Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC) Consortium
Biospecimen Standard Operating Procedures
Version 6 Date: 07.17.20

Contents

Comments: This is the proposed standard operating procedures for collection of biospecimens for the CPDPC. We have incorporated protocols from the EDRN, literature searches, and collaborating CPDPC centers SOP protocols. We also include examples of manufactures and catalog numbers for specific materials. In addition, biospecimen instructional videos are available on the CPDPC website for your reference.

Processing Videos Link: http://cpdpc.mdanderson.org/references.html

NOTE: All material/ supplies that have an asterisk (*) will be essential. All sites must use the exact material/ supply stated in the SOP.

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Blood Products

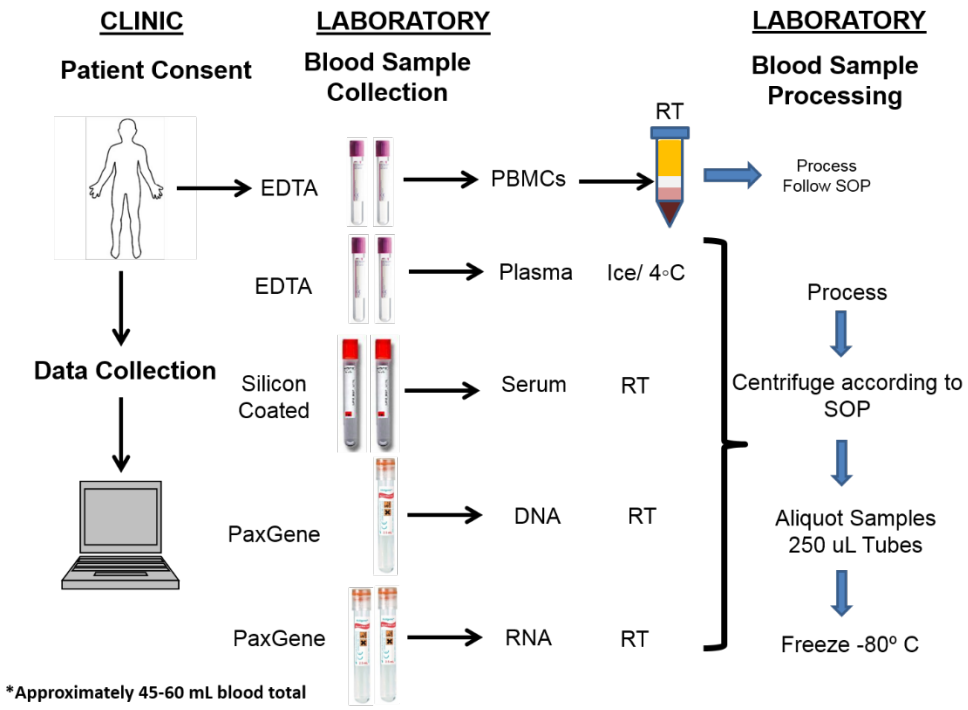


Figure 1. Blood Product SOP Workflow

Purpose

The purpose of this procedure is to outline the process for collecting, processing, and storing of blood specimens and derivatives for the CPDPC Consortium working groups and ancillary study proposals. All blood products are collected and processed within 4 hours.

Materials

1. Biospecimen form (at the end of the SOP)
2. 4 - EDTA tubes (7mL) for plasma and buffy coat [Becton Dickinson, 366450]*
3. 2 - Red top tubes (6mL), no additive, no clot activator, no SST (serum separator tubes) for serum [BD vacutainers catalog# 367815]*
4. 2 PAXgene® Blood RNA Tube (2.5 mL) Catalog No.762165*
5. 1 PAXgene® Blood DNA Tube (8.5 mL) Catalog No.761115*
6. Crushed ice if a delay is anticipated (Always available)
7. Appropriate racks to hold tubes in upright position
8. Refrigerated centrifuge
9. Transfer pipette [Southern Labware, 138080G or similar]
10. Volume adjustable pipette [Eppendorf, ETTR4423 or similar] to accommodate different volumes (i.e. 1uL, 250uL or 1.8mL)

11. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding (CDMC will provide, Request Form in Secured Website)
12. 50 Aliquot cryovials with screw top gasket closure [Fisher Scientific, 12565163N]
13. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider or similar]
14. Cardboard freezer storage boxes [VWR #89214-738 4" Cryogenic box or Divider 49 cell VWR #82021-122 or similar for RNA whole blood]
15. –80ºC Freezers with emergency backup generators

Additional supplies needed for the isolation of peripheral blood mononuclear cells (PBMCs) from buffy coat layer using the EDTA tubes collected and kept at Room Temperature (RT):

Supplies for Preferred/Primary Method of PBMC isolation from buffy coat layer

1. 50 mL conical tubes [Sigma Aldrich, CLS430828]
2. 15 mL conical tubes [Sigma Aldrich, CLS430791]
3. Ficoll Paque-Plus [Sigma Aldrich, GE17-1440-03]*
4. Phosphate-buffered saline sterile [Gibco, 14190-144]
5. Red blood cell ACK lysis buffer [ThermoFisher, A1049201]* or [Lonza, 10-548e]
6. Trypan Blue [Gibco, 15250-061]
7. Microcentrifuge tube [Costar, 3620]
8. Cell Freezing Media (Sigma) [Sigma Aldrich, C6164-50ML]*
9. "Mr. Frosty" freezing container [Nalgene, 5100-0001] [see attachment]
10. C-Chip Disposable Hemocytometer [NanoEnTek, DHC-N01]
11. Micropipette
12. LN2 Freezer for extended storage of samples.

Supplies for Alternative Method of PBMC isolation from buffy coat layer

13. Lymphoprep Separation Medium – STEMCELL Technologies Part #07801, Category #41-11-61-13, stable until manufacturer expiration at or below 20°C protected from light. HMMIS# 88151
14. Leucosep cell separation tubes (12 & 50 mL) (Fischer Scientific (Greiner Bio-One International), Vendor Cat#163290 & 227290)

1. Collection

1.1 Blood collection should be performed by a licensed phlebotomist, nurse, anesthesiologist, or medical doctor, or a clinical research coordinator trained in phlebotomy.
1.2 Collection should be performed in an adequate setting, e.g. in the phlebotomy room, or on the ward. Blood collection in the operating theater should be avoided, if possible.

2. Processing

2.1 Appropriate precaution should be taken for all patient blood samples as the infectious status of specimens cannot always be determined.
2.2 All centrifuge spins should be performed using lids that prevent aerosol release from blood samples.

Notes

- This SOP applies to the collection of serum, plasma, buffy coat, and whole blood.
- This SOP does not cover blood withdrawal techniques/procedures (qualified personnel should follow standard withdrawal procedures).
- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines.
- This SOP does not cover informed consent procedures.
- Definition: g force (G) = relative centrifugal force (RCF)

Plasma (EDTA tube) kept at 4ºC (refrigerated or on wet ice)

1. Collect approximately 7 mL blood in each of the two (2) EDTA blood collection tubes
2. Mix blood thoroughly after draw by inverting the tube 8 to 10 times.
3. Store vacutainer tubes upright at 4ºC (refrigerated or on wet ice) until centrifugation.
4. **Blood samples should be centrifuged, processed and in -80°C freezer within four hours of blood collection.**
5. Centrifuge at 1200g for 10 minutes at room temperature with the **BRAKE ON**. After centrifugation, the sample should separate into 2 layers: top layer is the plasma, and the bottom layer is the white (buffy coat) and red blood cells.
6. Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat from both EDTA tubes into a 15 mL conical tube.
    - Care should be taken when collecting the plasma to prevent disturbing the bottom layer which could result in contamination. NOTE: If one of the tubes are hemolyzed, do not pool.
7. Ensure sample homogeneity.
8. Aliquot using a pipet into 250 µL aliquots. After collecting (10) 250 µl aliquots divide the remainder of the samples into large volume, 1.8 mL aliquots (up to 6) and freeze. When requested, the 1.8 mL tubes should be completely thawed, vortexed and processed into 250 µL aliquots, labelled and logged into system. Place tubes in -80˚C freezer.
9. Care should be taken to prevent disturbing the bottom layer which could result in contamination.

Notes

- Plasma should be free of hemolysis (reddish in color). If the sample is hemolyzed (reddish in color), aliquot the non-hemolyzed sample first, and then finish with the hemolyzed sample.
- Freeze plasma specimens below –80ºC no more than 4 hours after the blood draw. Plasma aliquots can be temporarily (< 4 hours) refrigerated or stored on wet ice (4ºC) until able to freeze at –80ºC. Samples should not be thawed prior to shipping. (Will be shipped on dry ice. Refer to Shipping and Receiving SOP.)

Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated. For an on-line calculator tool, please refer to: http://www.changbioscience.com/cell/rcf.html or http://insilico.ehu.es/mini_tools/rcf_rpm.php

Plasma (EDTA tube) kept at RT for the isolation of PBMCs from the buffy coat layer

Preferred/Primary Method for the isolation of PBMCs from the buffy coat layer

1. Collect 7 mL of blood in each of the two (2) EDTA blood collection tubes.
2. Mix blood thoroughly after draw by inverting the tube 8 to 10 times.
3. Store vacutainer tubes horizontally at room temperature until centrifugation.
4. **Blood samples should be centrifuged, processed and in -80°C freezer within four hours of blood collection.**
    a. Centrifuge at 580g for 10 minutes with the **BRAKE ON** at room temperature.
5. After centrifugation, the sample should separate into 2 layers: top layer is the plasma, and the bottom layer is the white and red blood cells.
    a. Work in Biological Safety Cabinet (BSC) using aseptic technique.
    b. Using a transfer pipet, transfer the top plasma layer into a 50 mL conical tube and divide into (up to 10) 250 µL aliquots (label as plasma from buffy coat SOP).
    c. After collecting (10) 250 µl aliquots divide the remainder of the samples into large volume, 1.8 mL aliquots (up to 6) and freeze. When requested, the 1.8 mL tubes should be completely thawed, vortexed and processed into 250 µL aliquots, labelled and logged into system.
    d. Using a transfer pipet, combine and transfer the entire bottom layer of all the EDTA tubes into a 50 mL conical tube with appropriate label.
6. Rinse the blood collection tube (EDTA) with (10-15 mL) of PBS using a pipet in order to collect all possible cells and transfer this PBS to the 50 mL tube containing the remaining blood.
    a. Fill the 50ml tube with PBS to a volume of 35 mL.
    b. Re-cap the tube, and mix well by inversion 3-4 times.
7. Underlay 13 mL of Ficoll in the 50 mL conical tube using a sterile 10 mL plastic pipette placed beneath the blood at the bottom of the tube, and releasing very slowly. A clear Ficoll layer should form at the bottom of the tube.
8. Carefully cap the tubes and centrifuge them for 30 minutes at 800g with the **BRAKE OFF** to prevent mixing of the layers.
9. Following the spin, the layers will be separated into 3 layers as follows: TOP – PBS, MIDDLE – buffy layer containing PBMCs, BOTTOM – Ficoll, RBC, Granulocytes. (**See Figure 2**). Using a sterile 10 mL plastic pipette, carefully collect the Buffy coat layer and put in a fresh 50 mL tube. It is OK to collect all of the top PBS layer to maximize cell yield. After the cells are collected, dilute the sample to a total of 45-50 mL by adding PBS and centrifuge the sample at 580g for 10 minutes with the **BRAKE ON**.

