _____Fluorescence Units

CIT Enzyme Solution Volume actually used: _____ mL

Verified 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 stopcock and 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.
- 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 and Temperatures

			Start Time:		
Desired Temp. (°C)	**Desired Pressure (mm Hg)**	**Time (min)**	**Head Observed Pressure (mm Hg)**	**Tail Observed Pressure (mm Hg)**	**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 perfusion. Save all removed non-pancreatic tissue in the container from Section 5.9.

Post-perfusion trim finish time: _____

Performed by: _____ **Date:** _____

6.3 Trimmed Pancreas Weight

After perfusion is completed, weigh all removed tissue, suture material, cannulae, etc. in the container from Section 5.9. Record this weight in the table in Section 5.9, and calculate the Trimmed Pancreas Weight.

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 seven to eleven similar sized pieces of 1 to 1.5 inches length and place the pieces in a Ricordi digestion chamber. Place 6 to 8 marbles (See Section 7.0) 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, complete the following:

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

7.0 ENZYMATIC PANCREAS DIGESTION

SERVA Enzymes Pancreas Digestion Parameters

CANNULATED PANCREAS WEIGHT (g) (SECTION 5.9)	CHAMBER SIZE (mL)	CIT ENZYME SOLUTION VOLUME (mL)	MARBLE NUMBER	DIGESTION FLOW RATE	DILUTION FLOW RATE (mL/min)
< 100	600	350	6 – 8	First 5 minutes: 210 – 250 mL/min	210 – 250
100 – 125	600	400			
126 – 150	600	450		After first 5 min, 90 – 130 mL/min	
> 150	600, or divide the pancreas into two portions and perform two digestions.	500			

VitaCyte enzymes use one vial of each enzyme in 350 mL of CIT Enzyme Solution.

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/ampule,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 7.1.3. 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 7.1.3.

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	Ranges for Switching from Digestion to Dilution*
Amount of acinar 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).**

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: _____

7.1.4 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 7.1.3 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.
- 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 in Section 7.1.3.
- 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 in Section 7.1.3, and calculate the Total Dilution Time.

Verified by: _____ **Date:** _____

7.3 Remove the undigested pancreas material from the digestion chamber, weigh it, record the weight below, and in the table in Section 5.9. Calculate the weight of digested tissue in the table in Section 5.9.

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: _____ g

Estimate of undigested pancreatic tissue: _____%

Estimate of 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 with a wide mouth 10 mL pipet 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, 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

7.4.8 Bring the total re-suspended islets to 200 to 250 g or mL with CIT Purification Solution. Ensure that there are no clumps (dissolve if necessary). Record the volume or weight.

Total Suspension Volume or Weight: _____ mL or g (Circle One)

Verified by: _____ Date: _____

7.5 Pre-purification Islets Count

7.5.1 Re-suspend tissue evenly. Take one 100 µL sample for one pre-purification islets count.

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

Sample volume: _____ µL Total volume: _____ mL Dilution factor: _____

Pre-purification Islets Count & Calculations

Islets Diameter (µm)	Count	Factor	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**	
% Trapped Islets		**Sample Total IEQ**	
% Fragmented Islets		**Suspension Total IEQ**	
Technician's Initials			

Additional records are necessary if magnification calibration factors are used for individual microscopes.

Comments: _____

_____

_____

_____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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.

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, 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. 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. 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: There will be 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.

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 for each conical tube 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 this purification process did not yield a sufficient number of, and/or sufficiently pure, islets for transplant, and there is a substantial number of impure islets in the remaining tissue, follow one of the procedures in Section 9.0, for Supplementary Purification.

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 Bring the volume of the Supplementary Purification Islets 250 mL conical tube to 100 mL with CMRL 1066, Supplemented.

8.4.3 Take a 100 µL sample for counting. Dilute the Supplementary Purification Islets to approximately 250 mL with CMRL 1066, Supplemented. Lay the tube on its side at 2ºC to 8ºC while counts are performed.

