Collection, Broadcast, Packaging and Shipping of Non-Islet Biospecimens V.2

Version 1 is forked from Collection, Packaging and Cold Shipping of Fresh Non-Islet Pancreatic (Acinar) Tissue

Integrated Islet Distribution Program[1]

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VERSION 2

AUG 22, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgby71nvpk/v2

Protocol Citation: Integrated Islet Distribution Program 2023. Collection, Broadcast, Packaging and Shipping of Non-Islet Biospecimens. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgby71nvpk/v2 Version created by Integrated Islet Distribution Program

Protocol status: Working
We use this protocol and it's working

Created: Aug 22, 2023

Last Modified: Aug 22, 2023

PROTOCOL integer ID: 86818

Keywords: islets, acinar, peripancreatic lymph nodes, plasma, serum, ductal tissue, pancreatic tissue, spleen, Integrated Islet Distribution Program, IIDP, tissue distribution, human islets, biomaterial, biospecimen, Non-Islet Pancreatic (Acinar) Tissue (NIPT)

ABSTRACT

This SOP defines a standardized method for processing, broadcasting, packaging and shipping preparation of research quality non-islet biospecimens. These tissues may be matched or unmatched to the islet donor and are made available to approved investigators in the Integrated Islet Distribution Program (IIDP) sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Institute. This protocol is written to assist the participating islet isolation centers who are part of the IIDP. This protocol includes the order of the summarized processing of acinar tissue, however details can still be gleaned from the original *Collection, Packaging and Cold Shipping of Fresh Non-Islet Pancreatic (Acinar) Tissue*.

Note

Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387)

Note: The IIDP appreciates the professional input from Rita Bottino, PhD, Imagine Pharma; Dagmar Klein, PhD, University of Miami; and Peter Chlebeck, University of Wisconsin. Dr. Klein recommends the addition of protease inhibitors to the media for holding the acinar tissue until ready for shipment and in the shipping media. Recommendations from Dr. Bottino and Peter Chlebeck on the methods for the processing of blood products and ancillary tissues, included with the pancreas provided by the OPOs, for quality research samples for IIDP investigators has been invaluable to this protocol.

IMAGE ATTRIBUTION

Image and video of peripancreatic lymph nodes were graciously contributed by Dr. Rita Bottino, PhD., Imagine Pharma, Pittsburgh, PA.

GUIDELINES

Responsibilities:

- 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 of the participating centers.
- 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 requirements.

Definitions:

- ***Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):*** The IIDP is a program commissioned and funded by the NIDDK to provide quality human islets to the diabetes research community to advance scientific discoveries and translational medicine. The IIDP consists of the NIDDK Project Scientist and Program Official, 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.

- ***IIDP Coordinating Center (CC):*** Joyce Niland, Ph.D., IIDP Principal Investigator leads CC staff to coordinate the activities of the IIDP and assists the participating centers and investigators in the distribution of human islets.

- ***Islet Equivalent (IEQ):*** An IEQ is defined as an islet with a diameter of 150 microns. The number of IEQ in each size class is calculated by multiplying the number of islets by a conversion factor for each micron size.

- ***Approved Investigators***: Researchers who have requested islets from the IIDP for basic science studies and whose research protocols have been reviewed and approved by the IIDP.

- ***Islet Allocation System (IA):*** This is the online system administered by the IIDP to allow fair distribution of basic science islets to approved investigators. This interactive system, used by the IIDP Centers and the Approved Investigators, facilitates and tracks the distribution of human islets.

- ***Non-Islet Pancreatic (Acinar) Tissue (NIPT):*** During a standard human islet isolation, the digested tissue goes through a purification process that allows the majority of the isolated islets to be separated from the acinar and other pancreatic tissues. This is commonly done using density gradients and a COBE centrifugation processor. The lighter density tissues, including the majority of human islets, are collected from the lighter density gradients, leaving the remaining pancreatic tissue, in the COBE processor bag that still holds the denser gradients and tissue. This non-islet pancreatic tissue is commonly referred to as the “acinar” tissue, although in reality, it contains not only the acinar tissue but also connective tissue, blood vessel fragments, ductal fragments as well as imbedded islets.

MATERIALS

MATERIALS

Corning™ Ciprofloxacin Hydrochloride **Fisher Scientific Catalog #MT61277RG (Corning™ 61277RG)**

- 2% Chlorhexidine Solution or equivalent **Fisher Scientific Catalog #50-223-2770**
- Human AB Serum (ABS) HI **Gemini Bio-Products Catalog #100-512; Heat Inactivated**
- PIM(G)® (5 mL Glutamine/Glutathione) **Prodo Laboratories, Inc Catalog #PIM(G)®**
- PIM(T)® **Prodo Laboratories, Inc Catalog # PIM(T)®**
- MP Biomedicals™ Ciprofloxacin Hydrochloride or equivalent **Fisher Scientific Catalog #MP219902005**
- Sigma T9128-Trypsin Inhibitor from Glycine max (soybean) **Fisher Scientific Catalog #501784544**

Equipment

Phase 22 PCM Flex Pack

NAME

Phase 22 cold packs to maintain temperature at 22C

TYPE

TCP Reliable Manufacturing

BRAND

FMIS76000

SKU

https://www.cryopak.com/packaging-and-refrigerants/phase-change-materials/phase-22/

LINK

SPECIFICATIONS

•Most used for applications that need to maintain a controlled room temperature
•Phase change temperature is 22°C
•Provides thermal protection when shipping products between 15-30°C

Equipment

PETG Media Bottles with Closure

NAME

60 mL PETG Media Bottles with Closure

TYPE

Thermo Scientific™ Nalgene™ Square PETG Media Bott

BRAND

03-311-1W

SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033111W#?keyword=true

LINK

PETG Media Bottles

SPECIFICATIONS

Equipment

Media Bottles with Closure

NAME

125 mL PETG Media Bottles with Closure

TYPE

Thermo Scientific™ Nalgene™

BRAND

03-312-1

SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033121#?keyword=true

LINK

PETG Media Bottles

SPECIFICATIONS

Equipment

Square PETG Media Bottles NAME

250 mL PETG Media Bottles with Closure TYPE

Thermo Scientific™ Nalgene™ BRAND

03-312-3 SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033123#?keyword=03-312-3 LINK