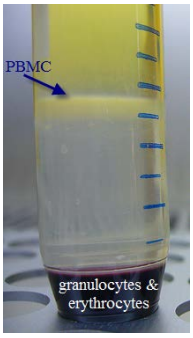


Figure 2. Buffy Coat SOP- 3 layers

10. After the spin, gently pour off the supernatant taking care not to disturb the cell pellet. Re- suspend the pellet in 10 mL of AKC Lysis Buffer and incubate for 2 minutes. Fill the 50mL tube with PBS to a volume of 50mL and centrifuge the sample at 580g for 5 minutes with the **BRAKE ON.**
11. Carefully remove the supernatant taking care not to disturb the pellet. Resuspend the pellet in PBS. For a small pellet, use 5 mL of PBS for counting so you don't have to re-concentrate after counting if the cell suspension is too diluted and falls below the minimum detection of the cell counter instrument being utilized. For large pellets, use up to 10 mL PBS for counting. Count using a suitable method. This may include an automated coulter counter, or manually via trypan blue exclusion on a hemocytometer. An example of this method is presented as follows:
    a. Take 10 µl of suspended pellet and mix with 90 µl of Trypan Blue (1:10) in a 1 or 0.5 mL microcentrifuge tube and count viable cells.

b. Calculations: { [# of cells * 10^4 * dilution factor (in this case 10)] / 4 }* volume of diluted PBMCs
c. Add enough PBS to cell suspension above to bring volume up to 25 mL.
d. Centrifuge the sample at 580 g for 5 minutes with the **BRAKE ON**.
e. After centrifugation, discard PBS by gently pouring it off and resuspend the pellet (this is your buffy coat containing PBMCs) in a suitable volume of Cell Freezing Media (Sigma) so that the cell concentration is between 3 X 10^6 – 5 X 10^6 cells per mL and transfer into labeled cryovials. (Follow the aliquot recommendations below):
    - The total volume is usually ~2 mL with a concentration range of 3 x 10^6 – 5 x 10^6 cells per mL of cell freezing media.
    - Multiple frozen aliquots of cells may be necessary. It is recommend to have at least 1ml of cells/ml in per cryovial at the cell concentration recommended (3 X 10^6 – 5 X 10^6 cells per mL)
    - If your total resuspended volume requires more than one cryovial, aliquot the cells in freezing media equally between cryovials, so that each cryovial has the same volume. (Example: if you have 3 mL of cells at the correct concentration, aliquot the 3 mL into three cryovials at 1 mL each.) Do NOT aliquot less than 500 uL per cryovial. It would be recommended to divide the volume equally to achieve more than 500 ul in each tube, as a concentration of range of 3 X 10^6 – 5 X 10^6 cells per mL.
    - Record the concentration of PBMCs, number of aliquots and the volume of each aliquot on the buffy coat sample acquisition form and in specimen manager. In specimen manager, record the PBMC concentration in the comments section
f. Place the tube into a "Mr. Frosty" (**See Figure 3**) freezing container (Nalgene) at room temperature and then place in -80°C freezer.
g. Remove Mr. Frosty from -80°C after 4-96hrs, place the cryovials in a cardboard freezer storage box (do not let vials thaw, ideally samples should be place in dry ice during transfer) and then placed in vapor phase of liquid nitrogen freezer for long-term storage.

Figure 3. Mr. Frosty
Nalgene 5100-0001

12. All samples (plasma, serum and buffy coat) should remain frozen and shipped on dry ice. (Refer to Shipping SOP.)

Alternative/ Secondary Method for the isolation of PBMCs from the buffy coat layer

1. Work in BSC using aseptic technique.
2. Gently invert blood tube several times until well mixed.
3. Using a biohazard wipe, remove the specimen cap and pour blood into appropriately labeled separation tube. Repeat this process for each blood sample, working with one at a time.
4. Centrifuge the separation tubes for 10 minutes at 1000 RCF at 20oC.
5. **Using a transfer pipet, transfer the top plasma layer into a 50 mL conical tube and divide into (up to 10) 250 µL aliquots (label as plasma from buffy coat SOP).**
6. Make certain not to disrupt the PBMC layer. This will help to prevent contamination of the PBMC's with platelets.

   a. After collecting (10) 250 µl aliquots divide the remainder of the samples into large volume, 1.8 mL aliquots (up to 6) and freeze. When requested, the 1.8 mL tubes should be completely thawed, vortexed and processed into 250 µL aliquots, labelled and logged into system.
7. Using a sterile transfer pipette, transfer PBMC layer to the labeled intermediate tube.
8. Add RPMI to a total volume of 13 mL. Invert gently until mixed.
9. Centrifuge at 250 RCF for 10 minutes at 20oC.
10. Aspirate the supernatant almost to the cell pellet (leaving cell pellet undisturbed).
    a. Use a sterile glass Pasteur pipette with an Erlenmeyer vacuum flask
11. After the spin, gently pour off the supernatant taking care not to disturb the cell pellet. Re- suspend the pellet in 10 mL of AKC Lysis Buffer and incubate for 2 minutes. Fill the 50mL tube with PBS to a volume of 50mL and centrifuge the sample at 580g for 5 minutes with the **BRAKE ON.**
12. To the intermediate tube containing cell pellet, add PBS up to the 10 mL mark.
13. Gently mix sample using a disposable sterile transfer pipette to re-suspend cells, create aliquot for cell counting.
    a. Perform cell counting on aliquot using AO/PI stain and automated counter
14. Centrifuge intermediate tube containing cells/PBS at 250 RCF for 10 minutes at 20oC.
15. Aspirate the supernatant almost to the cell pellet using a glass Pasteur pipette and Erlenmeyer vacuum flask.
16. Add a suitable volume of freezing medium (Sigma) to the cell pellet so that the cell concentration is between 3 X 10^6 – 5 X 10^6 cells per mL (See aliquoting recommendations in section 11e)
17. Gently re-suspend the cell pellet using a sterile transfer pipette.
18. Transfer no less than 500µl - 1 mL cell suspension to each of the labeled 1.8 mL cryovials.
    a. Isolated cells may be temporarily stored refrigerated for up to 4 hours <u>from the time of blood collection if it is necessary but delay to cryopreserve the cells (i.e. to go to step 19) should be avoided</u>.
19. Cryopreserve cells by placing the tube into a "Mr. Frosty" (See Figure 3) freezing container (Nalgene) at room temperature and then place in -80°C freezer.
20. Remove Mr. Frosty from -80°C after 4 - 96 hrs, place the cryovials in a cardboard freezer storage box (do not let vials thaw, ideally samples should be place in dry ice during transfer) and then placed in vapor phase of liquid nitrogen freezer for long-term storage.
21. Upon completion of controlled rate freezing, transfer cryovials into the vapor phase of liquid nitrogen freezer for long-term storage.

Note: A separation tube is a conical tube with a frit. We fill this separation tube with a separation gradient media similar to Ficoll. The frit simply allows a technologist to pour the blood in versus gently layering it over the media and more easily harvest the cells after the first centrifugation step. We have compared this technique to the Ficoll methodology and have observed comparable yields and viability results with a slight improvement in the %CV over the manual Ficoll process.

<u>Serum</u>

1. Collect approximately 6mL in each of the two (2) red blood collection tubes ("vacutainers").
   Note: Use red top (serum) tubes (silicon-coated)—no additives and not SST (serum separator tubes). These tubes, without additives, allow the red blood cells to form a clot. The clot also includes white blood cells, platelets etc. After centrifuging, the clot is at the bottom of the tube, and the serum is on top of the clot). The red top tubes do not have to be full to be used.
2. Filled red top blood collection tubes should sit upright after the blood is drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form. If the blood is not centrifuged immediately after the clotting time (30 to 60 minutes at room temperature), the tubes should be refrigerated or on wet ice (4ºC) for no longer than 4 hours.

3. Centrifuge at 1200g for 10 minutes with the **BRAKE ON** at room temperature.
4. Pull serum in 15mL conical tube and mix.
5. Aliquot the serum (the supernatant or upper layer) into the cryovials with appropriate labels. Aliquot volume is required to be 250 µl. After collecting ten (10) 250 µl aliquots divide the remainder of the samples into large volume aliquots, 1.8 mL (up to 6), freeze, and later, thaw when aliquots are requested for analysis. When requested, the 1.8 mL tubes should be completely thawed, vortexed and processed into 250µL aliquots, labelled and logged into system.
6. Close the caps on the cryovials tightly. This process should be completed within 1 hour of centrifugation.
   Note: Use all serum except the last 1/4 inch to avoid red blood cell contamination. If a gel-like mass is present, pierce gently with a pipette tip and re-centrifuge at 1200g for 5 minutes, then aliquot into desired amount. Aliquot as much as the serum yields. Serum should be free of hemolysis (reddish in color). If the sample is hemolyzed (reddish in color), discard.
7. Check that all aliquot vial caps are secure and that all vials are labeled.
8. **Freeze serum specimens–80ºC no more than 4 hours after the blood draw.** Serum aliquots can be temporarily refrigerated or stored on wet ice (4°C, < 4 hours) until able to freeze at –80ºC.
9. The samples should not be thawed prior to shipping. (Serum will be shipped on dry ice. Refer to Shipping and Receiving SOP.)

Whole Blood for DNA (PAXgene)

1. Collect approximately 8.5 mL of blood in one (1) 8.5 mL PAXgene Blood DNA tube.
2. Mix blood thoroughly after draw by inverting the tube 8 to 10 times.
3. Aliquot blood into cryovials with appropriate labels. Aliquot volume is required to be 1.7 mL.
4. Collect five (5) 1.7 mL aliquots.
5. Check that all aliquot vial caps are secure and that all vials are labeled.
6. Freeze whole blood specimens –80ºC.
7. Samples should not be thawed prior to shipping. (Samples will be shipped on dry ice. Refer to Shipping and Receiving SOP.)

Whole Blood for RNA (PAXgene)

1. Collect 5 mL of blood into two (2) of the 2.5 mL PAXgene Blood RNA tubes.
2. Mix blood thoroughly after draw by inverting the tube 8 to 10 times.
3. **Allow tubes to sit upright at room temperature for a minimum of 2 hours and maximum of 72 hours, then freeze.**
4. Freeze tube upright in -20°C freezer for at least 24 hours.
5. Place frozen tube in -80°C freezer until shipped.
6. Samples should not be thawed prior to shipping. (Samples will be shipped on dry ice. Refer to Shipping and Receiving SOP.)

Whole Saliva

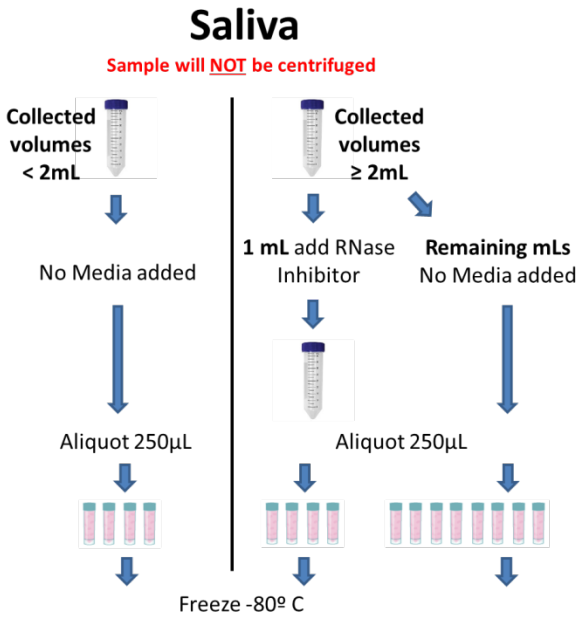


Figure 4. Saliva SOP Workflow

Purpose

The purpose of this procedure is to outline the process for collecting, processing, stabilizing and storing of saliva specimens for the CPDPC. All saliva is collected and processed within 4 hours.

Notes

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel much follow institutional biosafety guidelines.
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (at the end of the SOP)
2. Sterile saliva collection container [Corning, Inc. Falcon 50 mL Conical Centrifuge Tube, 14-959-49A, or similar]
3. RNnase inhibitor [SUPERase-IN RNase Inhibitor stock AM2694; Ambion, Austin, TX]*
    - Stock solution as supplied by manufacturer.
4. Picture of lemon [provided]
5. Crushed ice (always)
6. Transfer pipette [Southern Labware, 138080G or similar]
7. Volume adjustable pipette [Eppendorf, ETTR4423 or similar] to accommodate different volumes (i.e. 1uL, , 250uL or 1.8mL)
8. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding (CDMC will provide, Request Form in Secured Website)
9. Aliquot cryovials with screw top gasket closure [Fisher Scientific, 12565163N]
10. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider]

11. –80ºC Freezers with emergency backup generator.

1. Collection

1.1 The research participant will rinse mouth out with water.

1.2 Participant should avoid use of the following before giving a saliva sample

1.2.1 Brushing teeth/ eating meal > 1 hour

1.2.2 Mouthwashes or fluoride rinses > 1 hour

1.2.3 Smoking or chewing gum > 30 min

1.2.4 Alcoholic drink > 12 hours

1.3 **Unscrew cap and have participant hold a sterile Falcon 50 mL specimen collection tube that is seated in a cup of crushed ice.**

1.4 Ask participant to close mouth and think of favorite food or provide a visual stimulant such as lemons.

1.5 Ask participant to allow saliva to pool in mouth for approximately 1-2 minutes.

1.6 Tilt head forward.

1.7 Ask participant to gently push the (passive) drool into the Falcon collection tube with their tongue.

1.8 Obtain maximum volume of saliva in 5 minutes (approximately 2-3 mL of saliva apart from foam/bubbles). Try to keep bubbles to a minimum.