Verified by: _____ **Date:** _____

8.4.4 Count islets according to the institution's procedure in the Supplementary Purification Islets sample and record counts in the table below and attach spreadsheet. Indicate 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, proceed to Section 9.0.

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				
Total				
% Trapped				
Technicians' Initials				

Islets Lot Number: _____

Comments on Supplementary Purification: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Decided by: _____ **Date:** _____

8.5 Tissue Preparation for Re-purification

8.5.1 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.

8.5.2 Bring the Supplementary Purification Islets to approximately 250 mL with CIT Purification Solution and gently re-suspend them. Seal the tube and place it at 2ºC to 8ºC for 30 to 50 minutes while preparation for Supplementary Purification occurs. Then proceed to the Supplementary Purification.

Verified by: _____ **Date:** _____

9.0 ISLETS SUPPLEMENTARY PURIFICATION

If there is tissue insufficiently purified by the procedure described in Section 8.0, the tissue may be re-purified by the OptiPrep Supplementary Purification Procedure, Section 9.1, or the Biocoll Supplementary Purification Procedure, Section 9.2.

Describe the supplementary purification procedure to be used.

_____

_____

_____

Approved by: _____ **Date:** _____
Site Principal Investigator, or Designee

9.1 OptiPrep Supplementary Purification Procedure

9.1.1 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:** _____

9.1.2 COBE 2991 Procedure – Gradient and Tissue Loading

9.1.2.1 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.

9.1.2.2 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.

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

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

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

9.1.2.6 Pour 120 mL of the High Density Gradient into the left chamber of the gradient maker.

9.1.2.7 Pump the bottom layer into the COBE Bag then stop the pump.

9.1.2.8 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.

9.1.2.9 Begin loading the continuous density gradient into COBE bag.

- 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)
- 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.**

9.1.2.10 Start the COBE and ensure the centrifuge speed is 1800 to 2000 rpm.

Centrifuge Speed: _____ rpm

Recorded by: _____ **Date:** _____

9.1.2.11 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.

9.1.2.12 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.**

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

9.1.2.14 Load the Supplementary Purification Islets (Section 8.5) with the pump at a setting of 20 mL/min. Gently swirl the beaker to keep the tissue well suspended during the loading.

9.1.2.15 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.

9.1.2.16 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.

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

9.1.2.18 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.

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.**

9.1.2.19 Clamp the green line and allow the COBE to spin for 3 minutes. Record data on the Data Log for the Re-purification COBE run, below.

Verified by: _____ **Date:** _____

9.1.3 COBE 2991 Procedure – Tissue Collection

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

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

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

9.1.3.4 Collect the first 150 mL of effluent into the conical tube labeled "W1" (waste) and 12 X 25 mL fractions into the numbered conical tubes each pre-filled with 225 mL CMRL 1066, Supplemented, as described on the Purification Data Log for each respective COBE run.

9.1.3.5 Once the fractions are collected, stop the COBE and discard the COBE bag and tubing.

9.1.3.6 To evaluate each COBE fraction quickly, gently but thoroughly mix each fraction from step 9.1.3.4, 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 35 mm dish.

9.1.3.7 Stain each sample with dithizone according to the institution's procedure and observe for islets. Record observations on the Re-purification Data Log.

9.1.3.8 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 Re-purification Data Log. Discard the supernatant.

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 D: This is the disposition for each conical tube as defined in the column header.

OptiPrep Supplementary Purification Data Log

Layer	Medium	Amount
Capping Layer	CIT Cold Storage 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 Cold Storage 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, D: Discard (Circle One)
W	0	150				H M L D
1	225	25				H M L D
2	225	25				H M L D
3	225	25				H M L D
4	225	25				H M L D
5	225	25				H M L D
6	225	25				H M L D
7	225	25				H M L D
8	225	25				H M L D
9	225	25				H M L D
10	225	25				H M L D
11	225	25				H M L D
12	225	25				H M L D
Bag	0	95				D

Comments on supplementary purification: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

9.1.4 Combine fractions with purity of 30% or greater with the complimentary fractions from Section 8.3.10, and record the disposition of each fraction in the OptiPrep Supplementary Purification Data Log in Section 9.1.3.8. Discard fractions < 30% pure.