PETG Media Bottles SPECIFICATIONS

Equipment

Bottle Top Filters with PES Membrane or equivalent NAME

0.2 µm Bottle Top Filter TYPE

Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Di BRAND

09-741-09 SKU

https://www.fishersci.com/shop/products/nalgene-rapid-flow-sterile-disposable-bottle-top-filters-pes-membrane/0974109 LINK

0.2 µm membranes for sterile filtration; PES is the best membrane for cell culture fluids; lowest protein binding to maintain protein balance, lowest extractables to maintain media purity SPECIFICATIONS

Equipment

Durasorb™ Underpads

NAME

Underpads

TYPE

Covidien

BRAND

22-031-340

SKU

https://www.fishersci.com/shop/products/covidien-durasorb-underpads/22031340#?keyword=22+031+340

LINK

Wings™ Fluff Underpad 17 x 24 in.

SPECIFICATIONS

Equipment

Reclosable Zip Bags

NAME

Clear Reclosable Zip Bags

TYPE

RD Plastics

BRAND

19-130-6043

SKU

https://www.fishersci.com/shop/products/rd-plastics-clear-reclosable-zip-bags-6/191306043?keyword=true

LINK

PE (Polyethylene) Reclosable bag (8 x 10 in.)

SPECIFICATIONS

Equipment

Uline Corrugated Boxes

NAME

Corrugated Boxes (Inner Shipping Box)

TYPE

Uline

BRAND

S-4344

SKU

https://www.uline.com/Product/Detail/S-4344/Corrugated-Boxes-200-Test/7-x-5-x-5-Corrugated-Boxes?keywords=S-4344+7+x+5+x+5%22+Corrugated+Boxes

LINK

7 x 5 x 5" Corrugated Boxes

SPECIFICATIONS

Equipment

Insulated Foam Shipping Kit

NAME

Insulated Foam Shipping Kit

TYPE

Uline

BRAND

S-7359

SKU

https://www.uline.com/Product/Detail/S-7359/Insulated-Shippers-and-Supplies/Insulated-Foam-Shipping-Kit-8-x-6-x-9?keywords=S-7359

LINK

Insulated Foam Shipping Kit - 8 x 6 x 9"

SPECIFICATIONS

Equipment

Uline Cold Pack

NAME

Cold Pack

TYPE

Uline

BRAND

S-7361

SKU

https://www.uline.com/Product/Detail/S-7361/Insulated-Shippers-and-Supplies/Cold-Packs-8-oz?keywords=S-7361

LINK

Reusable, leakproof refrigerant gel stays colder than ice. Thick 4 mil poly. 6 x 4 x 3/4"

SPECIFICATIONS

Equipment

Uline Industrial Tape

NAME

Uline Industrial Tape - 2 Mil, 2" x 110 yds, Clear

TYPE

Uline

BRAND

S-423

SKU

https://www.uline.com/Product/Detail/S-423/Carton-Sealing-Tape/Uline-Industrial-Tape-2-Mil-2-x-110-yds-Clear?keywords=S-423

LINK

2 Mil, 2" x 110 yds, Clear. DURABLE: Industrial Tape stands up to heat, humidity and cold.

SPECIFICATIONS

Equipment

Custom Cold Chain Complete Card NAME

Temperature Monitoring system TYPE

Tip TEMPerature Products BRAND

WMSSEN044; SKU

https://www.tiptemp.com/ LINK

Custom Cold Chain Complete Card SPECIFICATIONS

Equipment

Tip TEMPerature Products NAME

WarmMark Ascending Temperature indicator TYPE

WarmMark Ascending Temperature indicator BRAND

WMSSEN009 SKU

https://www.tiptemp.com/Products/Rising-Time-Temperature-Indicating-Labels/WMSSEN009-WarmMark-with-Trigger-Temperature-37C-99F.html LINK

Maximum exposure above 37°C/99°F SPECIFICATIONS

Equipment

ColdMark Descending Temperature Indicator NAME

Descending Temperature Indicator TYPE

Tip TEMPerature Products BRAND

CLMSEN004 SKU

https://www.tiptemp.com/Products/Falling-Temperature-Indicating-Labels/CLMSEN004-Temperature-Label-ColdMark-2C-36F.html LINK

Falling Temp Monitor Changes Color Permanently; 2°C/36°F SPECIFICATIONS

Equipment

Long-Term Storage Cryogenic Tubes or equivalent NAME

Cryogenic Tubes - 2 mL TYPE

Thermo Scientific™ Nalgene™ BRAND

03-337-7D SKU

https://www.fishersci.com/shop/products/nalgene-general-long-term-storage-cryogenic-tubes/033377d LINK

Sterile, externally threaded Thermo Scientific™ Nalgene™ General Long-Term Storage Cryogenic Tubes, ideal for research and general lab storage. SPECIFICATIONS

Equipment

Long-Term Storage Cryogenic Tubes or equivalent

NAME

Cryogenic Tubes - 5 mL

TYPE

Thermo Scientific™ Nalgene™

BRAND

03-337-7H

SKU

https://www.fishersci.com/shop/products/nalgene-general-long-term-storage-cryogenic-tubes/033377h

LINK

Sterile, externally threaded Thermo Scientific™ Nalgene™ General Long-Term Storage Cryogenic Tubes, ideal for research and general lab storage.

SPECIFICATIONS

Equipment

Laser Labels

NAME

Uline Laser Labels - White, 8 1⁄2 x 11"

TYPE

Uline

BRAND

S-5045

SKU

https://www.uline.com/Product/Detail/S-5045/Laser-Labels/Uline-Laser-Labels-White-8-1-2-x-11?keywords=S-5045+Uline+Laser+Labels+-+White%2c+8+1%2f2+x+11%22

LINK

Box of 100 Single Labels, White, 8 1⁄2 x 11"

SPECIFICATIONS

SAFETY WARNINGS

Please see attached SDS (Safety Data Sheet) for hazards and safety warnings.