1.9 **Collect saliva for up to 5 min only.**

1.10 Replace cap on falcon tube.

2. Specimen processing

2.1 Keep the sample refrigerated or on wet ice (4ºC) until processed

2.2 **Collected saliva volumes will vary**

2.3 **For collected volumes < 2mL- Priority #1**

2.3.1 From collection falcon tube, aliquot 250 µl of the sample into cryovials with appropriate labels.

2.3.2 Do NOT centrifuge. Do NOT add any media.

2.3.3 Aliquot volume is required to be a minimum of 250 µl.

2.4 **For collected volumes ≥ 2mL- Priority #2**

2.4.1 Transfer **first** 1mL of saliva to a conical tube and add RNase inhibitor.

- Add SUPERase- IN RNase Inhibitor stock (AM2694; Ambion, Austin, TX) to saliva to a final concentration of 0.02 U/mL (1 µL of 20 U/µL stock per mL of saliva). Attached datasheet from Ambion.
- For example, if you collect 1mLs of saliva, add 1 µL of the 20 U/µL stock solution of SUPERase- IN

2.4.2 Mix tube thoroughly after adding inhibitor by capping the conical tube and inverting the tube 8 to 10 times.

2.4.3 From conical tube aliquot four (4), 250 µl of the sample into cryovials with appropriate labels.

2.4.4 Transfer the remaining saliva from collection falcon tube to cryovials with appropriate labels.

2.4.5 Aliquot volume is required to be a minimum of 250 µl.

2.4.6 Do NOT centrifuge. Do NOT add any media.

2.5 Check that all aliquot vial caps are secure and that all vials are labeled with barcodes.

2.6 Freeze whole saliva specimens below –80ºC no more than 4 hours after collection.

2.7 Samples should not be thawed prior to shipping. (Will be shipped on dry ice. Refer to Shipping and Receiving SOP.)

Urine

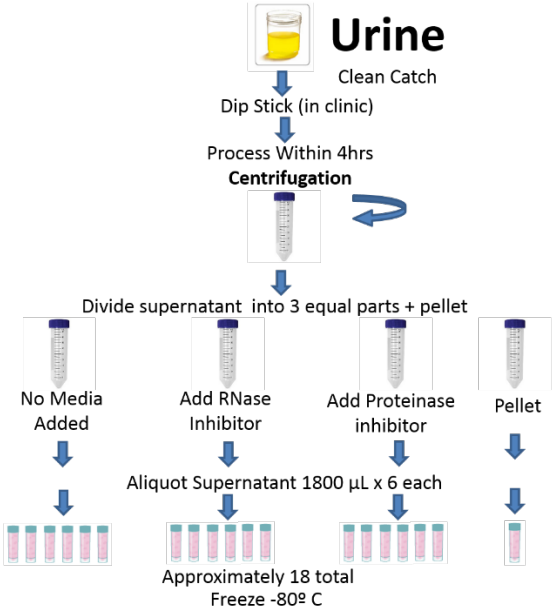


Figure 5. Urine SOP Workflow

Purpose

The purpose of this procedure is to outline the process for collecting, processing, and storing of urine specimens for the CPDPC. All urine is collected and processed within 4 hours.

Notes

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines.
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (at the end of the SOP)
2. Sterile urine collection container with a wide mouth and a leak-proof cap [Vendor: Cardinal Health, Catalog #: 13594-130]
3. Protease inhibitor stock [cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks 04693116001 Roche]*
    - For stock preparation=Dissolve 1 tablet in 2 ml of distilled water (25x concentration).
4. Rnase inhibitor [SUPERase-IN RNase Inhibitor stock AM2694; Ambion, Austin, TX]*
    - Stock solution as supplied by manufacturer.
5. Dipstick for urine analysis [Siemens multistix 10 SG reagent strips, SI2161 or similar]
6. Crushed ice if a delay is anticipated
7. Refrigerated centrifuge
8. Transfer pipette [Southern Labware, 138080G or similar]
9. Volume adjustable pipette [Eppendorf, ETTR4423 or similar] to accommodate different volumes (i.e. 1uL, 250uL or 1.8mL)
10. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding (CDMC will provide, Request Form in Secured Website)
11. Aliquot cryovials with screw top gasket closure [Fisher Scientific, 12565163N]
12. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider]
13. –80ºC Freezers with emergency backup generators
14. 50mL conical tubes [Sigma Aldrich, CLS430828]
15. 15mL conical tubes [Fisher Scientific, 14-959-53A or similar]
16. Clean Catch
17. Castile soap towelette [Fisher Scientific, 360994377 or similar]

1. Collection

1.1 Subjects will be instructed to be well hydrated with a full bladder for urine collection.

1.2 Urine will be collected in clinic.

1.3 Collect by clean catch at least ~50+ mL of urine into an approved Urine Collection Cup that measures volume and seal immediately and place on ice or in the refrigerator (4ºC).

1.4 Patient Instructions for Clean Catch Urine Sample

1.4.1 Wash your hands with soap and warm water.

1.4.2 Cleanse your urethral area with a castile soap towelette.

1.4.3 Urinate a small amount into the toilet bowl, then stop the flow of urine.

1.4.4 Hold the urine cup a few inches from the urethra and urinate until the cup is about half full.

1.4.5 You may finish urinating into the toilet bowl.

1.5 Discard the sample if it contains blood or if it severely cloudy.

1.6 Mix the sample by either swirling the cup or pipetting the urine up and down a couple of times.

1.7 In clinic or laboratory, decant a small amount of urine into a **separate container** to perform dipstick urine analysis and record all results. **NOTE:** Do not insert dipstick into urine container to avoid contamination of sample.

1.7.1 If specific gravity is lower than 1.001 or greater than 1.032, retest for accuracy and discard if confirmed.

1.7.2 If patient shows signs of infection per the urine dipstick (i.e. positive for nitrates and/or moderate leukocyte esterase), then do not collect the urine until infection has been cleared.

2. Processing

2.1 Keep the sample refrigerated or on wet ice (4ºC) until processed and complete processing within 4 hours of collection.

2.2 Fill a 50 mL conical tube with 36 mLs of urine from the sample collection container and centrifuge at 1200 g at 4ºC for 10 minutes with the **BRAKE ON** (make sure the centrifuge is at 4ºC before spin starts) If less than 36 mLs, then centrifuge the entire sample.

2.3 Aliquot the supernatant (upper layer) into 3 equal parts (if 36 mLs, aliquot into 12 mLs) each into (3) 15 mL conical tubes with appropriate labels.

2.3.1 1 conical tube should be fresh, no media added.

2.3.2 To 1 conical tube add Rnase inhibitor.

- Add SUPERase- IN RNase Inhibitor stock (AM2694; Ambion, Austin, TX) to urine to a final concentration of 0.02 U/mL (1 µL of 20 U/µL stock per mL of urine). Attached datasheet from Ambion.
- <u>For example, if you collect 12mLs of urine, add 12 µL of the 20 U/µL stock solution of SUPERase- IN</u>

2.3.3 To the last conical tube add Protease inhibitor.

- Stock solution is at 25x (1 tablet in 2 mL distilled water). Attached datasheet from Roche.
- <u>For example, if you collect 12 mLs of urine, add 480 µL of the 25X stock solution of Protease inhibitor</u>
- Calculation example for 10 ml sample:

$$(25x)\ (X\ mL) = (1x)\ (12\ mL)$$
$$X\ mL = (1x)\ (12\ mL)/\ (25x)$$
$$X\ mL = 480\ \mu L \text{ of a } 25x \text{ in your } 12mL \text{ tube of supernatant}$$

2.3.4 Place the pellet in a cryovial and freeze at –80ºC. If necessary resuspend the pellet in 10 µl of PBS or leftover urine.

2.3.5 Discard any excess sample.

2.4 Mix tube thoroughly after adding inhibitors by capping them and inverting the tube 8 to 10 times.

2.5 From conical tubes aliquot the sample into cryovials with appropriate labels. Aliquot volume is required to be 1800 µl. If possible collect 18 aliquots – 6 Fresh, 6 with RNase inhibitor, and 6 with Protease inhibitor.

2.6 Place the pellet in a cryovial and freeze at –80ºC. If necessary resuspend the pellet in 10 µl of PBS.

2.7 Check that all aliquot vial caps are secure and that all vials are labeled.

2.8 Freeze urine specimens below –80ºC no more than 4 hours after collection.

2.9 The samples should not be thawed prior to shipping. (Urine will be shipped on dry ice. Refer to Shipping SOP.)

Stool (Microbiome Profiling)

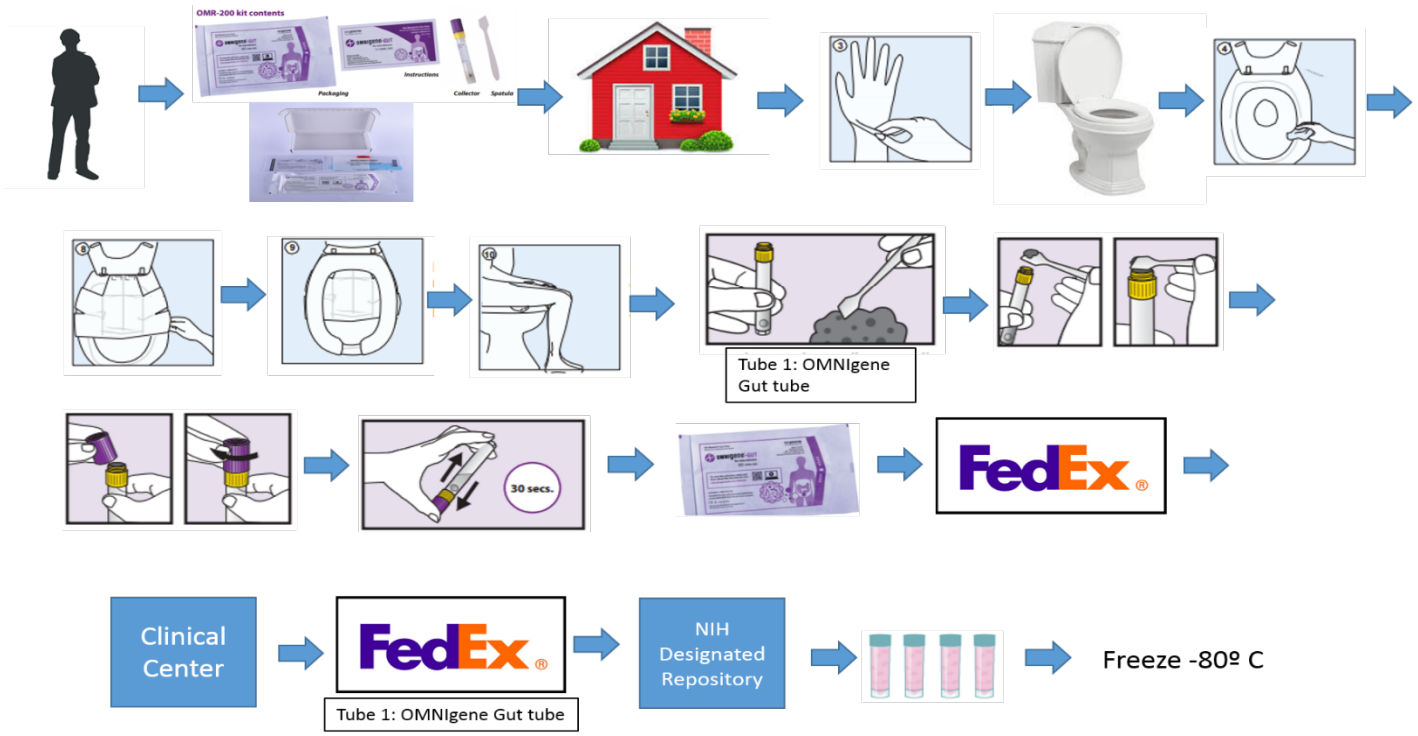


Figure 6. Stool Microbiome SOP Workflow

Purpose

The purpose **OMNIgene•GUT Kit** of this procedure is to outline process for collecting, processing, and storing of stool specimens for microbiome profiling for the CPDPC. **EasySampler® Stool Collection Kit** the

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel much follow institutional biosafety guidelines
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (at the end of the SOP)
2. Stool for Fecal Elastase: ALPCO; EasySampler® Stool Collection Kit; 58-EZSAMPLER*
3. Stool for Micrbiome Profiling and Long Term Storage: OMNIgene•GUT (OMR-200) [DNAgenotek, OMR200]*
4. Prepaid return shipping label and envelope container
5. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding (CDMC will provide, Request

Form in Secured Website

6. Aliquot cryovials with screw top gasket closure [Fisher Scientific, 12565163N]
7. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider]
8. –80ºC Freezers with emergency backup generators
9. Biohazard specimen transport bag [Fisher Scientific, 01-800-00 or similar]
10. 1 specimen shipping containers [Therapak, 56442, https://www.therapak.com/catalog/insulated_shippers]* [one for NIH designated repository (Baylor College of Medicine)]

1.0 Collection Method

1.1. The participant will be provided two kits for microbiome profiling: (1) EasySampler® [Stool Collection] Kit and (2) OMNIgene•Gut Kit for [Microbiome Profiling] and long term storage.
1.2. Participants will be instructed on how to the use the kits and how to return the sample to their clinical center. Instructional video on the CPDPC website will be shown and reviewed with the study participant.
1.3. Participant should avoid providing a stool sample if they have: a) active hemorrhoids b) menstrual bleeding, c) known stomach or colon infections, or d) traces of urine in stool sample
1.4. At home the participant will follow the EasySampler® and the OMNIgene•GUT (OMR-200) kit instructions. See below for more details.

