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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 14.5 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 17.3.

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
Certificates 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 Counts	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 37°C, 95% air, and 5% carbon dioxide and record the date and time.

Date and time High Purity Islets flasks placed in 37°C 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:** _____

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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 22°C, 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 22°C 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: _____

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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°C, 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.

	Islet 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:** _____

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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."

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:** _____

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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.

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 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:** _____

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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: _____

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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				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-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 PBR Part 1, 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"
- 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"

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- "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: _____**

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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 single T-75 flask after combination should be 100 mL. Combine by settling and removing supernatant as in Section 12.11, 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 the T-75 flasks after combination should be 100 mL each. Combine by settling and removing supernatant as in Section 12.11, 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 the T-75 flasks after combination should be 100 mL each. Combine by settling and removing supernatant as in Section 12.11, 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
Interim Certificate of Analysis & Certificate 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)			
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.

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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												

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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 PBR Part 1, Section 10.2 for guidelines

Total Final Product IEQ: _____

Total IEQ/g of trimmed pancreas (PBR Part 1, 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:** _____

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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:** _____

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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:

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

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

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:

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

FINAL PRODUCT T-75 FLASKS	Endotoxins Concentration (EU/mL)	Suspension Volume (mL)	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

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:** _____

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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

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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:** _____

18.0 PRODUCT DISPOSITION

Was this product transplanted? Yes No (Circle one)

If this product was transplanted, give the Patient 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 assure 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 Production Batch Record (both Part 1 and Part 2A) and verified that it is complete and accurate.

_____ Date: _____

Quality Unit Representative

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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 TEST	RESULT
5.8	Pancreas Biopsy MCP-1	
5.8	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: _____

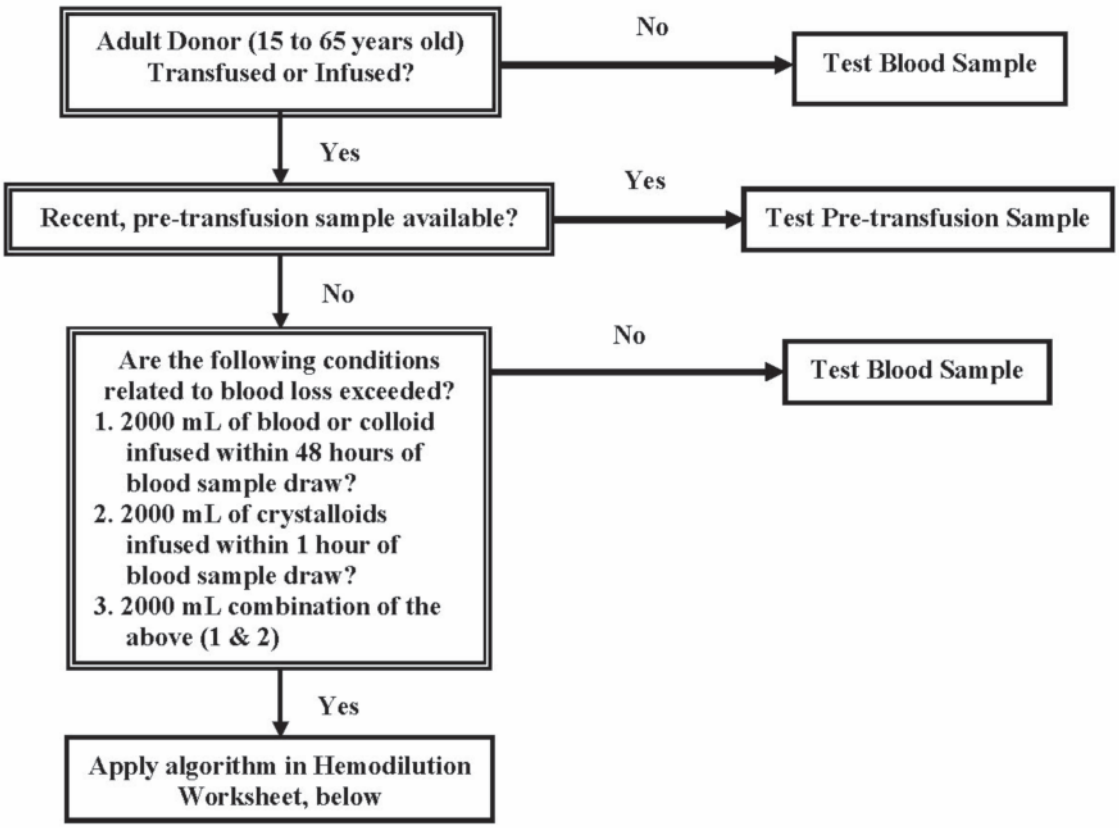
SAMPLES FROM MPBR SECTION	OPTIONAL TEST	RESULT
11.1	Pre-culture DNA Content	High Purity Islets: _____ µg DNA
11.1	Pre-culture Nuclei Measurement	nuclei
12.14	Post-culture DNA Content	High Purity Islets: _____ µg DNA
12.14	Post-culture Nuclei Measurement	nuclei
12.14	ATP/DNA Ratio	
12.14	OCR/DNA	nmol 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.

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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**

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: _____

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