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Note: Use this document only if "Islets Alone" are being manufactured.

11.0 ISLET CULTURE

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

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

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

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

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

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

Islets Lot Number: _____

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

Performed by: _____ **Date:** _____

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

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

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

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

Performed by: _____ **Date:** _____

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

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

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

Islets Lot Number: _____

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

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

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

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____

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

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

Performed by: _____ **Date:** _____

11.8 Media Change, 1st Culture Stop Date & Time

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

Performed by: _____ **Date:** _____

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

_____

_____

_____

_____

Inspected by: _____ **Date:** _____

Islets Lot Number: _____

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

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____

11.8.3 Equilibrate the CIT Culture Media at room temperature. Place each flask in the BSC, tilt each at a 45° 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 22℃, 95% air, and 5% carbon dioxide with the T-neck in the up position, and record the date(s) and time(s) that each purity level of islet product is placed in the incubator(s) in the table in Section 12.5 as the 2nd Culture Start Dates & Times.

Verified by: _____ **Date:** _____

12.0 ISLET PREPARATION FOR TRANSPLANT

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

Scheduled Islet Transplant Date: _____

Scheduled Islet Transplant Time: _____

Recorded by: _____ **Date:** _____

Islets Lot Number: _____

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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:** _____

Islets Lot Number: _____

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12.4 Before the scheduled transplant time:

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

Verified by: _____ **Date:** _____

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

Verified by: _____ **Date:** _____

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12.5 End of Culture

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

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

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

Yes No (Circle One)

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

Yes No (Circle One)

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

Name of Person notified: _____

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

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

_____

_____

_____

_____

_____

_____

Inspected by: _____ **Date:** _____

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

Name of Person notified: _____

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

12.7 Post-Culture Islet Recombination – High Purity Islets

12.7.1 Place all the High Purity Islets T-175 culture flasks at a 45° 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."

Islets Lot Number: _____

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 2°C to 8°C. Transfer the tissue to the "Islets – High Purity" T-75 flask.

Verified by: _____ **Date:** _____

12.8 Post-Culture Islet Recombination – Middle Purity Islets

12.8.1 Place all the Middle Purity Islets T-175 culture flasks at a 45° 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 2°C to 8°C. 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 45° 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.

Islets Lot Number: _____

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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 2°C to 8°C. Transfer the tissue to the "Islets – Low Purity" T-75 flask.

Verified by: _____ **Date:** _____

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

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

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.11 Wash Tissue in Preparation for Loading into Transplant Bags

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

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

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

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

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

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

12.12 The Final Product Composition Plan

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

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

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

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

Determined by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

Name of Person notified: _____

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

Islets Lot Number: _____

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

Post-culture Islets Counts

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

*Remaining Suspension Volume recorded in Section 12.11.5, above.

Islets Lot Number: _____

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Post-culture Islets Calculations

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

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

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Total Post-purification Islets Count: _____ IEQ

Total Post-culture Islets Count: _____ IEQ

Percent Change: _____%

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

Name of Person notified: _____

Notified by: _____

Date & Time Notified: _____

Islets Lot Number: _____

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12.14 Post-culture Sampling of High Purity Islets Suspension

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

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

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

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

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

Final Volume in one T-75 flask: _____ mL

Verified by: _____ **Date:** _____

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

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

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

Verified by: _____ **Date:** _____

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

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

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

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

Verified by: _____ **Date:** _____

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

12.17 Sampling and Testing of Final Product T-75 Flasks

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

Islets Lot Number: _____

Final Product Islets (Post-combination) Counts & Calculations

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

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

Total Final Product Islets Quantity: _____ IEQ

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

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

Islets Lot Number: _____

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

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

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

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

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

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

Sampled by: _____ **Date:** _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

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

Sampled by: _____ **Date:** _____

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

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

Verified by: _____ **Date:** _____

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

Performed by: _____ **Date:** _____

Islets Lot Number: _____

12.20 Calculation of Heparin Quantity Addition

****

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

****

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

Recipient Body Weight (Section 12.3): _____ kg

Heparin Concentration: _____ units/mL

Divide the heparin equally among the infusion bags.

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

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

Calculated by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

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

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

Labeled by: _____ **Date:** _____

Checked by: _____ **Date:** _____

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: _____

Islets Lot Number: _____

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.

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

Does each product infusion bag meet these criteria?

Bag #1: Yes No (Circle One)

Bag #2: Yes No (Circle One)

Bag #3: Yes No (Circle One)

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

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

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

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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13.0 CHECKLIST OF RECORDS FILED WITH THIS PRODUCTION BATCH RECORD

13.1 Required Solution and Media Preparation Records

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

Verified by: _____ **Date:** _____

13.2 Required Lists

MPBR SECTION	LISTS	PRESENT? YES	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 Endotoxins		
12.18.2	Pre-culture Sample Glucose Stimulated Insulin Release		

Verified by: _____ **Date:** _____

Islets Lot Number: _____

13.4. Supplementary Purification Records (if performed)

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

13.5 Additional Records

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

Verified by: _____ **Date:** _____

14.0 Pre-transplant Test Results

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

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

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

Islets Lot Number: _____

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

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

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

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

Verified by: _____ **Date:** _____

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

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

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

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

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

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

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

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

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

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

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

Verified by: _____ **Date:** _____

15.0 PRE-TRANSPLANT BATCH RECORD REVIEW AND INTERIM APPROVAL

After the completion of all activities and records of this manufacturing process to this point, and before transplant of this batch of islets, a qualified technician, and the Laboratory Director, Operations Manager, or designee, must review the Production Batch Record (both Part 1 and Part 2A) to verify that it is complete and accurate to this point.