NOTE: Stool Collection

- **The EasySampler Kit is being used to collect stool sample for the CPDPC.**

1.5. The research participant will be provided a stool collection kit.
1.6. The stool collection kit that the research participant will be provided includes:

A. EasySampler® Kit for stool collection:

1.6.1. EasySampler® instructions
1.6.2. 1 EasySampler® collection hat (paper portion)
1.6.3. 1 pair of disposable gloves
1.6.4. 1 Identification label
1.6.5. 2 collection spoons
1.6.6. 1 leak-proof primary container with brown screw cap
1.6.7. 1 leak-proof secondary container with absorbent material and white screw cap.
1.6.8. 1 padded envelope marked for mailing biological sample (stool)

NOTE: The Easy Sampler Kit primary and secondary containers will not be used to collect stool for microbiome. The OMNIgene•GUT kit contains a special tube with preservatives for stool collection.

NOTE: Microbiome Profiling

- **The OMNIgene•GUT collection kit is being used to stabilize and preserve stool for microbiome profiling and long term storage.**
  - For collecting stool for microbiome profiling and long term storage, the participant will use the OMNIgene GUT instructions below.

Stool for Microbiome Profiling and Long Term Storage Collection In brief: OMNIgene•GUT (OMR-200)

1.8 Collect stool sample using EasySampler Stool Collection Kit
1.9 Unscrew ONLY the purple cap from the OMNIgene•GUT (OMR-200) collection device and set aside.
1.10 Use spatula to collect a small amount of stool sample.
1.11 Transfer the stool into the yellow tube top.
1.12 Scrape horizontally across the yellow tube to level the sample and remove excess.
1.13 Screw the purple cap back onto the yellow tube top until tightly closed.
1.14 Shake the sealed tube for a **minimum of 30 seconds**.
1.15 The stool sample will be mixed with the stabilizing liquid in the tube.
Note: Not all particles will dissolve.
1.16 Participants will write their identification information in the purple tube top.
1.17 Place purple tube into biohazard bag.

Shipping from the participant

1.18 The participant will then insert the biohazard bag with the purple tube top in the mailing envelope and ship Therapak specimen container at ambient temperature to their clinical center as instructed.
[Insert Contact and Address]

2.0 Shipping from Clinical Center

2.1 The stool for microbiome profiling and long term storage will be shipped to NIH designated repository (CPDPC-Baylor College of Medicine) on a weekly basis (less than 14 days after collection) at ambient temperature in a Therapak specimen container.
2.2 Place the label "stool" on the Omni-gene tube and ship the remaining stool labels with the sample for microbiome processing at Baylor .
2.3 When preparing the package to Baylor, please complete the following forms and include a hard copy in the package and email an electronic copy to mcross@bcm.edu :

2.3.1 Complete Chain of Custody and Sample Inspection Form
- Fill out form with your institution's details
- Under Comments, add Shipment ID generated from Specimen Manager.

2.3.2 CMMR Metadata Capture Form
- Fill out the header with your institution's details
- On the spreadsheet only complete
- SampleID = Specimen ID
- SourceID = Subject Registration ID
- SampleSource will always be "Human"
- SampleType will always be "Stool"
- You do not need to complete the Box Position

2.4 Once the shipped label is created, go into Specimen Manager (under specimen collection) and enter the Specimen ID and shipment information. You can use the same container that patients used to ship the samples.

Ship packages to:
Center for Metagenomics and Microbiome Research
1 Baylor Plaza, Room 825E
Houston, Texas 77030

Re: CPDPC16-02 PROCEED STUDY

3.0 Processing: OMNIgene•GUT (OMR-200) - NIH designated repository (CPDPC-Baylor College of Medicine)

3.1 The kit can stabilize DNA at ambient temperature for 14 days, no cold chain required
3.2 Once the sample has been receive by Baylor College of Medicine, it will be divided in to 4-5 aliquots
3.3 Aliquot volume is required to be 100 mg.
3.4 Check that all aliquot vial caps are secure and that all vials are labeled.
3.5 Freeze stool specimens below –80ºC.
3.6 The samples should not be thawed prior to future shipping. (Stool will be shipped on dry ice. Refer to Shipping SOP.)

Stool (Fecal Elastase)

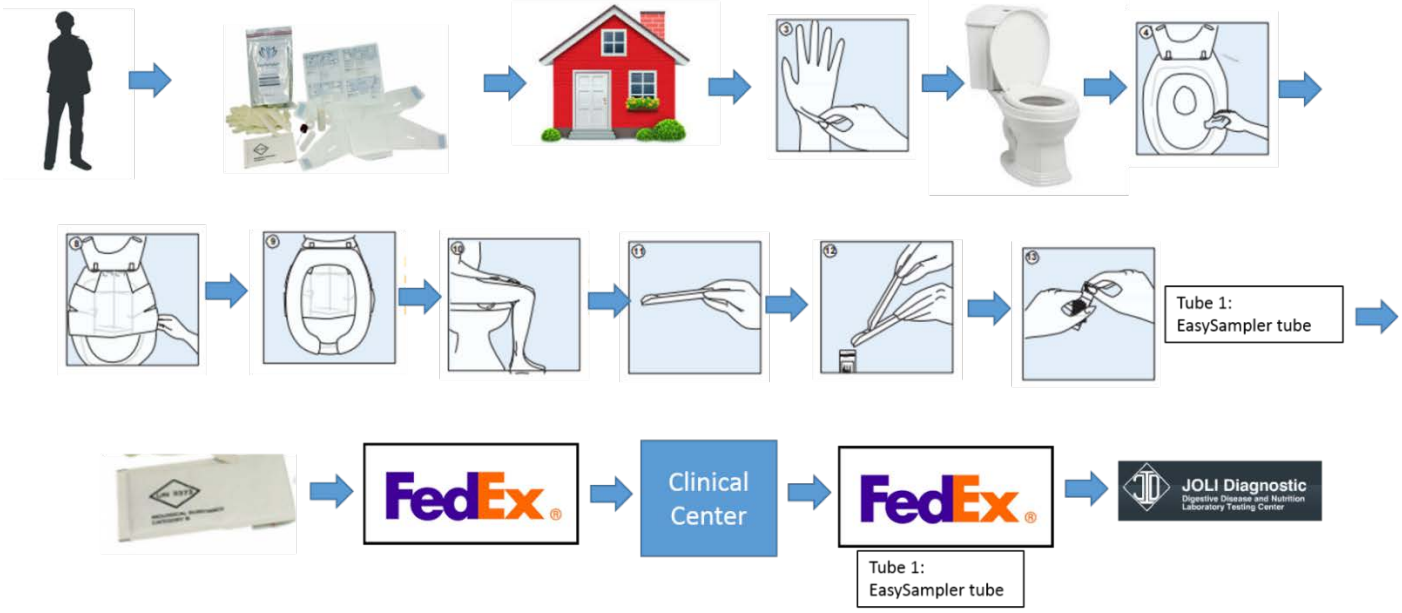


Figure 7. Stool Fecal Elastase SOP Workflow

EasySampler® Stool Collection Kit

Purpose

The purpose of this procedure is to outline the process for collecting, processing, and storing of stool specimens for the CPDPC.

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel much follow institutional biosafety guidelines
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (at the end of the SOP)
2. Stool for Fecal Elastase: ALPCO; EasySampler® Stool Collection Kit; 58-EZSAMPLER*
3. Prepaid return shipping label and envelope container
4. Cold Pak [Cypress Medical brand Fishersci # 19898725]*
5. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding (CDMC will provide, contact Request Form in Secured Website)
6. –80ºC Freezers with emergency backup generators
7. 1 specimen shipping containers [Therapak, 56442, https://www.therapak.com/catalog/insulated_shippers]* [one for JOLI Diagnostics]

1.0 Collection Method

NOTE: Stool Collection

- **The EasySampler Kit is being used to collect stool sample for the CPDPC.**
  - For collecting stool for fecal elastase, the participant will use the EasySampler kit instructions below.

1.1. The research participant will be provided a stool collection kit for (A) fecal elastase.
1.2. The stool collection kit that the research participant will be provided includes:

B. EasySampler® Kit for Fecal Elastase:

1.2.1. EasySampler® instructions
1.2.2. 1 EasySampler® collection hat (paper portion)
1.2.3. 1 pair of disposable gloves
1.2.4. 1 Identification label
1.2.5. 2 collection spoons
1.2.6. 1 leak-proof primary container with brown screw cap
1.2.7. 1 leak-proof secondary container with absorbent material and white screw cap.
1.2.8. 1 padded envelope marked for mailing biological sample (stool)

1.3. Participants will be instructed on how to the use the kits and how to return the sample to the clinical center.
1.4. Participant should avoid providing a stool sample if they have: a) active hemorrhoids b) menstrual bleeding, c) known stomach or colon infections, or d) traces of urine in stool sample
1.5. At home the subject will follow the EasySampler® kit instructions. See attached for more details.

Note: Stool for Fecal Elastase Collection In brief: ALPCO; EasySampler® Stool Collection Kit (Fecal / Pancreatic Elastase measurement) – JOLI Diagnostics

1.6. Collect stool sample in paper collection toilet hat.
1.7. Open the outer container (white top) and remove inner sample container (brown top).
1.8. Unscrew the brown top from container and set aside.
1.9. Collect a small amount of stool using paper spoon.
1.10. Scoop the stool into the brown top container.

1.11. Screw the brown top onto the container until tightly closed.
1.12. Insert brown top container (with sample) into white top outer container.
1.13. Screw the white top onto the container until tightly closed.
1.14. Place white top receptacle into padded envelope.

Shipping from the participant

1.15. The participant will then insert the tube in the mailing envelope and ship with 2 Cold Packs (one on top and one on bottom) in a Styrofoam box.
1.16. The Styrofoam box will be placed into an outer container, where the shipping label will be added.
1.17. Then ship to clinical center as instructed.
[Insert Contact and Address]

2.0 Shipping: Fecal / Pancreatic Elastase Measurement – JOLI Diagnostics

2.1 The stool for fecal elastase will be shipped to JOLI Diagnostics on a monthly basis.
NOTE: JOLI may accept samples from the clinical lab and/or the research lab. This may vary at your center. Please work with your clinical or research lab on transporting, billing and any paperwork you may need to drop off samples to them.

2.2 When sending samples to JOLI, complete the following:

2.2.1 For RESEARCH FECAL ELASTASE:
2.2.1.1 CPDPC can use JOLI FED EX Number: 201323118
2.2.1.2 Write: CPDPC PROCEED STUDY at top of JOLI Requisition (see attached)
2.2.1.3 Write correct Billing address and FAX Number on requisition
2.2.1.4 Will FAX results end of testing day

2.2.2 For Standard CLINICAL FECAL ELASTASE:
2.2.2.1 Send via your clinical laboratory
2.2.2.2 JOLI will bill normally

2.3 Make sure each sample has patient's name and DOB and/or MRN #.
2.4 Specimen Stability - Room temperature: 5 days; Refrigerated: 7 days; Frozen: 1 year
2.5 Do not add fixative or preservative.
2.6 Patients will ship samples on Cold Pak (Refrigerated) in Therapak containers.
2.7 Upon receiving samples, site will freeze the samples at -20 C to -70C until monthly shipment.
2.8 All collected samples will be shipped monthly on dry ice.
2.9 Pack in enough dry ice to last up to 48 hours.
2.10 Ship overnight by appropriate carrier (i.e. UPS/FedEx) for a weekday delivery (Ship Monday-Thursday only)

Ship Packages to:
JOLI Diagnostic Inc.
2451 Wehrle Drive
Williamsville, NY 14221

The results will be faxed and mailed to the clinical center following assay completion.

Pancreas Fluid

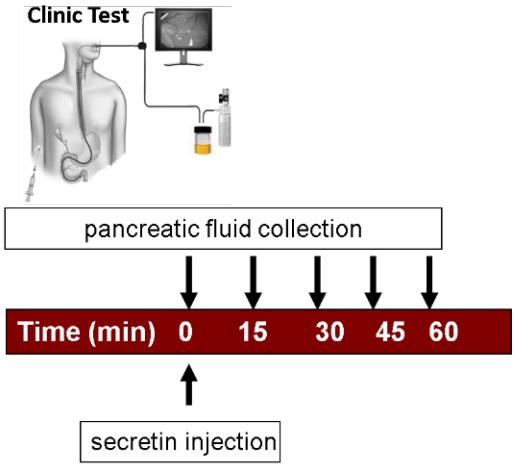


Figure 8. Endoscopic PFT Collection

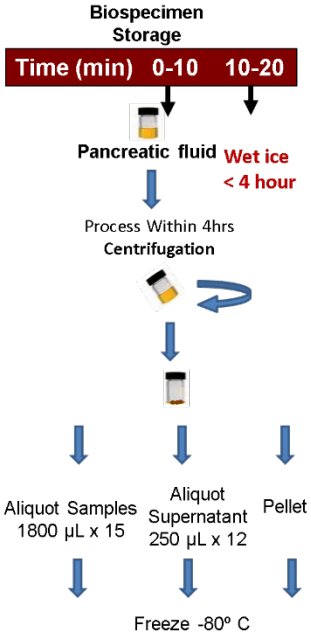


Figure 9. Pancreas Fluid SOP Workflow

Purpose

The purpose of this procedure is to outline the process for collecting, processing, and storing of pancreas juice specimens for the CPDPC. All pancreas fluid is collected and processed within 4 hours.

