Integrated Islet Distribution Program
City of Hope

STANDARD OPERATING PROCEDURE (SOP)
Flash Freezing Unclaimed Islets
(For IIDP Centers)
Version: SHP-002-04
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1.0 Objective

1.1 The objective of the Integrated Islet Distribution Program (IIDP) is to develop a standardized procedure for the freezing and storage of unclaimed islets. This procedure is necessary to provide centers that cannot distribute isolated islets on a certain date, to be able to freeze and store leftover islets to be used for genetic studies at a later date by some users. This may provide an opportunity for the isolating centers to be reimbursed for islets that cannot be placed through the algorithm.

1.2 A brief survey of islet users revealed that the availability of flash frozen islets would be a welcomed addition to the allocation system.

1.3 The immediate flash freezing of unclaimed islets will not only prevent the loss of these islets but may provide a less expensive and more advantageous method of islet distribution for investigators that need islets immediately after isolation for gene panel research, RNA expression, and other experiments where viable islets are not necessary.

2.0 Scope and Applicability

2.1 This SOP applies to the IIDP Coordinating Center (CC) and to any center using funds from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) that provides islets for basic research studies to IIDP approved investigators.

2.2 This SOP will require participation from all participating IIDP centers. The frozen islets will benefit any interested approved basic science islet users.

3.0 Responsibilities

3.1 It is the responsibility of the IIDP CC to both follow and ensure adherence to the procedures outlined in this SOP. In order to accomplish this, the IIDP CC will interact with the relevant personnel from each participating centers.

3.2 It is the responsibility of each IIDP center to follow the procedures listed in this SOP and to work to the best of their ability to follow all schedule requirements, labeling requirements, logging of samples, and shipment of samples.

4.0 Definitions

4.1 Integrated Islet Distribution Program (IIDP): The IIDP is a contracted program commissioned and funded by the NIDDK to provide quality human islets to the
diabetes research community to advance scientific discoveries to enhance treatments and find a cure. The IIDP consists of the NIDDK Project and Scientific Officers, the External Evaluation Committee and the CC at City of Hope (COH). The IIDP CC integrates an interactive group of academic laboratories including the subcontracted IIDP centers.

4.2 IIIDP Coordinating Center (CC): Joyce Niland, Ph.D. is the Principal Investigator for the IIDP CC and leads staff from the Department of Research Information Sciences at COH to coordinate the activities of the IIDP and assist the participating centers and investigators in the distribution of human islets.

4.3 Approved Investigators: Researchers who have requested islets from the IIDP for basic science studies and whose research protocols have been reviewed and improved by a subcommittee of the EEC.

4.4 Islet Allocation System (IA): The online system administered by the IIDP to allow fair distribution of basic science islets to approved investigators. This interactive system used by both the IIDP Centers and the Approved Investigators tracks not only the distribution of islets but the return of the shipping materials to the IIDP centers.

4.5 Standard Operating Procedure (SOP): The Standard Operating Procedure is detailed, written instructions to achieve uniformity of the performance of a specific function. In this SOP, there are attachments that will assist the IIDP in accomplishing the shipment of flash frozen islets. (See Attachments 8.1 through 8.4)

5.0 Materials

5.1 Unclaimed human islets of ≥80% purity after the completed run of the IIDP algorithm.

5.2 Hanks Buffered Salt Solution (HBSS) – no additives.

5.2 15 ml, 50 ml, or 250 ml conical tubes (depending on final volume of islet prep).
5.4 Corning® 2 mL External Threaded Polypropylene Cryogenic Vials, Self-Standing with Round Bottom (Product #430659) (*this brand and style of cryo tubes are recommended but not mandatory*)

5.5 Cryogenic markers or pencils for tube labeling.

5.6 Centrifuge with capabilities of spinning the appropriate sized conicals and the 2 ml cryogenic vials at 1000 rpm for 2 minutes.

5.7 Sterile pipettes, pipettors, and other appropriate sterile lab ware for manipulation of islet preparations.

5.8 Log sheet, IIDP Frozen Box Labels, and Flash Frozen Tissue Shipment Forms, Fed Ex Shipping Form.

5.9 Liquid nitrogen and appropriate vessels and equipment for safely freezing the unclaimed islets.

5.10 Appropriate sized Styrofoam shipping boxes and adequate dry ice.

6.0 Procedures

6.1 **Islet Isolation**: Perform islet isolation and distribute available islets through the IIDP distribution system.

6.2 **Flash freezing of unclaimed islets**: If at the end of the distribution deadline there are still unclaimed islets of greater than 80% purity, 10 aliquots of approximately 1,000 IEQ may be flash frozen and stored for future distribution using the following method.

6.2.1 Calculate the necessary volume of the islet suspension needed to provide approximately 1,000 IEQ per appropriate sized conical tube and divide left over islets needing to be frozen into the required number of tubes.