NOTE: At this point there will be one 250 mL conical tube for each Purity Level (High, Middle, Low Purity Islets).

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

9.2 Biocoll Supplementary Purification Procedure

9.2.1 Prepare the tissue by adding 150 mL of UW Solution to the Supplementary Purification Islets from Section 8.3.10.

Note: When using this Biocoll Supplementary Purification procedure, up to 45mL of packed tissue volume can be loaded on the COBE for each run. It is very important not to overload the COBE.

Note: The volume of UW Solution for each run remains constant, regardless of the volume of the packed tissue.

Volume of UW Solution used for each COBE run: _____ mL

Total Packed Tissue Volume: _____ mL

Number of COBE runs: _____

Packed Tissue Volume prepared for each COBE run: _____ mL

Performed by: _____ **Date:** _____

9.2.2 Incubate the tissue in UW solution for 30 minutes on ice or in the cold room, using the Maxi-rotator (or mix the tissue in the tube by swirling every 5 minutes).

Performed by: _____ **Date:** _____

9.2.3 Preparation of Biocoll Heavy (49% ficoll/51% UW Solution mixed) and Light (30% ficoll/70% UW Solution mixed) density gradients:

9.2.3.1 Pipette 66.3 mL of UW Solution into one sterile bottle. Label this Bottle with "**Heavy Gradient**," Islets Lot Number, date and time of preparation, and initials of preparer.

9.2.3.2 Pipette 98.0 mL of UW Solution into another sterile bottle. Label this Bottle with "**Light Gradient**," Islets Lot Number, date and time of preparation, and initials of preparer.

9.2.3.3 Pipette 63.7 mL of 1.10 g/mL Ficoll Gradient Solution into the bottle labeled "**Heavy Gradient**" and quickly swirl bottle to mix properly.

9.2.3.4 Pipette 42.0 mL of 1.10 g/mL Ficoll Gradient Solution into the bottle labeled "**Light Gradient**" and quickly swirl bottle to mix.

Performed by: _____ **Date:** _____

9.2.4 Set the COBE at 1500 rpm and Superout at 0. Press Start to start the COBE.

9.2.5 Add 110 mL of 1.10 g/mL Biocoll Gradient Solution to the first (front) beaker and start the peristaltic pump on the maximum setting.

9.2.6 After all the Biocoll Gradient Solution is loaded onto the COBE, press Superout, turn off the pump, unclamp the pump head, and turn Superout to 100.

9.2.7 When the Biocoll Gradient Solution reaches the beaker, quickly re-clamp the pump head. Stop the COBE and turn Superout back to 0. Change the COBE speed to 3,000 rpm. All air should now be out of the system.

9.2.8 Add 130 mL of Heavy Gradient to the front beaker. Unclamp the line between the beakers briefly and re-clamp to get all air out.

9.2.9 Add 140 mL of Light Gradient to the second (rear) beaker.

9.2.10 Turn the pump speed down to 20 mL/min on the peristaltic pump and turn magnetic stirrer on the lowest setting. Start the COBE. Start pump. Unclamp the line between the beakers.

9.2.11 When nearly all the Biocoll is loaded onto the COBE, tilt the magnetic stirrer forward to ensure all Biocoll is loaded. Before the last bit of Ficoll is loaded, stop the stirrer and begin to slowly add the suspended islets to the front beaker.

9.2.12 When all tissue has been added, rinse the conical which contained the suspended islets with 50 mL of HBSS, 1X, and add this volume to the front beaker.

9.2.13 When everything has been loaded onto the COBE, clamp the tubing above the bag, press Super-Out (set at 0), turn off the pump and unclamp the pump head.