NOTES

- This SOP does not cover juice extraction techniques/procedures (qualified personnel should follow standard extraction procedures).
- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines.
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (at the end of the SOP)
2. Sterile container for initial collection [Corning, Inc. Falcon 50 mL Conical Centrifuge Tube, 14-959-49A, or similar]
3. **Note: Do not use a Protease or RNA inhibitors for processing and storage - PENDING ANCILLARY STUDY RESULTS**
    - Protease inhibitor stock [cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks 04693116001 Roche]*
        - For stock preparation=Dissolve 1 tablet in 2 ml of distilled water (25x concentration).
    - Rnase inhibitor [SUPERase-IN RNase Inhibitor stock AM2694; Ambion, Austin, TX]*
        - Stock solution as supplied by manufacturer.
4. Crushed ice if a delay is anticipated
5. Transfer pipette [Southern Labware, 138080G or similar]
6. Volume adjustable pipette [Eppendorf, ETTR4423 or similar] to accommodate different volumes (1uL, 1.8mL, or 250uL)
7. Centrifuge
8. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding (CDMC will provide, Request Form in Secured Website)
9. Suction Specimen traps [40 mL Specimen Trap; MediChoice; 3583MST4000]
10. Aliquot cryovials with screw top gasket closure [0.5 mL, 1.8 mL, and 5 mL] [Fisher Scientific, 12565163N]
11. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider]
12. –80ºC Freezers with emergency backup generators
13. Secretin [ChiRhoStim®] [A minimum of 12 vials will be purchased by your research pharmacy]

1.0 Duodenal Fluid Aspiration (ePFT) – EGD or EUS Collection Method

1.1. Aspirate and discard all fluid from the stomach prior to pancreas juice collection.
1.2. Aspirate fluid from the duodenum and either discard or save as a baseline specimen, per protocol.
1.3. A test dose of human synthetic secretin 0.2 mcg (0.1 mL) is injected intravenously to test for possible allergies. If there are no signs of allergic reaction, ChiRhoStim® at a dose of 0.2 mcg/kg of body weight (but no more than the (maximum dose of 16 mcg) is injected intravenously over 1 minute.
1.4. Aspirate all specimens through the working channel of an endoscope into a suction specimen trap attached directly to the endoscope.
1.5. **Clinic Test**: Aspirate fluid from the duodenum into a suction trap continuously in separate, labeled timed aliquots: up to 60 minutes after secretin administration.
1.6. Aliquots (approximately 2-3 mL) from all time periods are sent to the clinical lab for bicarbonate analysis. PMID:19806083, PMID:15332022
1.7. **Biorepository storage**: Aliquots of fluid from the 0-10 minutes and 10-20 minute time periods are processed separately for biorepository storage as described below.

2.0 Pure Pancreatic Juice Collection (PPJ) – ERCP Collection Method

2.1 Pure pancreas juice (PPJ) is collected during ERCP. PPJ should only be collected under IRB- approved protocols and with informed consent.
2.2 Pancreatography should be performed prior to PPJ collection to assess for ductal abnormalities.
2.3 An ERCP cannula is positioned in the pancreatic duct. Place the catheter a short distance up the duct from

the ampulla.

2.4 Aspirate out contrast material prior to PPJ collection.

2.5 Administer secretin intravenously as described above (section 1.3).

2.6 Gently and continuously aspirate fluid from the pancreas duct via the ERCP cannula, repositioning the catheter as needed. Discard fluid that is likely contaminated by contrast material.

2.7 Collect PPJ at 5 minute intervals for 30 minutes. Place immediately on wet ice.

2.8 A one mL aliquot from each time point should be sent on wet ice to the local clinical lab for bicarbonate concentration. This aliquot should be placed in a plastic vial, not a cryovial. The vial to be used should be chosen in consultation with your local lab. An autoanalyzer should be used for this assay per cited reference.

2.9 Aliquots of fluid from the 0-10 (combined) minutes and 10-20 (combined) minute time period is processed separately for biorepository storage as described below.

2.10 Process remaining PPJ from each time interval as above (1.6; PMID: 19806083, PMID: 15332022).

3.0 Processing – Clinical Laboratory testing (3.2) and Biorepository storage (3.3 – 3.10)

3.1 After the pancreas juice has been extracted, it should be handled on wet ice (4ºC) while being aliquoted. (PMID: 20589857)

3.2 **Clinical lab test processing (up to 5 clinical testing time points)**: A 2-3 mL aliquot from each time point (0-10, 10-20, 20-30, 30-45 and 45-60 minutes) should be sent on wet ice to the local clinical lab for bicarbonate concentration. This aliquot should be placed in a plastic vial, not a cryovial. The vial to be used should be chosen in consultation with your local lab. An autoanalyzer should be used for this assay per cited reference (PMID: 19806083, PMID: 15332022).

3.3 **Biorepository storage (2 research testing time points):** pancreas juice is to be handled on wet ice as much as possible. Spin the pancreas juice collected from time points 0-10 minutes and time points 10-20 minutes at 1200g for 10 minutes at 4°C.

3.4 Place the two (2) pellets (time point 1 and time point 2 specimens) in a cryovial and freeze at –80ºC. If necessary resuspend the pellet in 10 µl of PBS.

3.5 The supernatant should be aliquoted (as detailed below), supernatant should be immediately snap frozen in liquid nitrogen or in a portable -80 C freezer. If snap-freezing is not possible, place aliquots on wet ice and transport to a –80ºC freezer as soon as possible (should be < 4 hour between collection and freezing). **No inhibitors are added.**

3.6 Specimens should be transferred from the suction trap to cryovials with screw top gasket closures.

3.7 From each time point (time point #1= 0-10 mins; time point #2= 10-20 mins collection), aliquot spun supernatant pancreas juice samples as outlined below:

- 3.7.1 250 µl x 12 vials of supernatant
- 3.7.2 1.8 mL x 15 vials of supernatant
- 3.7.3 2 Pellet vials; 1 vial per time point

3.8 **If there is excess pancreas juice divide the remainder of the sample into large volume aliquots, 1.8 mL, freeze, and later, thaw them when aliquots are requested for analysis.** When requested, the 1.8 mL tubes should be completely thawed, vortexed and processed into 250 µL aliquots, labelled and logged into system.

3.9 Check that all aliquot vial caps are tight and that all vials are labeled. Labels should specify date, time, test time (i.e. baseline, 0-10 minutes, 10-20 minutes) and subject ID.

3.10 Samples should not be thawed prior to shipping. (Will be shipped on dry ice or a Cryoport. Refer to Shipping and Receiving SOP.)

Pancreas Tissue

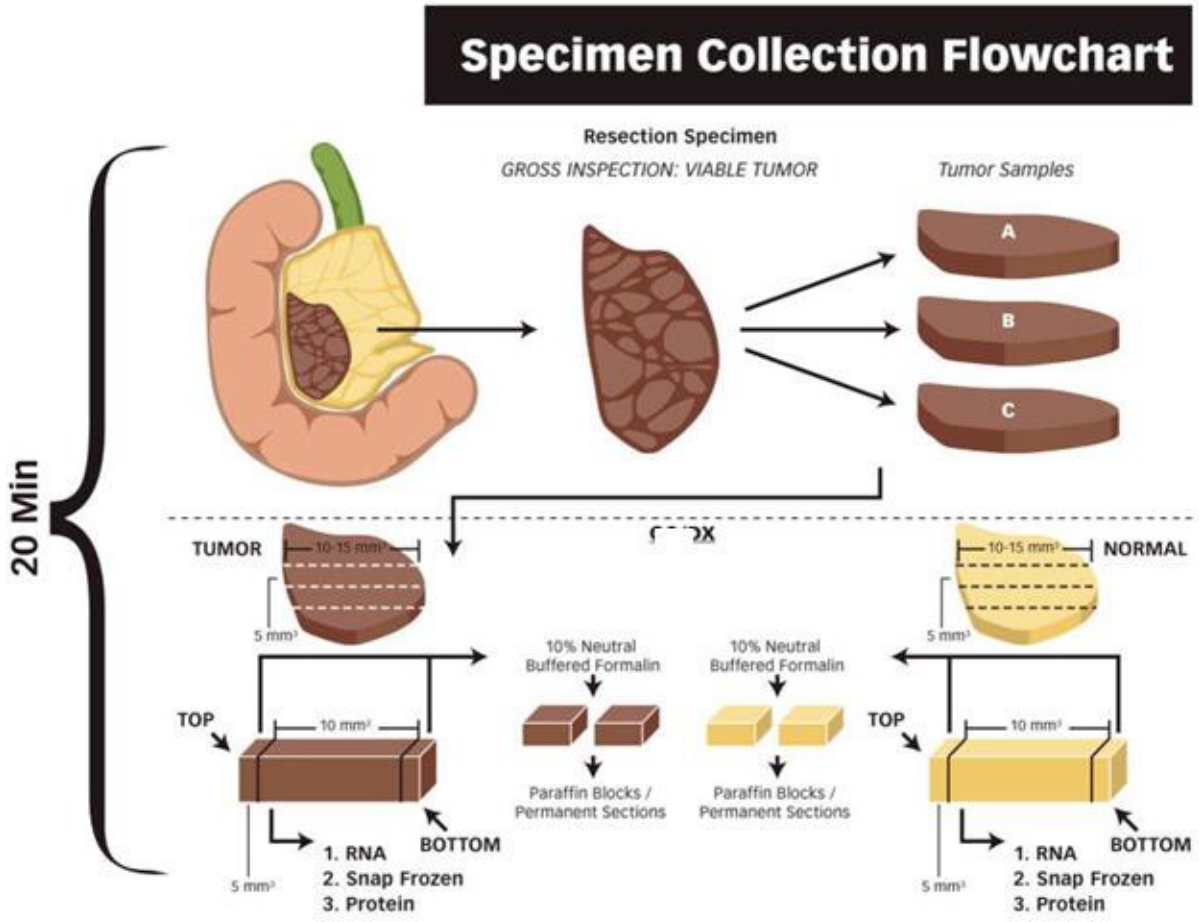


Figure 10. Pancreas Tissue SOP Workflow

Purpose

The purpose of this procedure is to outline the process for collecting, processing, and storing of tissue specimens for the CPDPC. All pancreas tissue is collected and processed within 4 hours.

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines.
- This SOP does not cover informed consent procedures.

Materials

1. Biospecimen form (CDMC will provide)
2. Liquid nitrogen or isopentane bath or crushed dry ice (for snap freezing)
3. Rnase inhibitor [SUPERase-IN RNase Inhibitor stock AM2694; Ambion, Austin, TX]*
4. Protease Inhibitor [cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks 04693116001 Roche]*
5. Neutral buffered formalin, universal molecular fixative, paraffin;
6. HistokinetteTM cassette [Micromesh™ Loose Biopsy Cassettes # B1000735AQ]
7. Digital Scale
8. Crushed ice if a delay is anticipated
9. Refrigerated centrifuge
10. Transfer pipette [Southern Labware, 138080G or similar]
11. Labels suitable for long-term freezer storage, and IDs printed using 20 barcoding(CDMC will provide, contact CDMC-labelrequest@mdanderson.org)
12. Aliquot cryovials with screw top gasket closure [Fisher Scientific, 12565163N]
13. Cardboard freezer storage boxes [VWR #89214-752 2" Cryogenic box, with drain slots, with grids, 81 cell divider]
14. –80ºC Freezers with emergency backup generators
15. Absolute alcohol

1. Surgical collection and processing

1.1. After excision of tissue from the patient, the specimen is placed in a sterile bag on ice. Ideally, time from excision to preservative immersion should be no more than 30-60 minutes.

1.2. After clinical needs are met, the tissue advocate will work with the pathologist to collect as large a sample as is reasonable.

1.3. Recommend at least a 1 cm slice of tumor and adjacent normal tissue. An effort should be made to harvest the samples away from necrotic, hemorrhagic, or fibrotic capsular tissue since these factors risk the purity and abundance of tumor cells.