Ciprofloxacin Hydrochloride Corning Cipro SDS-2014Jul22.pdf

MP Cipro_MSDS_199020_USA_EN.pdf

Precautionary statements:

- P280 - Wear protective gloves and eye/face protection
- P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P337 + P313 - If eye irritation persists: Get medical advice/attention.
- P273 - Avoid release to the environment.

GemCell™ U.S. Origin Human Serum AB GemCell Human Serum AB.pdf

GemCell™ human serum AB is collected from healthy male donors of the AB serotype at FDA-licensed facilities in the United States.

Hazardous Components:

- Biohazard contains human source material. Handle as though capable of transmitting infectious agents.
- Toxicity: Not Established.

Target Organs/Systems: Product could possibly irritate the skin, eyes and respiratory system. Do not ingest this product.

Trypsin inhibitor, from Glycine max (soybean) Trypsin MSDSAction.pdf

- Laboratory chemicals, Synthesis of substances
- Not a hazardous substance or mixture.
- Avoid dust formation. Avoid breathing vapours, mist or gas.
- For personal protection:Use equipment for eye protection. Handle with gloves. Respiratory protection is not required.

Preparation of Supplies

1 **The IIDP will provide each center with the following supplies necessary for donor matched or unmatched non-islet biospecimens shipping:**

- Gemini Human AB Serum (ABS) - Heat Inactivated (HI)

- Prodo Islet Media - Transport PIM (T)®
- Ciprofloxacin Hydrochloride. MP Biomedicals™ MP219902005 (1 gm bottles). (Cipro has a relatively short shelf life of 12-18 months, so the expiration date must be closely monitored)
- PETG Shipping bottles, Thermo Scientific Nalgene brand equivalent
- 2% Chlorhexidine solution (diluted to 1% with HBSS for disinfecting duodenum if needed)
- 2 mL & 5 mL Cryogenic Vials (cryo vials or cryo tubes)
- Ziplock Bags, 8" x 10", 2 mil
- Durasorb Underpads
- TipTemp Custom Cold Chain Complete Card
- Insulated shipping containers (6" x 4.5" x 3") (IIDP Histo shipping box - Uline S-7887)
- Phase 22 pouches - Cryopak: small, ambient temperature pouches @ RT (~24° C)/blood tube sent
- 8 ounce cold packs
- Shipping tape (2 mil)
- 8" x 11" Labels for outside of boxes (Cut in half to fit smaller box)

2 **Supplies provided by the IIDP Centers:**

- Non-Islet Biospecimens (NIB) for distribution
- 15 mL & 50 mL conical tubes
- <500mL of HBSS at 4°C
- Routine lab supplies for transferring ancillary tissue
- Markers for labeling
- IIDP Box Labels from IIDP template
- Tissue Shipment Forms
- Completed FedEx Shipping Labels

3 ***Receipt of Supplies:***

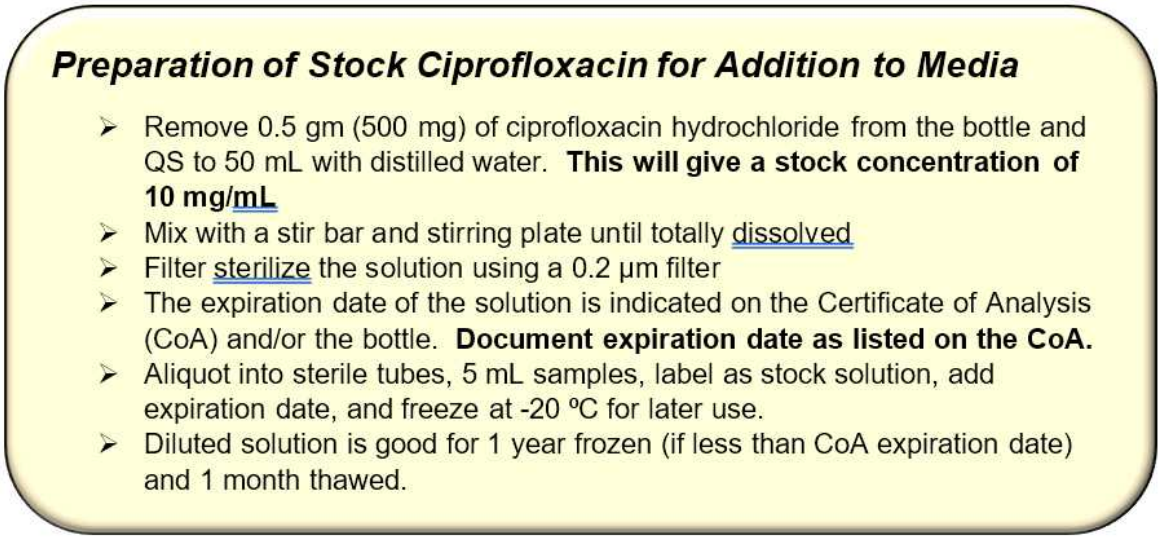
The majority of supplies should be stored in appropriate dry, temperature-controlled environments (room temperature 16 °C to 28 °C).

- The PIM(T)® should be stored, in the dark, between 2 °C and 8 °C upon receipt but is stable at room temperature.
- The Gemini AB serum vials should be stored at -5 °C to -20 °C in the dark.
- The Ciprofloxacin powder should be stored as indicated by manufacturer (MP Biomedicals at 4°C OR Corning at room temperature); however once prepared, the filter sterilized, stock solution aliquots should be stored at -5 °C to -20 °C.
- The Trypsin Inhibitor powder can be stored should be stored between 2 °C and 8 °C, but filter sterilized, stock solution aliquots should be stored at -5 °C to -20 °C.

Preparation of Stock Reagents

4 ***Preparation of Ciprofloxacin:***

Aliquots of Ciprofloxacin can be prepared prior to the isolation. Prepare the ciprofloxacin according to the directions below. This can be prepared ahead of an isolation and stored as indicated on Attachment: ***Cipro Stock Preparation Sheet*** (below). Filter sterilized suspension aliquots should be stored at -20 °C. The expiration date must be noted on tubes and closely monitored.