9.2.14 SLOWLY, unclamp the clamp above the COBE bag and start the timer.

Performed by: _____ **Date:** _____

9.2.15 Centrifuge for 5 minutes.

9.2.16 Prepare collection rod and line for fraction collection.

9.2.17 Prepare 12 X 250 mL conical tubes. Label them #1 through #12. Leave Tube #1 empty, and pre-fill Tubes #2 through #12 with 220 mL each of CMRL 1066, Supplemented.

Performed by: _____ **Date:** _____

9.2.18 After 5 minutes, slowly adjust the Superout up to 100 and begin collecting tissue into the conical tubes.

9.2.19 Collect 150 mL of effluent in Tube #1. Collect 30 mL of effluent in Tubes #2 through #12, to a total volume of 250 mL in each tube.

9.2.20 When all effluent has been collected, press Stop on the COBE.

Performed by: _____ **Date:** _____

9.2.21 To evaluate each COBE fraction quickly, gently but thoroughly mix each fraction from Section 9.2.19, then quickly transfer a 0.5 mL sample to one well of a 12-well microtiter plate.

9.2.22 Stain each sample with dithizone according to the institution's procedure and observe for islets. Record observations on the Biocoll Supplementary Purification Data Log for each COBE run, below.

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

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 D: This is the disposition for each conical tube as defined in the column header.

Biocoll Supplementary Purification Data Log, COBE Run #1:

#	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, D: Discard (Circle One)
1	0	150				H M L D
2	220	30				H M L D
3	220	30				H M L D
4	220	30				H M L D
5	220	30				H M L D
6	220	30				H M L D
7	220	30				H M L D
8	220	30				H M L D
9	220	30				H M L D
10	220	30				H M L D
11	220	30				H M L D
12	220	30				H M L D
Centrifuge Start Time				**Centrifuge Stop Time**		

Comments on purification: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

9.2.24 Repeat all steps for each COBE run.

Biocoll Supplementary Purification Data Log, COBE Run #2:

#	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, D: Discard (Circle One)
1	0	150				H M L D
2	220	30				H M L D
3	220	30				H M L D
4	220	30				H M L D
5	220	30				H M L D
6	220	30				H M L D
7	220	30				H M L D
8	220	30				H M L D
9	220	30				H M L D
10	220	30				H M L D
11	220	30				H M L D
12	220	30				H M L D
Centrifuge Start Time				**Centrifuge Stop Time**		

Comments on purification: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

9.2.25 Prepare three 250 mL conical tubes by adding 100 mL of CMRL 1066, Supplemented, to each and labeling them "High Purity," "Middle Purity," and "Low Purity."

9.2.26 Combine the islets fractions by transferring the pellets with 10 mL pipets into the three labeled 250 mL conical tubes according to their purity level: High Purity (≥ 70%), Middle Purity (69% to 40%), and Low Purity (39% to 30%). Discard fractions < 30% pure. 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.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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 has settled, remove the supernatant and re-suspend the final tissue in 100 to 200 mL of CIT Culture Media in a T-75 flask labeled with Lot Number, isolation date and Purity Level identification.

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 or attach spreadsheet, and calculate the Total Islet Number (IPN) and Total IEQ. The contents of these tubes 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												

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 trimmed pancreas (Section 5.8)				
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 14.5 and on the Interim Certificate 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 20,000 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 20,000 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 (10 mL/flask)	CIT Culture Media Volume (Section 10.2)	CIT Culture Media Added or Removed
Example 1 – High Purity	14	140 mL	100 mL	+ 40 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.

Date and time High Purity Islets flasks placed in 37ºC incubator: _____

Record this date and time in the table in Section 12.5.

Performed by: _____ **Date:** _____

The islets' culture must end (Section 12.5) between 36 and 72 hours of the start time. Calculate these dates and times.

Date and time of minimum culture: _____

Date and time of maximum culture: _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Notify the Site Principal Investigator, or designee, of these 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.

Date and time Middle and Low Purity Islets flasks placed in 22ºC incubator: _____

Record this date and time in the table in Section 12.5.