1.4. If tissue size and weight allows storage in separate pieces, prioritize in the following order before freezing for long-term storage.: (1) snap freezing; (2) RNA stabilizing solution followed by freezing; (3) Protease Inhibitor

1.5. Using sterile forceps and sterile disposable scalpels in a small sterile dish or container, the tissue should be divided into sections approximately 0.3cm by 0.3cm by 0.3cm and weighting between 100-200mg.
Note: Work with normal and tumor tissue separately. Change the scalpel, forceps, and dishes between normal and tumor samples

1.6. After the tissue has been sectioned, then cut approximately ~1mm from each end. The ends are preserved in 10% formalin and designated ends are associated with the central sections for histologic examination and quality control. Every section needs its own corresponding ends. (See below for more detail.)
Note: Before placing the section into its cryovial, it may be cut into smaller pieces to increase surface area and optimize freezing.

Fresh Frozen

1. Weigh tissue samples. Record.

2. Store tissue samples in an aliquot vial with screw top gasket closure with appropriate label.
3. Check that all aliquot vial caps are secure and that all vials are labeled.
4. Snap freeze in liquid nitrogen/isopentane bath/crushed dry ice within 15 minutes or as soon as possible.
5. Samples should not be thawed prior to shipping. (Will be shipped on dry ice. Refer to Shipping SOP.)

RNAlater

1. Weigh tissue samples. Record.
2. Store tissue samples in an aliquot vial with screw top gasket closure with appropriate label containing RNA stabilizing solution within 15 minutes of collection (or as soon as possible), and stored at 4°C for 24 hours
3. Tissue should not be thicker than 0.5cm in any single dimension. This will allow the solution to penetrate the entire tissue piece
4. After 24 hours (which allows thorough penetration of the tissue) remove the RNA stabilizing solution using a disposable pipet.
5. Check that all aliquot vial caps are secure and that all vials are labeled.
6. Freeze specimens below –80ºC.
7. Samples should not be thawed prior to shipping. (Will be shipped on dry ice. Refer to Shipping SOP.)

Protease Inhibitor

1. Weigh tissue samples. Record.
2. Store tissue samples in an aliquot vial with screw top gasket closure with appropriate label containing Protease Inhibitor within 15 minutes of collection (or as soon as possible), and then immediately snap freeze in liquid nitrogen/isopentane bath/crushed dry ice.

   Note: Do not fill they cryovial all the way with solution because when it freezes it will expand and burst.
3. Check that all aliquot vial caps are secure and that all vials are labeled.
4. Freeze specimens below –80ºC.
5. Samples should not be thawed prior to shipping. (Will be shipped on dry ice. Refer to Shipping SOP.)

Fixed Tissue/Histology Quality Control

1. All ends from each piece of tissue are Formalin-fixed, Paraffin-embedded (FFPE).
2. Recommend mirror-image sections of fresh-frozen piece when possible.
3. Tissue samples should be mounted flat in a cassette in a 1:10 (tissue: formalin) ratio of 10% neutral buffered formalin (NBF), within 1 hour.
4. The samples are stored at room temperature for at least 12 hours, but not longer than 72 hours.
5. The tissue is then transferred to paraffin embedding.

Endoscopic (EUS-FNA/Core Biopsy) Collection and Processing

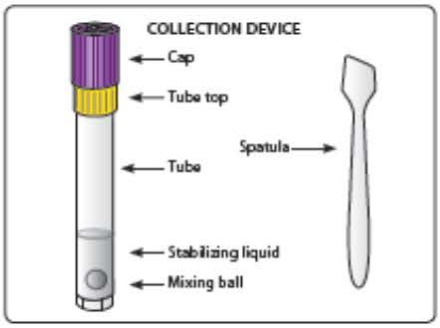
1. Research endoscopic aspirates or core biopsies of pancreatic tissue should be obtained and processed only under IRB-approved protocols and after obtaining informed consent.

   FNA specimens: each FNA pass obtained for research purposes should be placed in a 1.8 cc cryovial and snap-frozen on liquid nitrogen or at -80C. Multiple specimen may be placed in one cryovial.

   Core biopsy specimens: from each needle pass, split the tissue specimens, placing half in a 1.8 cc cryovial with no additives and the other half in a 1.8 cc vial containing absolute alcohol. Snap freeze both vials.

OMNIgene·GUT

For microbiome



Preparation:

- **Empty your bladder before beginning the collection.**
- **Collect fecal sample free of urine or toilet water.**
- **Toilet paper or tissues may be required.**

Summary and explanation of the kit:

OMNIgene·GUT provides the materials and instructions for collecting and stabilizing microbial DNA from a fecal sample.

Warnings and precautions:

- FOR EXTERNAL USE ONLY.
- Do NOT remove the yellow tube top from the tube.
- Do NOT spill the stabilizing liquid in the tube.
- Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
- If fecal sample is liquid or donor has diarrhea wait until the next bowel movement to collect the sample.
- Small items may pose a choking hazard.

Storage: room temperature storage (15°C to 25°C) pre- and post-collection.

Ship in accordance to applicable regulations covering transport of biological specimens.
See MSDS at www.dnagenotek.com

Label legend:

	Collect sample by (Use by)
REF	Catalog number
	Manufacturer
15°C-25°C	Storage instructions
	Caution, consult instructions for use
LOT	Lot number

USER INSTRUCTIONS

Read all instructions prior to collection

Procedure:

1 While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set aside for later use.

IMPORTANT:
Do NOT remove the yellow tube top.
Do NOT spill the stabilizing liquid in the tube.

2 Use the spatula to collect a small amount of fecal sample.

3 Transfer the fecal sample into the yellow tube top. Repeat until the sample reaches the top and fills it completely.

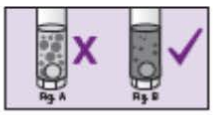
IMPORTANT: Do NOT push sample into the tube.

4 Scrape horizontally across the tube top to level the sample and remove any excess.

Wipe exterior of tube and top with toilet paper or tissue as needed.

5 Pick up the purple cap with the solid end facing down and screw onto the yellow tube top until tightly closed.

6 Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.



7 The fecal sample will be mixed with the stabilizing liquid in the tube; not all particles will dissolve.

IMPORTANT: Continue shaking if large particles remain as shown in Figure A.

Place spatula in original packaging or wrap in toilet paper and discard in garbage.

Send the sample for processing following the delivery instructions supplied by the kit provider.

DNA genotek

Superior samples
Proven performance

Made in Canada
DNA Genotek Inc.
3000 - 500 Palladium Drive
Ottawa, ON, Canada K2V 1C2

Toll-free (North America): 1.800.613.6354
Tel.: +1.613.723.5757 • Fax: +1.613.723.5067
info@dnagenotek.com
www.dnagenotek.com

Australian Sponsor: Emergo Australia, Level 20, Tower II, Darling Park, 201 Sussex Street, Sydney, NSW 2000 Australia

PD-PR-00442 Issue 5/2016-01

OmnigeneGUT Kit Instructions

(Link: http://www.dnagenotek.com/US/pdf/PD-PR-00442.pdf)

ambion
by life technologies

SUPERase•In™ RNase Inhibitor

Catalog Number AM2694, AM2696
Pub. No. 4393876 **Rev.** E

Contents	Quantity	Storage conditions
SUPERase•In™ RNase inhibitor, 20 U/µL	Cat. no. AM2694: 2,500 Units	Store at –20°C. *Do not store in a frost-free freezer.*
	Cat. no. AM2696: 10,000 Units	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product Description

Nuclease-free SUPERase•In™ RNase Inhibitor is a protein-based ribonuclease inhibitor which noncovalently binds and inactivates a wide variety of RNases in a range of temperature (37–65°C) and pH (5.5–8.5) conditions. This product is distinct from ANTI-RNase Inhibitor (Cat. nos. AM2690, AM2692) and placental RNase Inhibitor protein (Cat. nos. AM2682, AM2684) in that it inactivates RNases I and T1 in addition to RNases A, B and C. Both ANTI-RNase Inhibitor and SUPERase•In™ RNase Inhibitor are distinct from RNase Inhibitor protein in that they have more robust interactions with RNAses, and do not release active RNases in the absence of DTT or other reducing agents. SUPERase•In™ RNase Inhibitor does not interfere with the activities of SP6, T7, and T3 RNA Polymerases, MMLV Reverse Transcriptase, or Taq DNA Polymerase.

Unit (U) definition: SUPERase•In™ RNase Inhibitor at 1 U/µL will block the degradation of 0.1 µg/µL labeled RNA by 2.5 pg/µL of RNase A, 2.5 pg/µL of RNase I and 0.0075 U/µL of RNase T1, for 4 hours at 37°C, in 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA. Analysis is by denaturing PAGE.

Storage buffer (*not included*): 2 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.4 in 50% glycerol.

Using Superase In RNase Inhibitor

Use SUPERase•In™ RNase Inhibitor at a final concentration of 1 U/µL to prevent RNA degradation in applications including cDNA synthesis, RT-PCR, *in vitro* transcription, *in vitro* translation, preparation of cell lysates, RNA isolation and storage, and in any application where ribonuclease inhibitor protein is used. In experiments requiring intact RNA, (e.g., transcription), avoid denaturation of the SUPERase•In™ protein by heat, SDS or other detergents, urea, etc., which could release active RNases. The addition of DTT is not recommended for storage.

Note: Heating samples containing SUPERase•In™ RNase Inhibitor and DTT to 95°C may cause RNA to hang up in the wells of denaturing urea/polyacrylamide gels. Load directly onto gels without heating.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Print

Roche

For life science research only.
Not for use in diagnostic procedures.

cOmplete

Protease Inhibitor Cocktail Tablets provided in *EASYpack*

For the complete inhibition of proteases during extractions from animal and plant tissues or cells, yeast and bacteria

Cat. No. 04 693 116 001

20 tablets

Do not eat

Version 04
Content version: September 2014

Store at +2 to +8°C

1. What this Product Does

1.1 Properties

cOmplete tablets inhibit a broad spectrum of serine, cysteine and metalloproteases as well as calpains. Due to the optimized composition of the tablets they show excellently inhibition effects and are therefore very well suited for the protection of proteins isolated from animal tissues, plants, yeast and bacteria. cOmplete contains both irreversible and reversible protease inhibitors.

1.2 Contents

20 individually packed cOmplete Protease Inhibitor Cocktail Tablets in foil blisters. Each tablet is sufficient for a volume of 50 ml solution.

1.3 Stability

- The tablets are stable at +2 to +8°C, stored dry, until expiration date.
- The stock solution is stable for 1–2 weeks, stored at +2 to +8°C, or at least for 12 weeks at −15 to −25° C.

1.4 Application

Used for the inhibition of serine, cysteine, and metalloproteases in bacterial, mammalian, yeast, and plant cell extracts.

cOmplete contains both reversible and irreversible protease inhibitors. Therefore, we recommend the addition of cOmplete to all stock buffers and solutions normally protected with protease inhibitors and not only during the initial purification steps.

cOmplete contains EDTA (18.5 mg/tablet yield 1 mM solution of EDTA in 50 ml). Therefore, the extraction buffer should not contain divalent cations like Ca^{2+}, Mg^{2+} or Mn^{2+}, otherwise the inhibition of the metalloproteases might be incomplete. If the protein of interest will be purified by IMAC (immobilized metal- chelate affinity chromatography), *e.g.* Poly-His tagged recombinant proteins, EDTA has to be eliminated (*e.g.* by dialysis) prior to the chromatography.

Alternatively, the product cOmplete, EDTA-free can be used (see table "Ordering Information"). These tablets are identical to cOmplete, with the only difference that no EDTA or other chelating agent are present.

2. How to Use this Product

2.1 Handling

Carefully push the tablet through the foil packaging using the base of your thumb (and not yout finger nail) to prevent the breakage of tablets.

Do not eat the tablets.

2.2 Preparation of Working Solutions

One tablet cOmplete is sufficient for the inhibition of the proteolytic activity in 50 ml extraction solution. If very high proteolytic activity is present, one tablet should be used for 25 ml extraction buffer. The tablets can be added directly to the extraction medium. Alternatively a stock solution (25 × conc.) can be prepared.

If it is necessary to inhibit proteolytic activity in a smaller volume we recommend to use cOmplete, Mini (1 tablet for 10 ml extraction solution, see "Ordering Information"). The composition of the cOmplete Mini tablet is identical to the normal cOmplete tablet, therefore comparable results are achieved with both product types.

2.3 Stock solution (25 × conc.)

Dissolve one tablet cOmplete in 2 ml redist. H_2O or in 100 mM phosphate buffer, pH 7.0.

3. Results

In extracts from animal tissues mainly serine, cysteine and metalloproteases are found; in plant extracts serine and cysteine proteases dominate. For bacterial extracts serine and metalloproteases are typical (1). cOmplete tablets inhibit efficiently serine, cysteine and metalloproteases in a broad range.

Occasionally, aspartic proteases ("acid proteases") can interfere upon isolations from animal tissues. These proteases, however, exhibit pronounced activities only at low pH values. If extraction or single isolation steps have to be performed at this pH range the addition of pepstatin* is recommended to inhibit aspartic protease activity as a precaution.

Typical values for the inhibition of different proteases and protease mixtures by cOmplete are shown in table 1.