If not prepared previously as a Stock Reagent, then record Reagent Preparation Details on ***Cipro Stock Preparation Sheet.***

Cipro Stock Preparation Sheet (v2023.05.25).docx

5 **Preparation of Trypsin Inhibitor (used for Fresh Acinar Tissue only):**

Aliquots of Trypsin Inhibitor can be prepared prior to the isolation. The T9128 Trypsin Inhibitor from Glycine Max (soybean) – lyophilized powder should be stored between 2 °C and 8 °C upon receipt. Trypsin Inhibitor can be prepared ahead of an isolation and stored **PIM(T)®** as indicated in attached ***Trypsin Stock Preparation Sheet*** *(below)*. Filter sterilized suspension aliquots should be stored at -20 °C. The expiration date must be noted on tubes and closely monitored.

Preparation of Stock Trypsin Inhibitor for Addition to Media:

- Weigh 1 gm (1000 mg) of trypsin inhibitor from the bottle and QS to 100mL with serum free PIM-T (no additives). This will give a stock concentration of 10 mg/mL
- Mix with a stir bar and stirring plate until totally dissolved
- Allow to stand on ice at least 30 min before filter sterilizing
- Filter sterilize the solution using a 0.2 µm filter
- The expiration date of the solution is indicated on the Certificate of Analysis (CoA) and/or the bottle. **Document expiration date as listed on the CoA.**
- Aliquot into sterile tubes, 5 mL samples, label as stock solution, add expiration date.
- Store and freeze at -20ºC for later use. Stock is stable at -20ºC.
- Inhibitor must be added fresh to the medium before culture or shipment. Add 5 mL of Trypsin Inhibitor stock to each 500 mL bottle of PIM(T)®. This will give a ***final working concentration of 100 µg/mL*** of Trypsin Inhibitor in the media.

If not prepared previously as a Stock Reagent, then record Reagent Preparation Details on attached ***Trypsin Stock Preparation Sheet.***

Trypsin Stock Preparation Sheet (v2023-05-25).docx

Preparation of Working Solutions

6 **Preparation of PIM(T)® Complete for shipment**

- Prepare one 500 mL bottle of PIM(T)®
- Add 5 mL of thawed PIM(G)® (L-Glutamine media)
- Thaw and add 12.5 mL of AB serum (2.5% v/v)
- Thaw and add 0.5 mL of prepared ciprofloxacin sterile aliquot per go to step #4.
- Once all additives have been added to the bottle of PIM(T)®, it is now referred to as **PIM(T)® Complete.**
- Record Reagent Preparation Details on ***attached PIM(T)® Solution Preparation Sheet.***

PIM-T Solution Preparation (v2023.05.25).docx

Register Pancreas

7 As soon as a pancreas has been accepted from the OPO, log into the secure IIDP website and register the date, time, and disease state of the pancreas.

Receipt and Storage of Non-Islet Biospecimens (NIB)

8 ***Receipt of Pancreas and Biospecimens***
Open shipping box from OPO and inspect all available samples for processing and shipment to IIDP recipients.

8.1
1. Determine what was included with the pancreas from OPO.
   - # red-top tubes for serum
   - # yellow-top tubes for plasma
   - # lavender-top tubes for plasma
   - Spleen piece (in tube or small container or still attached *en bloc* to the pancreas)
   - Pancreas with duodenum

   Note: Discard unidentified lymph nodes if included in box
2. Hold red top tubes at 15-25 °C until clots have formed (~ 30-45 minutes).
3. Centrifuge all blood tubes at 3000 x g for 10 minutes at 4°C.
4. Remove tubes; transfer serum (from red tops) and/or plasma (from yellow/lavender tops) to 15 mL conical tubes. Serum from redtop tubes my be combined into conical tubes for holding. Plasma from yellow and lavender tops can be combined into conical tubes for holding.
5. Store all samples at 2-8 °C until NIB deadline has been reached.

Create Isolation Record

9 Once pancreas has been confirmed as acceptable for processing, open IIDP secure website and click on ***Start Isolation - Create Isolation Record*** for the pancreas that that was registered in Step 7 go to step #7 .
- *The information on this page will generate the RRID#s for each tissue type for this isolation*

9.1 Insert information in the ***New Isolation Record*** for Covid-19 results.

9.2 ***Check each Non-Islet Biospecimens (NIB) intended to be offered*** with this isolation. This should include both the materials received from the OPO in the box with the pancreas, as well as the other tissue that will be procured from the pancreas itself (peri-pancreatic lymph nodes and frozen pancreas pieces) or through the islet isolation process (ductal cells, acinar).

- *The tissues checked will be offered to researchers in the NIB Broadcast.*
- *If there is a problem during the recoveries of any of the tissues, there will be a chance to revoke the offer on the confirmation page, after the deadline has been reached.*

9.3 Enter UNOS ID, the center's Islet Isolation ID #, and minimal donor information.

- *All information entered on this page will be pre-populated into the rest of the broadcast system.*

9.4 When information on page is completed, click the ***Broadcast Non-Islet Biospecimens button*** at the bottom of the page, to create the Isolation Record.

Broadcast of Non-Islet Biospecimens(NIB)

10 Begin the broadcast of non-islet biospecimens on the ***Broadcast Non-Islet Biospecimen (NIB) page*** of the "IIDP Secure Access Center" website.

10.1 Enter Isolation information (1), Pancreas Procurement Information (2), and Donor Information (3), including HLA (4), Serology (5), Covid Testing (6), Terminal Hospital Stay (7), and Diabetes Information (8) on IIDP secure website.

- *All data entered for the isolation and donor information will be auto-populated to the subsequent "Islet Broadcast".*

10.2 Continue to next page of NIB Broadcast.

- Complete cross clamp and cold ischemia questions (1-3).
- Recheck the chart for which NIB will be offered (4) based on receipt from the OPO (blood samples, spleen pieces) and anticipated tissues made available from the pancreas/isolation process (acinar tissue, frozen pancreatic pieces, peri-pancreatic lymph

nodes, pancreatic ductal tissue, duodenal tissue, and spleen samples if *en bloc* to the pancreas).