Performed by: _____ **Date:** _____

11.8 Media Change

11.8.1 After 12 to 24 hours remove all the flasks from the incubators and record the date(s) and time(s) that each purity level of islet product is removed from the incubator(s) in the table in Section 12.5.

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:** _____

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: _____

Islets Lot Number: _____

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.

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 and the Biological Safety Cabinets (BSCs) according to the institution's procedure.

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 Remove all the islet product flasks from the incubator(s) and record the date and time in the table below.

		High Purity Flasks	Middle Purity Flasks	Low Purity Flasks	Recorded by	Verified by
1st Culture Start	Date					
	Time					
1st Culture Stop	Date					
	Time					
1st Culture Time (Hours)						
2nd Culture Start	Date					
	Time					
2nd Culture Stop	Date					
	Time					
2nd Culture Time (Hours)						
Total Culture Time (Hours)						

Is the date and time of the 2nd Culture Stop within the dates and times of minimum and maximum culture calculated in Section 11.6?

Yes No (Circle One)

If it is not, immediately notify the Principal Investigator, or designee.

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

If the Site Principal Investigator, or designee, is notified, 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:** _____

Islets Lot Number: _____

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 High, Middle and Low Purity Islets flasks

12.10.1 Allow the tissue to settle in the corner of the High Purity T-75 flask for 3 to 5 minutes.

12.10.2 Gently aspirate the tissue into a 10 mL glass pipet.

12.10.3 Allow the tissue to settle in the pipet while holding it vertically for 3 to 5 minutes.

12.10.4 Estimate the Settled Tissue Volume from the pipet and record data on the table in Section 12.12.

12.10.5 Re-suspend the tissue in the T-75 flask.

12.10.6 Repeat steps 12.10.1 to 12.10.5 for the Middle and Low Purity islets flasks.

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 100 to 200 mL in CIT Transplant Media after the second wash.

12.11.6 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.

Verified by: _____ **Date:** _____

12.12 The Final Product composition is based on the Settled Tissue Volume and the Gram Stain results recorded in the table, below. Determine and record which flasks to combine, 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)	Gram Stain Result*	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			

*The 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: _____

Deviation Number: _____

12.13 Take two 100 µL samples of each purity level and perform counts and calculations. Attach spreadsheet 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												

Islets Lot Number: _____

Post-culture Islets Calculations

	High Purity	Middle Purity	Low Purity	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 trimmed pancreas (Section 5.8)				
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 Certificate of Analysis and Section 20.0, as required.

SAMPLE QUANTITY	REQUIRED FOR CERTIFICATE OF ANALYSIS	SAMPLE REMOVED (mL)
Suspension, 400 IEQ	Post-culture Glucose Stimulated Insulin Release	
	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, 500 IEQ	ATP/DNA	
Suspension, 3 X 100 IEQ	Nuclei Measurement*	
Suspension, 5,000 IEQ	OCR/DNA*	
Suspension, 5,000 IEQ	Molecular Profiling*	
Suspension, 500 IEQ	Islets Fraction*	
	Total Volume Removed from High Purity Islets Suspension	
	Volume of High Purity Islets Suspension Before Sampling (Section 12.13)	
	Remaining Volume of High Purity Islets Suspension	

*Note: Follow instructions in the Laboratory Manual for preparation and shipment of samples.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.15 Label with at least 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" or "Human Islet Product"
- Islets Lot Number
- Donor Identification (UNOS or DDD) Number
- Donor Blood Type
- Total IEQ in Bag
- "Bag X of Y"
- Recipient Name
- Recipient Medical Record Number
- 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
- "Contains Heparin, Total Units: _____"

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

Make three identical labels for each bag. Place one on the bag, one in the space below, or on the back of this page, if necessary, and send one with the product bag with an instruction to affix it to the recipient's medical record chart.

Labeled by: _____ **Date:** _____

Checked by: _____ **Date:** _____

12.16 Combine the Islets Suspensions

12.16.1 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. The volume in the single T-75 flask after combination should be 100 mL. Combine by settling and removing supernatant as in Section 12.11, above, as necessary.

Final Volume in one T-75 flask: _____ mL

Verified by: _____ **Date:** _____

12.16.2 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. The volume in each T-75 flask after combination should be 100 mL. Combine by settling and removing supernatant as in Section 12.11, above, as necessary.

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

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

Verified by: _____ **Date:** _____

12.16.3 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. The volume in each T-75 flasks after combination should be 100 mL. Combine by settling and removing supernatant as in Section 12.11, above, as necessary.

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:** _____

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.18 Sampling and Testing of Final Product containers

12.18.1 Estimate the Tissue Volume in the final product containers

- Allow the tissue to settle in the corner of T-75 flask #1 for 3 to 5 minutes.
- Gently aspirate 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.
- Re-suspend the tissue in the T-75 flask.
- Repeat these steps for other T-75 flasks.

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

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

Verified by: _____ **Date:** _____

12.18.2 Sample the suspension(s) in the final product T-75 flask(s) [Sample the supernatant(s) for the Endotoxin test only] 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 and 20.0, and on the Certificates of Analysis, as indicated.

Note: **Samples for Islets Identity and Quantity are not taken here for purity levels (High, Middle, and/or Low) that have not been combined with other purity levels for transplant. Results of the Post-culture Identity and Counts samples taken in Section 12.13, are used for the Certificates of Analysis.**

SAMPLE TYPE & QUANTITY	TESTS	SAMPLE REMOVED (mL)			TESTING LAB
Required for Certificates of Analysis		T-75 #1	T-75 #2	T-75 #3	
2 X 100 µL/Each Final Product T-75 Flask	Islet Identity and Quantity	(A)			
100 IEQ/Each Final Product T-75 Flask	Viability	(B)			
1 mL of Supernatant/Each Final Product T-75 Flask	Endotoxins	(C)			
Required for Certificate of Analysis					
3 mL/Each Final Product T-75 Flask	Sterility 21 CFR 610.12	(D)			
Required Product Characterization, For Information Only					
1,000 IEQ/Each Final Product T-75 Flask	Cell Composition	(E)			University of Miami*
500 to 1,000 IEQ/Each Final Product T-75 Flask	MCP-1 & Tissue Factor	(F)			Uppsala University Hospital, Sweden*
Optional Product Characterization, For Information Only					
2,000 IEQ/Each Final Product T-75 Flask	β-cell Viability	(G)			
Total Volume Removed (mL) (H) = Σ (A) through (G)		(H)			
Remaining Suspension Volume (mL) (J) = 100 mL – (H) Required in Section 14.3, below		(J)			
Sampled by:	Date:	Verified by:			Date:

*Note: Follow instructions in the Laboratory Manual for preparation and shipment of samples for Cell Composition, and for MCP-1 and Tissue Factor.

Islets Lot Number: _____

12.19 Perform counts and calculations (Portions of product that are not combined with other portions are not counted again. Their values from Section 12.13 are used.)

Final Product Islets Counts

	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			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												

Final Product Islets Calculations

	Final Product T-75 Flask #1	Final Product T-75 Flask #2	Final Product T-75 Flask #3
Final Product IPN			
Final Product IEQ			
Comments			

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

Total Final Product IEQ: _____

Total IEQ/g of trimmed pancreas (Section 5.8): _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.20 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:** _____

12.21 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.

****

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

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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, 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,	MEDIA	PRESENT? YES	PRESENT? NO
5.4	B01	CIT Digestion Solution		
5.9	B11	CIT Enzyme Solution		
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 (Supplementary Purification, if 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	PRESENT? 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	PRESENT? NO
12.11.6	Gram Stain		
12.18.2	Final Product Viability		
12.18.2	Final Product Endotoxin		
12.18.2	Pre-culture Sample Glucose Stimulated Insulin Release		

Verified by: _____ **Date:** _____

13.4 Deviation and Discrepancy Investigation Reports

Ensure that all Deviation and Discrepancy Reports related to this Batch Record are attached and approved according to the institution's procedures.