Protease resp. protease mixture	Enzyme concentration (mg/ml)	pH-value	% inhibition after immediate addition to the protease	% inhibition after 60 min incubation (protease + cOmplete) at +15 to +25° C
Pancreas-extract	0.015 0.03	7.8 6.5	87% 88%	99% 96%
Pronase	0.0015 0.003	7.8 7.0	88% 90%	99% 95%
Thermolysin	0.0008	7.8	99%	100%
Chymotrypsin	0.0015	7.8	97%	97%
Trypsin	0.0002	7.8	93%	89%
Papain	1.0	6.5	95%	73%

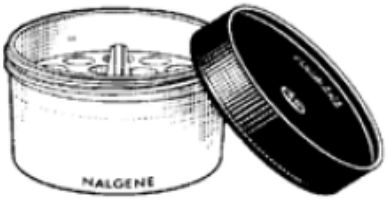
Table 1: Inhibition of different proteases by cOmplete Protease Inhibitor Tablets.
One cOmplete tablet was added per 50 ml incubation solution. Proteolytic activity was determined with the Roche Diagnostics Universal Protease Substrate (casein, resorufin-labeled*). When extractions or single-step isolations are necessary in the acid pH range, simply include pepstatin* along with cOmplete tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at room temperature.

Personal research communications indicate that acetylcholinesterase is strongly inhibited by cOmplete. Butyrylcholin-esterase is inhibited to a lesser extent.

* *available from Roche Diagnostics*

Thermo SCIENTIFIC | NALGENE

Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers



Cat. No. 5100-0001, -0036, -0050
Polycarbonate container, high-density polyethylene closure, tube holder, foam insert

The Nalgene Mr. Frosty Cryo 1°C Freezing Containers are inexpensive, reusable and convenient devices for freezing biological samples at the recommended controlled rate of cooling, -1°C/minute. Isopropyl alcohol is necessary to achieve the recommended rate of cooling.

DIRECTIONS:

1. Remove high-density polyethylene tube holder and foam insert from polycarbonate unit. **Discard foam insert.**
2. Add 100% isopropyl alcohol to the fill line on the Mr. Frosty container. **DO NOT overfill.**
3. Carefully replace tube holder.
4. Place tubes containing sample into holes in tube holder.
5. Place unit in bottom of -70°C to -80°C mechanical freezer. Leave undisturbed for a minimum of 4 hours.
6. Remove frozen tubes from unit and place in a permanent, long-term storage freezer (e.g. -130°C or below).

NOTE: DO NOT store full jar on its side. Store at room temperature when not in use. Replace alcohol every fifth use. Clean with non-abrasive, non-alkaline detergent such as Thermo Scientific Nalgene L-900 Detergent (Cat. No. 900-4000). The tube holder floats with tubes in place and can be used as a floating rack when thawing samples.

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

Shipping and Receiving

Frozen Shipment Instructions

1) Retrieve all samples stored from boxes.

2) Once samples are ready to be physically shipped from a site to insert receiving lab name, they must be "electronically shipped" in the IIMS Specimen Manager system and an e-mail notification will be sent to the receiving lab to notify them of an incoming shipment. The receiver will be provided with a sample list and courier tracking number to track the physical location of the samples.

   Note: The IIMS Specimen Manager tracking system is accessed via the CPDPC secure Website at https://oneaccessportal.mdanderson.org

3) Use insulated shipping boxes, with an inner foam box and outer cardboard box. Examples of an appropriate box would be: ThermoSafe item #355, which is available from VWR as item #15714-500 or Fisher Scientific as item #03-530-17.

4) Pack the samples according to Standard UN 3373 (IATA Shipping Instructions 650) for "Biological Substance, Category B", i.e. triple packaging with two water tight and pressure safe layers with absorbent material in between. Triple packaging consists of the following:

   a. A leak proof primary receptacle
   b. A leak proof secondary packaging
   c. An outer rigid packaging of adequate strength for its capacity, mass and intended use

5) Each completed package must pass the IATA drop test from a height of not less than 1.2 m. For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging.

6) Marking Requirements: Packages containing UN3373 materials must be clearly marked with the proper shipping name of "Biological substance, Category B" with the characters being at least 6 mm high. Packages must also have the mark illustrated in Packing Instruction 650 clearly and legibly displayed on the external surface of the outer packaging adjacent to the proper shipping name. The UN3373 mark must be in a square on point configuration (diamond shaped) with each side being a minimum of 50 mm (or 2 inches) in length with the UN3373 characters being at least 6 mm in height. In addition, UN1845 stickers will also be provided for use in accordance with IATA regulations for shipping dry ice.

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

Biological substance, Category B

7) Include sufficient dry ice for the planned shipping time, and include enough dry ice to protect the samples in the event of a one-day delay of transit. Thawed samples cannot be used for this research. ***Please do not try to save shipping costs by putting less dry ice in the package.***

   Dry Ice: Use minimum 15 pounds (7kg) for each box. Use more for larger boxes, proportional to the size of the box**.**

8) Containers must be large enough to hold sufficient dry ice to ensure samples arrive frozen.

9) Boxes of samples are shipped in one box at a time (this doesn't mean that only one box may be shipped to the receiving lab at a time).

   Entering the box number into SM (manual or scan) will display the sample IDs of the samples that are supposed to be in the box that is about to be shipped. Always take a moment to perform the following steps to ensure the samples in the box matches the data that will be generated on the shipping list. Have dry ice nearby to place in the boxes while out of the freezer (required). Do not let samples sit for more than 1-2 minutes at room temperature. No freeze-thaw cycles are allowed for the samples.

10) Check that the number of samples in the box is the same as the number of samples listed on the sample/shipping list:
    a. If not, do a quick but careful inventory. Make sure not to let the samples get warm and thaw.
    b. If there are extra samples in the box that are not on the list, pull them out for later evaluation. DO NOT SHIP SAMPLES THAT ARE NOT ON THE SHIPPING LIST.

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

11) Spot-check a few of the samples. Pull a couple of samples from the box, check to see if they are on the shipping list.
    a. If not, the wrong box may have been pulled from the freezer.
    b. If not, carefully inventory the box (See Item 11a).
    c. Verify box contents with the use of a "box map" (in addition to the shipping list).

	1	2	3	4	5	6	7	8	9
A	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18
C	19	20	21	22	23	24	25	26	27
D	28	29	30	31	32	33	34	35	36
E	37	38	39	40	41	42	43	44	45
F	46	47	48	49	50	51	52	53	54
G	55	56	57	58	59	60	61	62	63
H	64	65	66	67	68	69	70	71	72
I	73	74	75	76	77	78	79	80	81

12) Do not ship the samples until the number on the shipping list matches to the number of samples in the box. In SM enter the shipping date and tracking number for courier.

Print copies of the packing lists for the contents of all boxes and file in the site's research records. Include a paper packing list of the shipment contents for all boxes shipped. Once samples are electronically shipped in the IIMS SM, their status will be updated as "in transit".

Specimen Shipping Address & contact information

All specimens must be shipped via Priority Overnight for delivery the next day. DO NOT ship on days that would cause the specimens to arrive on a weekend or holiday. Contact the insert receiving lab name for any questions related to anticipated delivery dates (for example the observed holidays at insert receiving lab name). Ship specimens to:

Attention: Enter receiving lab contact personnel name

Insert address telephone number and email of receiving lab/contact person

Priority Overnight Carrier Examples:
- a. UPS
- b. FedEx, FedEx® UN 3373 Pak and FedEx® Clinical Pak
- c. World Courier
- d. DHL

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

2.8 Specimen Receiving

Shipped specimen samples will be received by the insert receiving lab name. The insert receiving lab name will follow the steps below to acknowledge the specimen receipt and track the location of the specimens within the insert receiving lab name:

1) Log into the SM tracking system and "electronically receive" each box one at a time.
2) Quickly check the contents of each box against the packing list or box map for that particular box.
3) Once all aliquots are stored at insert receiving lab name, their new storage locations should be updated into the SM tracking system; including the box and freezer.
4) When boxes have to be moved to another location (i.e. freezer farm facility), their new location must be updated in the SM tracking system.
5) Details of the above processes are provided in the specimen manager training.

Note: The IIMS Specimen Manager tracking system is accessed via the CPDPC secure Website at https://oneaccessportal.mdanderson.org

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

Subject ID: _____

Place barcode label here

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____
(mm/dd/yyyy)

1.2 Time Collected: _____:_____
(24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____
(initials)

1.5 Processing Start Time: _____:_____
(24h clock 00:00)

1.6 Processing End Time: _____:_____
(24h clock 00:00)

1.7 Time plasma aliquots were placed in -80°C freezer: _____:_____
(24h clock 00:00)

Tube	Collected	Hemolyzed	Instructions	Aliquots	[Receiving Lab use]
1) 7mL EDTA	☐ Yes ☐ No	☐ Yes ☐ No	From a 15mL conical tube, aliquot a volume of 0.25mL into 10 cryovials. The remaining volume is to be aliquoted into 1.8mL aliquots 6 cryovials, and store at –80°C. All aliquots may not be filled depending on the amount of plasma obtained. Refer to CPDPC Biospecimen SOP for complete processing instructions.	**Plasma** 10 - 0.25 mL # generated: _____	# received: _____
2) 7mL EDTA	☐ Yes ☐ No	☐ Yes ☐ No		Additional up to 6 1.8 mL # generated: _____ Other: # generated: _____ _____ mL	# received: _____ # received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	**Date aliquots received by Receiving Lab**	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

Include a copy of this completed form in the shipment with the associated specimen/s. Keep the original of the completed form in the study research records at your site.

CPDPC16-02 PROCEED PLASMA SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED BUFFY COAT SAMPLE ACQUISITION FORM

Subject ID: _____

Place barcode label here

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____ (mm/dd/yyyy)

1.2 Time Collected: _____:_____ (24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____ (initials)

1.5 Processing Start Time: _____:_____ (24h clock 00:00)

1.6 Processing End Time: _____:_____ (24h clock 00:00)

1.7 Time plasma from buffy coat were placed in -80°C freezer: _____:_____ (24h clock 00:00)

1.8 Buffy Coat Processing Protocol used: ☐ Preferred/ Primary Method ☐ Alternative/Secondary Method

1.9 Buffy Coat Concentration _____ cell/mL (~3 - 5 million cells per mL)

1.10 Time Mr. Frosty (buffy coat aliquots) were placed in -80°C freezer: _____:_____ (24h clock 00:00)

1.11 Date and Time Mr. Frosty (buffy coat aliquots) were transferred to liquid nitrogen freezer (vapor phase): _____ (mm/dd/yyyy) _____:_____ (24h clock 00:00)

Tube	Collected	Hemolyzed	Instructions	Aliquots	[Receiving Lab use]
1) 7mL EDTA	☐ Yes ☐ No	☐ Yes ☐ No	Aliquot a volume of 0.25mL into 10 cryovials labelled plasma from buffy coat. The remaining volume is to be aliquoted into 1.8mL aliquots up to 6 cryovials all labelled as plasma from buffy coat, and store at –80°C. All aliquots may not be filled depending on the amount of plasma from buffy coat obtained. Refer to CPDPC Biospecimen SOP for complete processing instructions.	**Plasma from buffy coat** 10 - 0.25 mL # generated: _____ Additional up to 6 - 1.8 mL # generated: _____ Other: _____ mL # generated: _____ **Buffy Coat/Pellet** # generated: _____ 8 - (500uL-1mL) Volume per cryovial _____mL	# received: _____ # received: _____ # received: _____ # received: _____
2) 7mL EDTA	☐ Yes ☐ No	☐ Yes ☐ No			

Date aliquots shipped to Receiving Lab	Shipped by:	Date aliquots received by Receiving Lab	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

Include a copy of this completed form in the shipment with the associated specimen/s. Keep the original of the completed form in the study research records at your site.

CPDPC16-02 PROCEED BUFFY COAT SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED SERUM SAMPLE ACQUISITION FORM

Subject ID: _____

Place barcode label here

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____
(mm/dd/yyyy)

1.2 Time Collected: _____:_____
(24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____
(initials)

1.5 Red Top tube(s) are to clot at room temperature prior to processing (≥30mins ≤60mins): Amount of time: ____ mins
(initials)

1.5 Processing Start Time: _____:_____
(24h clock 00:00)

1.6 Processing End Time: _____:_____
(24h clock 00:00)

1.7 Time aliquots were placed in -80°C freezer: _____:_____
(24h clock 00:00)

Tube	**Collected**	**Hemolyzed (If Yes, discard sample)**	**Instructions**	**Aliquots**	**[Receiving Lab use]**
1) 6mL Red Top	☐ Yes ☐ No	☐ Yes ☐ No	Aliquot a volume of 0.25mL into 10 cryovials. The remaining volume is to be aliquoted into 1.8mL aliquots (up to 6) and stored at –80°C. All aliquots may not be filled depending on the amount of serum obtained. Refer to CPDPC Biospecimen SOP for complete processing instructions.	**Serum** 10 - 0.25 mL # generated: _____ Additional up to 6 - 1.8 mL # generated: _____ # generated: _____ Other: _____ mL	# received: _____
2) 6mL Red Top	☐ Yes ☐ No	☐ Yes ☐ No			# received: _____ # received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	**Date aliquots received by Receiving Lab**	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

Include a copy of this completed form in the shipment with the associated specimen/s. Keep the original of the completed form in the study research records at your site.