10.3 Complete NIB Broadcast forms:

- Choose anticipated NIB shipment date (5).
- Choose anticipated islet shipment date (6).
- Add NIB Broadcast date and time deadline (7). (*This deadline should be approximately the anticipated time of the end of islet purification process)*
- Click on ***NEXT→*** screen.

10.4 Review data. If all information is complete and confirmed, broadcast NIB by clicking the ***Broadcast Non-Islet Biospecimens Now*** button.

Proceed with Retrieval of Additional NIB and Islet Isolation Pro..

11 Obtain pancreas container to retrieve additional NIB tissues and prepare to begin the islet isolation process while NIBs are being offered to interested investigators.

11.1 Proceed with pancreas processing according to center protocols

- Obtain PIM(T)® Complete prepared in Step 6 go to step #6. Add 20 mL of PIM(T)® Complete into a 50 mL conical tube and hold at 2-8 °C.
- Locate the **peri-pancreatic lymph nodes** in the connective tissue surrounding the pancreas (PowerPoint link below).
- Remove up to 10 peri-pancreatic lymph nodes and place in prepared 50 mL conical.

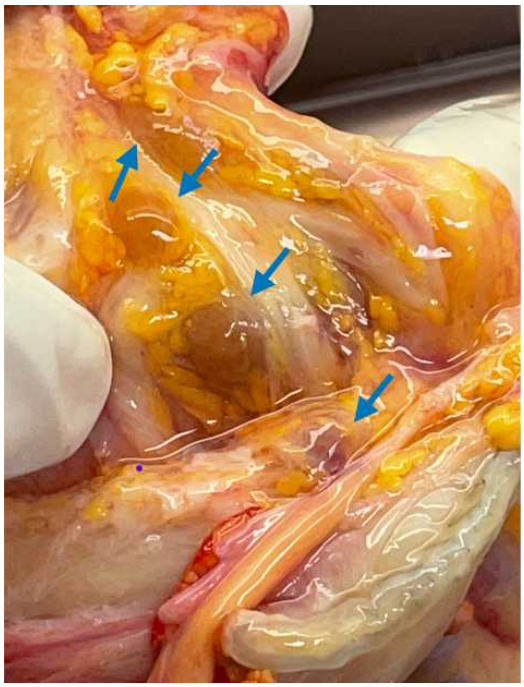


Image of peri-pancreatic lymph nodes

Peri-Pancreatic Lymph Nodes - IIDP_RB_3_28_23 revised 4_12_23 bjo.pptx

(Powerpoint attachment and image above provided by Dr. Rita Bottino, Imagine Pharma)

11.2 Remove four (4), thumbnail sized (1cm x 1cm x 2mm <2 mm), **pieces of pancreas** from near cannulation point to avoid unnecessary leakage of collagenase. Place pancreas pieces into a 50 mL conical with PIM(T)® Complete. Hold at 2-8 °C until end of NIB deadline.

11.3 Take pancreas **histology** sample (1cm x 1cm x 1cm) from near cannulation point to avoid leakage of collagenase and place in NBF container for fixation. Close with suture or clamp if necessary. Follow ***Standard Operating Procedure Preparing Pancreas Section for Histology*** for detailed procedure.

11.4 Remove **spleen**, if still attached to pancreas. Place in PIM(T)® Complete, in sterile container. Hold at 2-8 °C until end of NIB deadline.

11.5 Carefully remove **duodenum** from pancreas. Place back into Nalgene jar that held the pancreas. Hold at 2-8 °C until end of NIB deadline. Continue with islet isolation through purification process.

11.6 After all of the pancreatic digest has been collected, if **ductal tissue** was requested through the NIB Broadcast, open the Ricordi/Digestion chamber and place remaining undigested tissue in a large sterile petri dish or dissection pan. Add cold PIM(T)® Complete to the tissue. Hold at 2-8 °C until end of NIB deadline.

♦ *Note: Some times the ductal tree is completely digested during the digestion process and no remnants can be found. If no ductal remnants can be discerned in the remaining tissue, discard the material as biohazardous waste and cancel all ductal tissue acceptances in the IIDP secure website.*

NIB Broadcast Deadline / Processing and Preparation for Ship...

12 The NIB broadcast deadline should coincide with the end of the purification process, allowing the center to determine the requested need for **acinar** tissue in order to remove and wash only the tissue needed for distribution. The **acinar** harvest and packaging can be followed in **Step 13** below.

Following NIB broadcast deadline, determine the specific tissues requested and amounts and ***process NIB as soon as possible to preserve quality for interested investigators.***

- Check the results of the NIB Broadcast and determine which, how much, or how many samples of the offered tissue have been requested.
- ***If there are no requests for specific NIBs, the specific tissue can be discarded in biohazard waste.***
- Label appropriate tissue containers with researcher's name, #RRID of the specific tissue, and the date of the isolation.
- Specific packaging instructions for cold tissues can be found in **Step 15** of this document or in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***.
- Centers can choose the order of biospecimen fulfillment to investigators, however acinar tissue should be processed the same day as the isolation, and shipped at the next FedEx Pick up time due to risk of autodigestion.
- Fresh biospecimens should be shipped the day of, or the day following, the islet isolation.
- All biospecimens should be divided and handled under a laminar flow hood using sterile technique.
- Cold samples can be combined into a single package if more than one sample is requested for a single researcher.
- Frozen samples can be combined into a single package if more than one sample is requested for a single researcher.
- If islets are requested and will be shipped the following day, all but fresh acinar tissues samples, can be held and shipped with the islets if approved by the researcher. (Fresh acinar should be sent on the day of isolation or with the next FedEx pick up time due to the danger of autodigestion.)

♦ *Note: The health and handling of the islet preparation should always take the priority in the IIDP distribution process. However, the NIB tissue is also important to the recipients and the*

acinar tissue, because of it's capacity to secrete harmful enzymes, should be kept diluted and cold at all times.

12.1 Red Top Tubes for Serum (2x10mL Red Top Tubes)

Serum can be used for antibody studies and can be received either cold or frozen.