Verified by: _____ **Date:** _____

14.0 Pre-transplant Test Results

14.1 From the tests conducted on the samples from Section 12.18, enter the results in the table below.

FINAL PRODUCT T-75 FLASKS	#1	#2	#3	TOTAL
Settled Tissue Volume (mL)*				
Suspension Volume (mL)*				
Islets Identity (Yes/No)*				
Islets Equivalents (IEQ)				
Islets Quantity (IEQ/kg)* (Calculate in Section 14.2, below)				
Islets Concentration (IEQ/mL Tissue)* (Calculate in Section 14.3, below)				
Viability (%)*				
Endotoxins Concentration (EU/mL)				
Endotoxins (EU/kg Recipient Weight)* (Calculate in Section 14.4, below)				

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

14.2 From the Islets Equivalents in Section 14.1, above, and the Recipient Body Weight (kg) in Section 12.3, above, calculate the Islets Quantity (IEQ/kg) in each T-75 Flask and their sum, and record the results in the table above:

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

FINAL PRODUCT T-75 FLASKS	Islets Equivalents (IEQ)	Recipient body Weight (kg)	Islets Quantity (IEQ/kg)
1			
2			
3			

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

Verified by: _____ **Date:** _____

14.3 From the Islets Equivalents and the Settled Tissue Volumes in Section 14.1, above, calculate the Islets Concentration in each T-75 Flask and their sum, and record the results in the table above:

Islets Equivalents (IEQ) = Islets Concentration (IEQ/mL Tissue)
Settled Tissue Volume (mL)

FINAL PRODUCT T-75 FLASKS	Islets Equivalents (IEQ)	Settled Tissue Volume (mL)	Islets Concentration (IEQ/mL)
1			
2			
3			

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

Verified by: _____ **Date:** _____

14.4 From the Endotoxins Concentration (EU/mL) in Section 14.1, the Suspension Volume (mL) in Section 12.18.2 (J), and the Recipient Body Weight (kg) in Section 12.3, above, 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, and record the results in the table above:

Endotoxins Concentration (EU/mL) X Suspension Volume (mL) = EU/kg Recipient Weight
Recipient Body Weight (kg)

FINAL PRODUCT T-75 FLASKS	Endotoxins Concentration (EU/mL)	Suspension Volume (mL)	Recipient Body Weight (kg)	EU/kg
1				
2				
3				

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

Verified by: _____ **Date:** _____

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

HIGH PURITY LEVEL	INSULIN CONCENTRATIONS		
	LOW GLUCOSE	HIGH GLUCOSE	STIMULATION INDEX
PRE-CULTURE SAMPLE (SECTION 11.1)			

Report this result on the Interim Certificate of Analysis.

Recorded 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 Notify the clinical team that the islets are ready for transplant.

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

16.2 Custody Transfer Record

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

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

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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 12.18.2) 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
#1		
#2		
#3		

If there is a positive result, 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

HIGH PURITY LEVEL	INSULIN CONCENTRATIONS		
	LOW GLUCOSE	HIGH GLUCOSE	STIMULATION INDEX
POST-CULTURE SAMPLE (SECTION 12.14)			

Report this result on the Certificate of Analysis.

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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		
12.18.2	Final Product Sterility		

Verified by: _____ **Date:** _____

Islets Lot Number: _____

18.0 PRODUCT DISPOSITION

Was this product transplanted? Yes No (Circle one)

If this product was transplanted, give 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)
12.18.2	Cell Composition (Laser Scanning Cytometry & Immunofluorescence)	FPTF #1, β-cells: _____ % γ-cells: _____ % α-cells: _____ % PP-cells: _____ % FPTF #2, β-cells: _____ % γ-cells: _____ % α-cells: _____ % PP-cells: _____ % FPTF #3, β-cells: _____ % γ-cells: _____ % α-cells: _____ % PP-cells: _____ %
12.18.2	Final Product MCP-1	FPTF 1: _____ FPTF 2: _____ FPTF 3: _____
12.18.2	Final Product Tissue Factor	FPTF 1: _____ FPTF 2: _____ FPTF 3: _____