We have reviewed the Production Batch Record (both Part 1 and Part 2A) and verified that it is complete and accurate to this point.

_____ Date: _____
Qualified Technician

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

Islets Lot Number: _____

16.0 ISLET PRODUCT CUSTODY TRANSFER

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

Name of person notified: _____

Notified by: _____

Date & Time Notified: _____, _____

16.2 Custody Transfer Record

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

Performed by: _____ **Date:** _____

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

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

Recipient Identity Correct? Yes No (Circle One)

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

17.0 POST-TRANSPLANT TEST RESULTS & REPORTS

17.1 Sterility Test Results

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

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

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

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

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

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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

Name of Physician Notified: _____

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

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

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

Report the Mean Index on the Certificate of Analysis.

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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

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

Verified by: _____ **Date:** _____

18.0 PRODUCT DISPOSITION

Was this product transplanted? Yes No (Circle one)

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

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

_____

_____

_____

_____

Recorded by: _____ **Date:** _____

19.0 POST-TRANSPLANT BATCH RECORD REVIEW AND FINAL APPROVAL

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

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

_____ Date: _____

Qualified Technician

_____ Date: _____

Laboratory Director, Operations Manager or designee

A qualified representative of the institution's Quality Unit must review the entire Production Batch Record (both Part 1 and Part 2A) and verify that it is complete and accurate

I have reviewed this entire Batch Production Record (both Part 1 and Part 2A) and verified that it is complete and accurate.

_____ Date: _____

Quality Unit Representative

Islets Lot Number: _____

20.0 Product Characterization Test Results (For Information Only)
Record results of the following tests in the table below. File copies of the raw data with this PBR. "FPTF" means Final Product T-75 Flask.

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

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

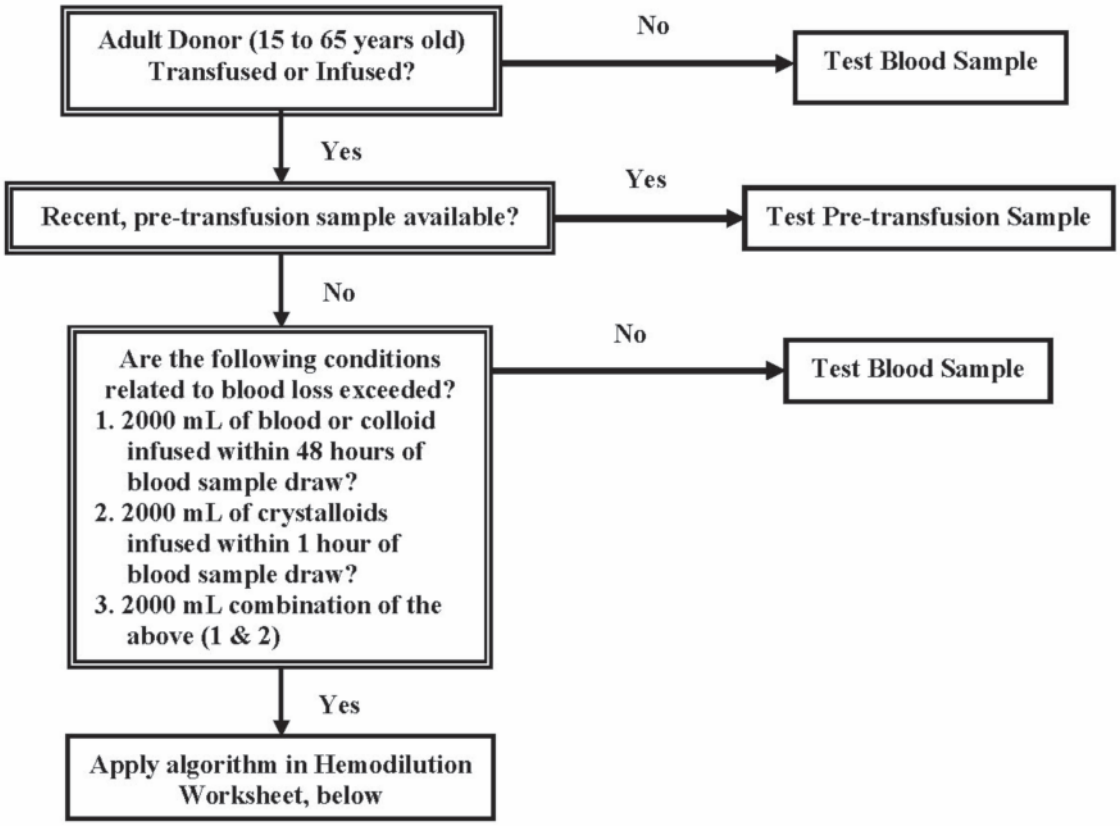
Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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.

Islets Lot Number: _____

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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: _____

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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: _____

Islets Lot Number: _____

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