1. Check broadcast records for the number of researchers and milliliters of serum requested.
2. Label required 5 mL cryo vial with requesting researchers' names, RRID# of the serum, and date.
3. Divide the serum being held from Step 8.1 go to step #8.1 into separate 5 mL cryo vials to fulfill as many of the requests as possible. The samples should be maintained at 2-8 °C while handling and until packaging for shipment.
   • *If no serum is requested, tubes can be disposed of in biohazard waste.*
4. Place tubes in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card***, as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***. ♦ *Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.*
5. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print Tissue Shipment Forms (TSF), affix shipping labels, hold for pick up by carrier.

12.2 Yellow and/or Lavender Top Tubes for Plasma (1-2): 5 mL ACD Yellow TOP Tubes; (1-2) 5 mL EDTA Lavender Top Tubes

Plasma is the liquid component of blood when an anticoagulant is added to the whole blood allowing the platelets, the clotting factor, to remain in the blood but is inactivated. Acid citrate dextrose (ACD) and Ethylenediaminetetraacetic acid (EDTA) are both anticoagulants.

1. Check broadcast records for the number of researchers and milliliters of plasma requested. • *If no plasma is requested, tubes may be disposed of in biohazard waste.*
2. Label required tubes with requesting researchers' names, RRID# of plasma, and date.
3. Divide the plasma being held from Step 8.1 go to step #8.1 into separate 5 mL cryo vials to fulfill as many of the requests as possible. The samples should be maintained at 2-8 °C while handling and until packaging for shipment.
   • *If no serum is requested, tubes can be disposed of in biohazard waste.*
4. Place tubes in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card***, as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***. ♦ *Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.*
5. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

12.3 Spleen Samples

Spleen samples are usually used to provide B and T lymphocytes for researchers.

1. Check broadcast records for the number of researchers and grams of spleen tissue requested.
2. Label required 15 mL conical tubes with requesting researchers' names, RRID# of spleen tissue, and date.
   • *If no spleen pieces are requested, tissue may be disposed of in biohazard waste.*
3. Recover spleen piece from OPO or container it was placed in if sent *en bloc* to pancreas. Divide spleen samples into ~5 gm (1x1x5cm) pieces and place requested number of pieces into labeled tubes with cold PIM(T)® Complete for each researcher requesting tissue (if 20 gm requested, then four 5 gm pieces should be added to that researcher's conical). The samples should be maintained at 2-8 °C while handling, and until packaging, for shipment.
4. Place tubes in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card***, as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***. ♦ *Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.*
5. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

12.4 Peri-Pancreatic Lymph Nodes

Peripancreatic lymph nodes are a part of the immune system containing the antigen presenting cells (APCs), B and T lymphocytes for researchers, and cloning expansion, can take place in these nodes.

1. Check broadcast records for the number of researchers and number of peripancreatic lymph nodes that were requested. • *If no lymph nodes are requested, tissue may be disposed of in biohazard waste.*
2. Label required 5 mL cryo vials (for ≤ 2 nodes) or 15 mL conical tubes (for >2 nodes) with requesting researchers' names, RRID# of the lymph nodes, and date.
3. Sequester the number of nodes specifically requested by each researcher, and place into labeled tubes, with cold PIM(T)® Complete, for each researcher requesting tissue. The samples should be maintained at 2-8 °C while handling, and until packaging for shipment.
4. Place tubes in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card,*** as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islet*** . ♦
   Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.
5. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

12.5 Pancreatic Ductal Tissue

Pancreatic ductal cells, which are sodium bicarbonate secreting epithelial cells, line the ducts that carry enzymes made by the acinar cells to the the duodenum. Pancreatic ductal cells can be manipulated in culture to form beta cells.

1. Tease the tissue with forceps to spread out and determine if you have the remnants of the ductal tree (skeleton). if large pieces of pancreas are still attached to the tree, remove as much additional pancreatic tissue as possible.
2. Check broadcast records for the number of researchers and the amount of ductal tissue requested.
   • *If no ductal tissue is requested, tissue may be disposed of in biohazard waste*
3. Label required 5 mL cryo vials with requesting researchers' names, RRID# of ductal tissue, and isolation date.
4. Cut the ductal tree in 1-3 cm pieces, for the number specifically requested by each researcher, and place into labeled vials, with cold PIM(T)® Complete for each researcher requesting tissue. The samples should be maintained at 2-8 °C while handling and until packaging for shipment.
5. Place vials in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card***, as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***.
   ♦ *Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.*
6. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

12.6 Frozen Pancreatic Pieces

- Flash frozen pieces of pancreata can be used as control DNA for the islets and additional biospecimens obtained through the IIDP.

1. Check broadcast records for the number of researchers requesting flash frozen pancreas pieces.
   • *If no frozen pancreas pieces are requested, tissue may be disposed of in biohazard waste.*
2. Label required 2mL cryo vials with requesting researchers' names, RRID# of pancreas pieces, and date with cryo marker.
3. Prepare a small container of liquid nitrogen using all necessary safety procedures and equipment including cryogenic gloves, apron, safety goggles.
4. Recover the tube containing the pieces of pancreas dissected pre-isolation. Remove each piece by holding the edge gently with forceps, rinse in cold HBSS (without serum), pat dry on gauze or paper towel, place in labeled cryo tube, and carefully place in LN_2.
5. Allow vials to remain in LN_2 or transfer to cryo freezer <-80 °C or LN_2 storage tank <-196 °C until ready to package on dry ice and ship to researcher.
6. Confirm shipments on Broadcast Confirmation List. Center ships out samples at agreed upon date with investigator; Ship on dry ice in islet shipping box (Uline S-7359) with approximately 5 pounds of dry ice. ***(Do not include the inner liner (S-4344) used to ship***

islets). Add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

12.7 **Duodenal Tissue**

Due to possibility of contamination of the bowel contents, this step is performed outside of the islet isolation hood and/or lab and will most likely be processed last, unless extra staff can process concurrent with the isolation. Duodenal tissue can be used for donor DNA or other experiments designed by the researcher.