6.2.2 Label the appropriate number of cryo tubes with Isolation #, Unos # and date using a cryo marker or pencil and set aside. All tubes should have a final aliquot of approximately 1,000 IEQ.

6.2.3 Spin conical tubes containing the islets at 1000 rpm for 2 minutes at 4°C.

6.2.4 Carefully evacuate supernatant from the islet pellet.

6.2.5 Add 1ml of HBSS to conical tube, gently resuspend the islet pellet and transfer islet suspension to a 2 ml labeled cryo tube. Add another 0.5 ml of
HBSS to conical and rinse to capture remaining islets. Transfer to the cryo tube. Repeat with another 0.5 ml rinse.

6.2.6 Spin cryo tube at 1000 rpm for 2 minutes.

6.2.7 Evacuate supernatant, leaving a dry pellet.

6.2.8 Carefully place cryo tubes in a liquid nitrogen bath taking all safety precautions to protect you from being burned by liquid nitrogen (mask, gloves, tongs, and apron)

6.2.9 Safely transfer the vials into a labeled container and store in a -80°C or -135°C freezer or in a Liquid Nitrogen Storage Tank until shipment.

6.3 **Cataloguing of frozen islets:** Complete the first nine columns of the IIDP Cryo Storage Log Sheet. A separate line should be used for each tube frozen. *(This log sheet is made available for in-house use and center convenience (Attachment 8.2. It does not have to be returned to the IIDP.)*

6.3.1 The log sheet should be completed as the islet aliquots are removed.

6.4 **Broadcast of frozen aliquots:** The flash frozen islets can be broadcast at any time after the original isolation has been broadcast and distributed and all islets greater than 80% purity still remain. The broadcast is not time dependent and interested investigators can claim these islets at any time after broadcast until all have been distributed.

6.4.1 Log into the IIDP database using your ID and Password.

6.4.2 As you log into the Dashboard, click on “Frozen Islets” on the Navigation Menu on the left hand side of the page. Click on “Broadcast Frozen Islet Offer”.

6.4.3 When the list of your center’s isolations appears, click on the Broadcast button of the isolation for which you wish to broadcast the flash frozen islets. A new screen will ask you a few questions about the timing of when the islets were frozen, how many vials are available (the total number of islets will automatically be populated at 1,000 IEQs per vial) and the purity of the frozen samples *(the purity must be greater than 80% to be useful as flash frozen samples).*

6.4.4 Once the form is completed, click on Broadcast and the information will be entered into the database system. At this point an email will be sent to
all possible investigators. You will see a confirmation notice that the broadcast has been successful.

6.4.5 The investigators will then enter the system and, if interested, will accept the offers. (This is similar to the cultured islet broadcasts but the flash frozen islets are not time dependent.)

6.4.6 The broadcasting center will be alerted by email when the flash frozen preparations have been accepted. At this point, the center staff should go back into the system to the “All Frozen Islet Offers” and look for “New Request” under the column Frozen Islets Accepted indicating an accepted offer. Note: Older isolations may have aliquots of 5,000 or 10,000 IEQs.

6.4.7 To ship the Flash Frozen aliquot, the center staff should click on the red “Confirm Shipment” button on the row that has the “New Request” indicator. The “Confirm Recipients” screen will appear and should be completed by center staff. Click Edit to insert the tracking number and the amount being shipped. After submission, the “Inventory List” will be populated with the appropriate information and emails will be sent to notify acceptance by the center staff with the shipping information to both the investigators and the center staff who did the broadcast. Once a recipient is confirmed, click on the “Print Tissue Shipment Forms” and include the appropriate form with each shipment. (Note: The center must click on the Print Tissue Shipment Form (TSF) in order to complete the distribution process and trigger the email distribution.)

6.5 Shipping of frozen aliquots: Islets should be maintained below -80°C until ready for shipment.

6.5.1 A completed Flash Frozen Tissue Shipment Form should accompany each sample shipment. Approximately five pounds of dry ice should fill a small, thick Styrofoam shipping box. (Sample sized box is listed in the supply list for reference.) Multiple tubes of 1,000 – 10,000 IEQ can be sent in a single box if necessary to satisfy the number of requested islets. The tubes should be placed in the middle of the dry ice. The IIDP Frozen Sample Labels should be placed on two sides of the box and the Dry Ice regulation label should be completed before shipping.
6.5.2 Samples should be sent priority overnight shipping by FedEx with appropriate boxes checked on the FedEx form for Dry Ice shipments. *(No materials will be returned by the investigators.)*

6.5.3 User Feed Back forms should be completed on line by the investigators.

7.0 References:


8.0 Attachments

8.1 Supply List

8.2 Flash Frozen Log Sheet