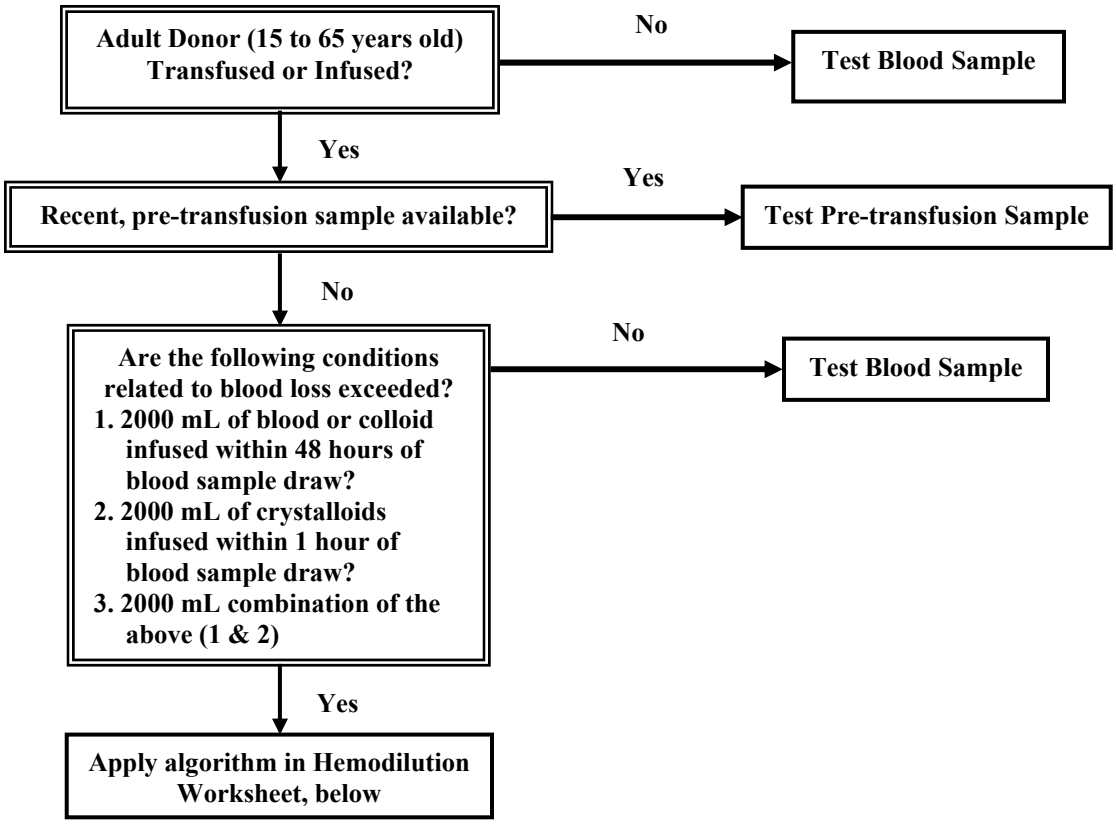
SAMPLES FROM MPBR SECTION	OPTIONAL PRODUCT CHARACTERIZATION	RESULT
11.1	Pre-culture DNA Content	High Purity Islets: _____ µg DNA
11.1	Pre-culture Nuclei Measurement	nuclei
12.14	Post-culture DNA Content	High Purity Islets: _____ µg DNA
12.14	Post-culture Nuclei Measurement	nuclei
12.14	ATP/DNA Ratio	
12.14	OCR/DNA	nmol O_2/min/mg DNA
12.14	Molecular Profiling	
12.14	Islet Fraction	
12.18.2	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: _____