1. Check broadcast records for the number of researchers requesting duodenal tissue. • *If there are no requests for duodenal tissue, it may be discarded in biohazard waste.*
2. Label required 15 mL conical tubes with requesting researchers' names, RRID# of duodenal tissue, and date.
3. Tissue should be maintained at 2-8 °C during prepping process.
4. The duodenal sample should be opened longitudinally and serially washed in two containers of 1% chlorhexidine (2% chlorhexidine stock diluted half and half with HBSS) to guarantee all contents of the duodenum are removed.
5. Divide the duodenal tissue into 5-10 cm pieces to satisfy the requests. Add a piece of duodenum to each labeled tube and fill with cold PIM(T)® Complete.
6. Place tubes in one quart size zip-lock bag, and package as for islets, ***minus the Tip Temp Cold Chain Card***, as described in ***Packaging and Cold Shipping of Human Islets SOP, Step 3 Packaging Islets***.
   Note: Cold samples can be combined into a single package if more than one sample is requested for a single researcher. Also, if islets are requested and will be shipped the following day, samples can be held and shipped with the islets if approved by the researcher.
7. Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, print TSF, affix shipping labels, hold for pick up by carrier.

Collection of Acinar/Non-Islet Pancreatic (Acinar) Tissue (NIPT...

13 ▪ Complete information regarding the collection of ***Acinar/Non-Islet Pancreatic (Acinar) Tissue (NIPT)*** will be available as freshly isolated [Collection, Packaging and Cold Shipping of Fresh Non-Islet Pancreatic (Acinar) Tissue and/or flash frozen [Packaging and Shipping of Flash Frozen Non-Islet Pancreatic (Acinar)Tissue].

13.1 Obtain PIM(T)® Complete prepared in Step 6 go to step #6 .

13.2 The NIPT (the tissue remaining in the COBE purification bag) should be transferred under sterile conditions, into two 250 mL conical tubes, as soon as feasible during the islet isolation process. Top with HBSS, cap, invert to mix and dilute Ficoll, and centrifuge at

180 x g, 4°C, 00:02:00 .

13.3 Obtain the Trypsin inhibitor from Step 5 go to step #5 . Thaw aliquot.

13.4 After centrifugation, calculate the pellet size, evacuate supernatant. If deadline has not yet been reached and the total amounts of NIPT accepted is not yet known, suspend up to 20 mL of pellet to 10 times the pellet volume with **PIM(T)® Complete plus inhibitor (200 mL)**. Lay conical of the NIPT suspension on its side at 4 °C to avoid pelleting of NIPT while waiting to confirm acinar shipments.

♦ *Note: As interest in the program expands and recipient requests increase, more tissue may need to be held.*

Freshly Isolated Acinar/NIPT: Preparation for Shipping

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1. Check broadcast records for the number of researchers and the amounts of acinar cells that were requested.
   • *If no acinar tissue is requested, tissue may be disposed of in biohazard waste.*
2. Calculate the amount of PIM(T)® Complete needed. Complete that which is needed for all shipments. Add 1 mL of Trypsin inhibitor stock to each 100 mL of PIM(T)® Complete for NIPT shipping (~150 mL of media/1 mL NIPT total requested). This will give a final working concentration of 100 µg/mL of Trypsin inhibitor in the media. Resuspend only the amount of NIPT needed to complete the requests in 10x the volume of PIM(T)® Complete plus inhibitor. *Example: If there is one request for 3 mL and two for 1.5 mL, only resuspend 6 mL of NIPT in 60 mL of* ***PIM(T)® Complete plus inhibitor****. This will save on the cost of the inhibitor and media.*
3. Label required bottles with requesting researchers' names, RRID# of acinar tissue, and date.
4. Prepare appropriate amounts of fresh NIPT into proper sized shipping vessel from the diluted samples in Step 13.5 above.

- For 0.5 - 0.75 mL of requested NIPT pellet, add 5 - 7.5 mL of NIPT suspension to 60 mL bottle for shipment.
- For 0.76 – 1.5 mL of requested NIPT pellet, add 7.6-15 mL of NIPT suspension to125 mL bottle for shipment.
- For 1.6 – 3.0 mL of requested NIPT pellet, add 16 - 30 mL of NIPT suspension to 250 mL bottle for shipment.
- For 3.1 – 5.0 mL of requested NIPT pellet, add 31 - 50 mL of NIPT suspension to 500 mL

bottle for shipment. Any sterile, empty PIM media bottle can be used for shipping these large amounts of NIPT.

- Distribute appropriate volume of NIPT into shipping bottles.

14.1 1. Top off the volume of each shipping vessel with ***PIM(T)® Complete plus Trypsin Inhibitor***, stored at 2-8 °C up to the halfway point of the neck of each shipping bottle

- 60 mL bottle - 75 mL of media plus NIPT
- 125 mL bottle - 167 mL of media plus NIPT
- 250 mL bottle - 300 mL of media plus NIPT
- 500 mL bottle - 550 mL of media plus NIPT (Use sterile, empty PIM Media bottle for shipping.)

14.2 Keep the shipping bottles with NIPT in the refrigerator 2-8 °C until ready for packaging.

Packaging of Fresh NIPT

15 Confirm shipments on Broadcast Confirmation List, add Tracking Numbers, and print TSF. Stage all materials needed for packaging the NIPT and ensure all ice packs and CryoPak pouches are at the proper temperature. ♦ *Note: Cold samples of other NIB can be combined into a single package if more than one sample is requested for a single researcher.*

15.1 For **LIVE CELLS only (Fresh Islets, Acinar, and NIPT)**, the Cold Chain Complete card is shipped with the WarmMark indicator attached. For other biospecimens, it is not necessary and a waste of resources. Store the card at least (5°C/9°F) below the **activation temperature of the adhered WarmMark of** 37 °C (therefore less than 32 °C). Room temperature is acceptable.

Attachment 2-Cold Chain Complete Handling Instructions 2020.pdf

15.2 The ColdMark is provided separately because it is always active and should be stored at least 2°C/50°F above **activation temperature of ColdMark of** 2 °C (therefore more than 12 °C). Room temperature is acceptable.

15.3 When ready to use, *adhere the ColdMark indicator to the card and attach the WarmMark*. **ACTIVATE** the WarmMark by folding up and pulling out the tab until completely removed.

Cold Chain Complete Card

15.4 Immediately place the Cold Chain Complete Card (TipTemp Indicator) and the acinar shipping bottles together into a Ziploc bag and seal.

Note

Do not place ColdMark indicators on or near -8 °C frozen cold packs while preparing to ship to prevent accidental triggering of cold indicator.

15.5 Line the inner cardboard (#4434) box with absorbent pad.

Attachment 3-NIPT Packaging Tips.pdf

Attachment 4-Packaging NIPT for Cold Shipping.pptx

15.6 Place the bagged shipping bottle(s) containing the NIPT in the Ziploc bag into the absorbent pad lined, inner cardboard box.

15.7 Surround bottle(s) with six 8 °C cold packs, one on bottom, one on each of the four sides and one on top.

15.8 Mark the direction for upright bottle. Seal the inner box with shipping tape and stand upright.

15.9 Place one 8 °C CryoPak pouch and one -20 °C frozen ice pack (stored in the freezer) in the bottom of the Styrofoam box.

15.10 Place the inner cardboard box containing the NIPT on top of the pouch and pack, ensuring that the bottle inside as well as the inner cardboard box, is lying on its side to ensure the maximum surface area for the acinar tissue to settle and centered in the shipping container.
♦ ***Note: The inner box and bottle are opposite of the way islet tissue is shipped.***

15.11 Place one 8 °C CryoPak pouch and one -20 °C frozen ice pack on the top of the inner box.

15.12 Add a second layer of cold/frozen packs on top, one 8 °C CryoPak pouch and one -20 °C frozen ice pack, on top of the first layer. ♦ *Note: All packs should be placed in an alternating method, to ensure two of the same packs are not next to each other in the configuration.*

15.13 Place the Styrofoam lid on top.

15.14 Place Tissue Shipment Form(s) on top of Styrofoam lid.

15.15 Close the flaps of the shipping box and seal with shipping tape. Attach "Cold Live Cells" label to box.

Attachment 5-Generic Institution Box Label IIDP.pdf

15.16 Attach FedEx shipping label for priority overnight shipment.

Preparation and Packaging of Frozen Acinar/Non-Islet Pancre... 6m

16
- As investigator's requests are determined through the broadcast system for ***Flash Frozen Acinar/Non-Islet Pancreatic (Acinar) Tissue (NIPT)***, prepare appropriate amounts of tissue into proper sized consolidation vessels for freezing from the diluted samples in ***Collection of Acinar-NIPT for Distribution (Fresh and Frozen) Step 13.4*** go to step #13.4 above. Label each conical for identification during transfer. Label corresponding 5mL cryogenic tube (cryotube) with acinar RRID #, date, amount of requested NIPT tissue and recipient name and hold until ready for final transfer.
- For 0.5 – 1.0 mL of requested NIPT pelleted tissue, add 5 - 10 mL of NIPT suspension to 15 mL conical.
- For 1.1 – 2.0 mL of requested NIPT pelleted tissue, add 11-20 mL of NIPT suspension to 50 mL conical. ***Collect an additional 2 mL acinar sample in labeled cryotube (with the specific Acinar RRID#) for IIDP DNA genotyping at Stanford HIGI Lab (Step 16.8).**
- For 2.1 – 3.0 mL of requested NIPT pelleted tissue, add 21-30 mL of NIPT suspension to 50 mL conical.
- For 3.1 – 4.0 mL of requested NIPT pelleted tissue, add 31-40 mL of NIPT suspension to 50 mL conical.

16.1 Centrifuge all samples at 180 x g, 4°C, 00:02:00 to pellet the acinar tissue. Remove the supernatant and wash the tissue with HBSS without serum by topping each conical with appropriate amount of HBSS. This will remove the serum (protein) from the acinar tissue. Resuspend tissue. Repeat the centrifugation 180 x g, 4°C, 00:02:00. 4m

16.2 1. Remove supernatant. Wet pipet and transfer each pellet to corresponding labeled 5 mL cryogenic tube, rinsing original tube with HBSS without serum to ensure all tissue is 2m

transferred. Centrifuge cryogenic vials at 180 x g, 4°C, 00:02:00 to pellet the acinar tissue. Remove all supernatant to ensure a dry, serum-free pellet.

16.3 Freeze pellet by placing tube in liquid nitrogen per your center's protocol.

Safety information

1. Carefully place cryogenic vials in a liquid nitrogen bath taking all safety precautions to protect you from being burned by liquid nitrogen (mask, gloves, tongs, and apron).

Safely transfer the vials into a labeled container and store in a -80 °C or -135 °C freezer or in the vapor phase of a liquid nitrogen storage tank until shipment.

16.4 The shipping center will enter the NIPT to be shipped (Confirm Recipient screen) and will enter FedEx tracking numbers Broadcast System. In addition, the shipping center should process the shipment by using the on-line FedEx process and click the prompt to alert the recipient of the FedEx tracking number. This will help the recipient investigator follow their shipment through the FedEx system and free up time for the centers and the IIDP. The investigators will have the FedEx tracking numbers listed in their History of Acinar Tissue Offers table.

16.5 Once a recipient is confirmed, click on the "Print Tissue Shipment Forms" and include the appropriate form with each shipment.

Note

Note: The center must click on the Print Tissue Shipment Form (TSF) in order to complete the distribution process and trigger the email distribution.

16.6

Note

Frozen NIPT should be maintained below -80 °C until ready for shipment.

1. A completed Flash Frozen Tissue Shipment Form should accompany each sample shipment. Fill a small, thick Styrofoam shipping box with dry ice. Multiple vials of NIPT can be sent in a single box as necessary to satisfy the number of requested NIPT. The vials 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.

16.7 Samples should be sent priority overnight shipping by FedEx with appropriate boxes checked on the FedEx form for dry ice shipments.

16.8 ***Store the additional labeled 2mL sample for DNA genotyping at -80C for bulk shipments to IIDP Stanford HIGI lab, once per quarter.**