CPDPC16-02 PROCEED SERUM SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED PAXgene DNA/RNA SAMPLE ACQUISITION FORM

Place barcode label here

Subject ID: _____

FOR DNA:

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____ (mm/dd/yyyy)

1.2 Time Collected: _____:_____ (24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____ (initials)

1.5 Date and time PAXgene for **DNA** tubes were aliquoted: _____ (mm/dd/yyyy) _____:_____ (24h clock 00:00)

1.6 Date and Time **DNA** aliquots were placed in -80°C freezer: _____ (mm/dd/yyyy) _____:_____ (24h clock 00:00)

FOR RNA:

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____ (mm/dd/yyyy)

1.2 Time Collected: _____:_____ (24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____ (initials)

1.5 Date and Time **RNA PAXgene** tubes were placed in -80°C freezer: _____ (mm/dd/yyyy) _____:_____ (24h clock 00:00)

Tube	Collected	Instructions	Aliquots	[Receiving Lab use]
1. 8.5mL PAXgene DNA	☐ Yes ☐ No	Aliquot a volume of 1.7mL from DNA PAXgene tube into 5 cryovials.	**DNA** 5 – 1.7 mL # generated: _____	# received: _____
2. (2) 2.5mL PAXgene RNA	☐ Yes ☐ No	Freeze RNA PAXgene tubes. Refer to CPDPC Biospecimen SOP for complete processing instructions.	**RNA** 2-RNA PAXgene tubes # generated: _____	# received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	Date aliquots received by Receiving Lab	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

Include a copy of this completed form in the shipment with the associated specimen/s. Keep the original of the completed form in the study research records at your site.

CPDPC16-02 PROCEED PAXgene DNA/RNA SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED SALIVA SAMPLE ACQUISITION FORM

Subject ID: _____

Place barcode label here

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____
(mm/dd/yyyy)

1.2 Time Collected: _____:_____
(24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____
(initials)

1.5 Processing Start Time: _____:_____
(24h clock 00:00)

1.6 Processing End Time: _____:_____
(24h clock 00:00)

1.7 Time aliquots were placed in -80°C freezer: _____:_____
(24h clock 00:00)

Draw Tube	Collected	Instructions	Aliquots		[Processing Lab use]
1. 50mL conical tube	☐ Yes ☐ No	Collect maximum volume of saliva in 5 minutes. Depending on volume collected, please reference Saliva collection/processing SOP for instructions.	4 - 0.25mL no media added	# Collected: _____	# Received: _____
			4 - 0.25mL RNA inhibitor	# Collected: _____	# Received: _____
					# Received: _____
			Other: _____ mL	# Collected: _____	# Received: _____
					# Received: _____

Date collection tube shipped to Receiving Lab	Shipped by:	**Date collection tube received by Receiving Lab**	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

Include a copy of this completed form in the shipment with the associated specimen(s). Keep the original of the completed form in the study research records at your site.

CPDPC16-02 PROCEED SALIVA SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED URINE SAMPLE ACQUISITION FORM

Subject ID: _____

Place barcode label here

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

1.1 Date Collected: _____ (mm/dd/yyyy)

1.2 Time Collected: _____:_____ (24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____ (initials)

1.5 Processing Start Time: _____:_____ (24h clock 00:00)

1.6 Processing End Time: _____:_____ (24h clock 00:00)

1.7 Time aliquots were placed in -80°C freezer: _____:_____ (24h clock 00:00)

Tube	Collected	Instructions	Aliquots	[Receiving Lab use]
1. 50mL conical tube	☐ Yes ☐ No	Collect 18- 1.8mL aliquots: 6 unprocessed after centrifuge, 6 w/ RNA later, & 6 w/ proteinase inhibitor. 1 cryovial w/ pellet. Refer to CPDPC Biospecimen SOP for complete processing instructions.	6 – 1.8mL fresh no media # generated: _____ 6 – 1.8mL w/ RNA # generated: _____ 6 – 1.8mL w/ Proteinase Inhibitor # generated: _____ *1 - w/ pellet # generated: _____ Other: # generated: _____ _____ mL	# received: _____ # received: _____ # received: _____ # received: _____ # received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	Date aliquots received by Receiving Lab	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

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CPDPC16-02 PROCEED URINE SAMPLE ACQUISITION FORM

Lab Personnel: If not already performed, please record the results below and return it to the study coordinator to enter into the database.

Urine dipstick result negative for infection (Elevated nitrates and/or leukocytes)? ☐ Yes ☐ No
Urine dipstick results:

If specific gravity is lower than 1.001 or greater than 1.032, retest for accuracy and discard if confirmed. If patient shows signs of infection per the urine dipstick (i.e. positive for nitrates and/or moderate leukocyte esterase), then do not collect the urine until infection has been cleared

Tests and Reading Times-

- **Leukocytes** ☐ Negative ☐ Trace ☐ Small + ☐ Moderate ++ ☐ Large +++
  2 minutes
- **Nitrite** ☐ Negative ☐ Positive (any degree of uniform pink color)
  60 seconds
- **Urobilinogen** ☐ Normal 0.2 ☐ Normal 1.0 ☐ 2 ☐ 4 ☐ 8
  60 seconds
- **Protein** ☐ Negative ☐ Trace ☐ 30+ ☐ 100++ ☐ 300+++ ☐ 2000 or more++++
  60 seconds
- **pH** ☐ 5.0 ☐ 6.0 ☐ 6.5 ☐ 7.0 ☐ 7.5 ☐ 8.0 ☐ 8.5
  60 seconds
- **Blood** ☐ Negative ☐ Non-hemolyzed Trace ☐ Non-hemolyzed moderate ☐ Hemolyzed Trace ☐ Small+ ☐ Moderate++ ☐ Large+++
  60 seconds
- **Specific Gravity** ☐ 1.000 ☐ 1.005 ☐ 1.010 ☐ 1.015 ☐ 1.020 ☐ 1.025 ☐ 1.030
  45 seconds
- **Ketone** ☐ Negative ☐ Trace 5 ☐ Small 15 ☐ Moderate 40 ☐ 80 ☐ 160
  40 seconds
- **Bilirubin** ☐ Negative ☐ Small + ☐ Moderate ++ ☐ Large +++
  30 seconds
- **Glucose** ☐ Negative ☐ 100 ☐ 250 ☐ 500 ☐ 1000 ☐ 2000
  30 seconds

CPDPC16-02 PROCEED URINE SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED STOOL SAMPLE ACQUISITION FORM

Subject ID: _____

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

Place barcode label here

1.1 Date Subject Collected: _____
(mm/dd/yyyy)

1.2 Time Subject Collected: _____:_____
(24h clock 00:00)

1.3 Clinic: _____

1.4 Received By: _____
(initials)

1.4 Date Received By: _____
(mm/dd/yyyy)

BAYLOR WILL COMPLETE:

1.5 Processing Start Time: _____:_____
(24h clock 00:00)

1.6 Processing End Time: _____:_____
(24h clock 00:00)

1.7 Time aliquots were placed in -80°C freezer: _____:_____
(24h clock 00:00)

Tube	Collected	Instructions	Aliquots	[Receiving Lab use]
1. Yellow top transfer tube	☐ Yes ☐ No	Aliquot a volume of 100mg into 4-5 cryovials. Freeze cryovials below -80°C. Refer to CPDPC Biospecimen SOP for complete processing instructions.	(4-5) – 100mg # generated: _____	# received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	Date aliquots received by Receiving Lab	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

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CPDPC16-02 PROCEED STOOL SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED PANCREATIC FLUID SAMPLE ACQUISITION FORM

Subject ID: _____

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

Place barcode label here

1.1 Specimen Type: ☐ Pure pancreatic juice (PPJ) ☐ Duodenal aspirates

1.2 Date Collected: _____ (mm/dd/yyyy)

1.3 Time Collected: _____:_____ (24h clock 00:00)

1.4 Clinic: _____

1.5 Collected By: _____ (initials)

1.6 0-10 Min Time: _____:_____ (24h clock 00:00)

1.7 10-20 Min Time: _____:_____ (24h clock 00:00)

1.8 0-10 Time Point Processing Start Time: _____:_____ (24h clock 00:00)

1.9 0-10 Time Point Processing End Time: _____:_____ (24h clock 00:00)

1.10 10-20 Time Point Processing Start Time: _____:_____ (24h clock 00:00)

1.11 10-20 Time Point Processing End Time: _____:_____ (24h clock 00:00)

1.12 Time aliquots were placed in -80°C freezer: _____:_____ (24h clock 00:00)

Tube	Collected	Instructions	Aliquots		[Receiving Lab use]
1) 0-10 Min. time point	☐ Yes ☐ No	For each time point aliquot a volume of 0.25mL into 12 cryovials, aliquot a volume of 1.8mL into 15 cryovials, aliquot the pellets into (2) 1.8mL aliquot and store at –80°C. Any excess should be aliquoted into 1.8mL cryovials and frozen. Refer to CPDPC Biospecimen SOP for complete processing instructions. **No protease or RNA inhibitor**	12 (0-10) - 0.25 mL	# generated: _____	# received: _____
			15 (0-10) – 1.8 mL	# generated: _____	# received: _____
			Additional up to 6 - 1.8 mL	# generated: _____	# received: _____
			(0-10) – 1 Pellet	# generated: _____	# received: _____
2) 10-20 Min. time point	☐ Yes ☐ No		12 (10-20) – 0.25mL	# generated: _____	# received: _____
			15 (10-20) - 1.8 mL	# generated: _____	# received: _____
			Additional up to 6 - 1.8 mL	# generated: _____	# received: _____
			(10-20) – 1 Pellet	# generated: _____	# received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	**Date aliquots received by Receiving Lab**	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

CPDPC16-02 PROCEED PANCREATIC FLUID SAMPLE ACQUISITION FORM

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

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ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments:

CPDPC16-02 PROCEED PANCREATIC TISSUE SAMPLE ACQUISITION FORM

Subject ID: _____

Time Point: ☐ Baseline Follow Up: ☐ Year 1 ☐ Year 2 ☐ Year 3 ☐ Year 4 ☐ Year 5

Place barcode label here

1.1 Date Collected: _____ (mm/dd/yyyy)

1.2 Time Collected: _____:_____ (24h clock 00:00)

1.3 Clinic: _____

1.4 Collected By: _____ (initials)

1.5 How was the specimen obtained: ☐ Surgical ☐ Endoscopic

1.6 Indication for surgery/ biopsy: ☐ Known pancreatic malignancy ☐ Suspected pancreatic malignancy ☐ CP ☐ Suspected CP ☐ AIP evaluation ☐ Other

1.6 Type: ☐ Normal ☐ Mass

1.7 Number of Lesions Prepared: _____

1.8 Time tissue were placed in -80°C freezer: _____:_____ (24h clock 00:00)

Type	Collected	Instructions	Aliquots		[Receiving Lab use]
1) Normal Tissue	☐ Yes ☐ No	For normal and tumor tissue, cut into 100mg. Store in snap frozen, RNAlater, Protease inhibitor, and FFPE. Refer to CPDPC Biospecimen SOP for complete processing instructions.	1-Normal Tissue Snap Frozen	# generated: _____	# received: _____
			1- Normal Tissue RNAlater	# generated: _____	# received: _____
			1- Normal Tissue Protease Inhibitor	# generated: _____	# received: _____
			6- Normal Tissue Formalin	# generated: _____	# received: _____
2) Tumor Tissue	☐ Yes ☐ No		1-Tumor Tissue Snap Frozen	# generated: _____	# received: _____
			1- Tumor Tissue RNAlater	# generated: _____	# received: _____
			1- Tumor Tissue Protease Inhibitor	# generated: _____	# received: _____
			6- Tumor Tissue Formalin	# generated: _____	# received: _____

Date aliquots shipped to Receiving Lab	Shipped by:	**Date aliquots received by Receiving Lab**	Received by:
_____ (mm/dd/yyyy)	_____ (initials)	_____ (mm/dd/yyyy)	_____ (initials)

NAME OF PERSON PROCESSING/SHIPPING SAMPLE:	CONTACT EMAIL AND PHONE NUMBER:
COMMENTS:	RECEIVING LAB COMMENTS:

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CPDPC16-02 PROCEED PANCREATIC TISSUE SAMPLE ACQUISITION FORM

ALIQUOT STORAGE

Sequence #	Volume Collected	Freezer	Rack	Shelf	Box # (Position)	Comments

Comments: