Standard Operating Procedures for Biospecimen Collection, Processing, and Storage: From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

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Abstract

High quality and well-annotated biorepositories are needed to better understand the pathophysiology and biologic mechanisms of chronic pancreatitis (CP) and its consequences.

We report a methodology for the development of a robust standard operating procedure (SOP) for a biorepository based on the experience of the Clinical Centers within the Consortium to study Chronic Pancreatitis, Diabetes and Pancreas Cancer Clinical Centers (CPDPC), supported by the National Cancer Institute and the National Institute for Diabetes and Digestive and Kidney Diseases as a unique multidisciplinary model to study CP, diabetes, and pancreatic cancer in both children and adults. Standard operating procedures from the CPDPC centers were evaluated and consolidated. The literature was reviewed for standard biorepository operating procedures that facilitated downstream molecular analysis. The existing literature on biobanking practices was harmonized with the SOPs from the clinical centers to produce a biorepository for pancreatic research. This article reports the methods and basic principles behind the creation of SOPs to develop a biorepository for the CPDPC. These will serve as a guide for investigators developing biorepositories in pancreas research. Rigorous and meticulous adherence to standardized biospecimen collection will facilitate investigations to better understand the pathophysiology and biologic mechanisms of CP, diabetes, and pancreatic cancer.

Keywords

Standard operating procedures; pancreas; biospecimens; biorepository

INTRODUCTION

Well annotated and characterized human biospecimens are needed to identify potentially diagnostic, prognostic, and predictive biomarkers. Through the acquisition of a cohort of well characterized patients and associated bio specimens (blood, urine, saliva, stool,
pancreatic and duodenal juice, stools and when feasible pancreatic tissue), the proposed clinical research network will provide the resources and collaborative opportunities necessary for achieving many of the research objectives identified by the Chronic Pancreatitis, Diabetes and Pancreas Cancer Clinical Centers (CPDPC). The CPDPC is supported by the National Cancer Institute (NCI) and the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) as a unique multidisciplinary model to study chronic pancreatitis, diabetes, and pancreatic cancer in both children and adults. Differences in specimen collection, processing, and storage methods can become a considerable source of error in studies that relate to the discovery, development, and validation of biomarkers. This trend is particularly true for biospecimens collected for pancreas research in which biomarker development is lacking and greatly needed. Thus, it is essential that the procedures for collection, handling, processing and storage of biospecimens be tested, standardized, and carefully documented to optimize biological sample use for pancreas research.

Our goal in developing this standard operating procedure (SOP) is to limit heterogeneity in specimen collection, handling, and processing, while minimizing short and long-term biospecimen degradation and pre-analytical variability. These SOPs will serve as a valuable resource for the entire scientific community of researchers performing pancreatic research within and outside of the CPDPC.

**OBJECTIVES**

The CPDPC established a biospecimen committee, composed of clinicians, translational researchers, and basic scientists, to determine solid practices and create evidence-based SOPs for biospecimen collection, processing, and storage.

During the development of SOPs for the CPDPC, previous efforts were reviewed from all the academic centers. These SOPs were used as a starting point in the present endeavor. The individual SOPs were reviewed in great detail to identify areas of uniformity and discrepancy.

The expertise and existing specific practices of pancreatic researchers within the CPDPC and investigators outside the CPDPC were used to fill in knowledge gaps and produce SOPs that are specific to pancreatic biospecimens. The committee desired to develop SOPs not only for routine biospecimens (blood, DNA), but also for an expanding biorepository that includes urine, saliva, peripheral blood mononuclear cells (PBMC), RNA, stool, and pancreas fluid, an outline which had not been developed previously. To address areas without clear, supporting literature, such as how to handle and store pancreas fluid, ancillary experiments were designed to fill in knowledge gaps and will be reported in future publication(s). All procedures were approved by the CPDPC Steering Committee.

**MEETINGS**

The group met weekly by webinar for 18 months. Outside experts were consulted as needed and brought into discussions.
RESOURCES UTILIZED

Initially, CPDPC centers provided their own SOPs, which were reviewed and discussed by the group. The breadth of specific sample types was considered and expanded to include all potentially relevant biospecimens. Among studied protocols were publicly available SOPs from other established large-scale biorepositories, such as the Early Detection Research Network (EDRN) and the North American Pancreatitis Study (NAPS) Consortium. The NAPS also provided video links that demonstrated blood collection and processing.

PROCESS

Consensus opinion regarding best practices was obtained, and the individual SOPs were combined into one working SOP document. Additional information was gathered to address discrepancies and areas of uncertainty among SOPs from different sites. Translational and basic science researchers provided their expertise on the details of procedures for each unique specimen type.

PREPARATION OF WRITTEN SOPS

After extensive discussion and debate regarding the ideal scientific approach, real-world clinical specimen considerations, and equipment processing and accessibility, a consensus method was agreed upon; the SOPs were then scribed accordingly. The SOPs were then tested in clinical settings at various CPDPC sites, and modifications were made as necessary to assure that uniform strict compliance would be possible at all participating centers.

TRAINING

Online training modules, including video demonstrations of biospecimen processing, were developed by the CPDPC Biospecimen Committee. The CPDPC sites were required to watch and sign-off on specific biospecimen collection, handling and processing. https://cpdpc.mdanderson.org/references.html

BIOREPOSITORY-SPECIFIC DATA ELEMENTS

Biomarker expression levels can be influenced by many factors, including diurnal rhythm, sample type, sample preservative method, as well as the time and temperatures from specimen collection to storage. These data elements can be commonly overlooked in the development of prospective clinical trials for reasons such as limited access to processing laboratories. Table 1 contains the CPDPC list of important biospecimen data elements that account for sample processing variabilities. In general, we recommend that biospecimens are frozen at −80°C in less than 4 hours from the time of collection and are kept on wet ice until frozen. Exceptions include blood samples to be processed for PBMCs, for which the ethylenediaminetetraacetic acid (EDTA) plasma tubes are to be kept at room temperature to maximize cell yield. Strict adherence to this processing methodology intends to improve the quality of human biospecimen research by providing researchers with standardized information to better interpret, compare, and replicate experimental results on biospecimen from the CPDPC biobank.
CENTRIFUGATION OF SAMPLES

Centrifugation is a common processing technique for liquid biospecimen, allowing separation of more dense components such as cellular debris. The precipitate (pellet) and the supernatant, both of which may be of interest to investigators, are stored. We considered whether specimens should be frozen without centrifugation or processed immediately with separate preservation of the supernatant aliquots and pellet. The specific details of the centrifugation method such as relative centrifugal force (‘g’ value), time, temperature, and use of a brake to end the process were also considered. To improve compliance across sites, we favored a uniform protocol for these factors wherever possible. In general, the samples are kept on wet ice (except for the plasma tubes for PBMC isolation) until frozen, and centrifuge times are short. Our SOPs generally allow for use of a brake except where disruption of the pellet or interphase can occur, as in the case of plasma, particularly if the buffy coat is to be preserved. Consensus agreement was to process saliva samples unspun.

CONSIDERATIONS IN SPECIFIC SPECIMEN TYPES

The complete details of specimen collection and processing are outlined in Table 2. The actual granular details of the specimen collection and processing can be found at https://cpdpc.mdanderson.org/references.html. In brief, samples are processed within 4 hours of collection. Most samples will be subjected to centrifugation prior to preservation, and both the supernatant and the pellet should be saved when possible. Processed biological samples are stored (in standard 2 ml cryovials) for future use at −80°C in 10–15 aliquots of 250 μl and, if large volumes are generated (i.e., urine) 10–15 individual aliquots of 1.8 ml. Review of existing SOPs and the literature revealed a number of options, each with potential positive and negative considerations. Our final SOP reflects a consensus opinion that biospecimen collection and processing need to be simple and clinically relevant in order to promote adherence to the SOPs, uniform sample collection and quality, and eventual widespread clinical application.

**Blood**

Peripheral blood samples provide an accessible source of material that can be useful for not only biomarker discovery but also genetic and immunologic studies. This material can be a valuable source of numerous blood components, including serum, plasma and cellular material. Ideally, blood draws should be collected after 8–12 hours of fasting and prior to anesthesia. We wanted to minimize the amount of blood drawn for research purposes to between 45–60 ml. In arriving at this decision, we carefully considered the current health status of the participants, other blood work they might be receiving, and institutional review board limitations at the different institutions. See Figure 1 for more details.

**Plasma**

In accordance with the EDRN, we chose to use EDTA for collection of plasma (purple top tube), as it seems to interfere the least with numerous types of assays.
**PBMCs**

In this procedure, the supernatant (plasma) is removed and preserved, and the remaining cellular portion is diluted with phosphate-buffered saline (PBS) and subjected to density gradient centrifugation for 30 minutes at 805g with the brake off (to avoid disruption of the buffy layer). After, it is underlayed with Ficoll-Paque. This technique maximizes the yield of PBMCs from the blood.\textsuperscript{18,19} Cells are carefully isolated from the resulting buffy layer (above the Ficoll) and washed with PBS. The cells undergo centrifugation again at 582g for 10 minutes with the brake on. To remove any remaining red blood cell (RBC) contamination, the cells undergo a brief RBC lysis procedure and are washed then centrifuged again. Prior to cryopreservation, PBMCs are resuspended in a dimethyl sulfoxide (DMSO)-based cell freezing media, stored for 24 hours in a controlled temperature freezing container and then stored at −80°C. Following this, the frozen tubes are transferred to the vapor phase of liquid nitrogen for long term storage. This protocol is one of the most technically demanding in the SOP and requires trained lab personnel.

**Serum**

Again, in agreement with the EDRN and extensive literature reviews it was decided to use red top (serum) tubes (silicon-coated) with no additives and not serum separator tubes (SST) for serum collection.\textsuperscript{20} These tubes will allow the red blood cells to form a clot, and the absence of additives will introduce the least amount of variance to any downstream analyses. Blood collected for serum needs to sit at room temperature for 30 to 60 minutes to allow a clot to form.

**Blood DNA and RNA**

Because nucleic acids, particularly RNA, are subject to degradation we use PAXgene tubes containing reagents that immediately stabilize DNA or RNA.

**Urine**

Urine, like blood, has been widely used in clinical laboratory testing because it is readily collectible, can be procured in a non-invasive fashion, is low-cost, and yields large volume samples.\textsuperscript{15} Urine samples give clinical insight into metabolized products excreted into the urine in healthy and diseased patients.\textsuperscript{21} The most clinically relevant method is a random “clean catch”. This is the standard type of urine specimen collection even for bacterial culture and sensitivity testing because it reduces the incidence of external cellular and microbial contamination. The container used has been selected due to its compatibility with proteomic studies that will be performed with the samples from the cohorts established by this consortium. To reduce the effects of enzymatic/cellular activities, urine samples should remain on ice or refrigerated at 4°C until processed and spun in a refrigerated centrifuged.\textsuperscript{22} See Figure 2 for more details.

**Saliva**

Saliva samples can be used in DNA studies when blood sampling is not desirable, to analyze oral microbiomes, and to measure biomolecules such as free hormones.\textsuperscript{23} Saliva collection is quick, uncomplicated, and non-invasive. Artificially stimulating saliva flow by asking the
subject to chew gum or paraffin or by presenting visual stimulation can increase salivary gland secretion; however, these external stimuli can result in changes in the concentrations of the components of saliva. For these reasons, our consortium chose “passive” drool as the best SOP for saliva collection. Saliva samples should remain on ice at all times and be immediately aliquoted to prevent the degradation of molecules and the growth of bacteria or other microorganisms. If more than 2 mls of saliva are collected, SUPERase• In™ RNase Inhibitor is added to 1 ml of the unspun saliva. See Figure 3 for more details.

Stool

Stool samples can be analyzed to identify metabolites, cellular components, nucleic acids and proteins contributed by substances that are undigested, cellular material from anywhere in the gastrointestinal tract, including the pancreatic ductal epithelium, and bacteria that make up the gastrointestinal tract microbiome. Participants collect stool at home with a commercially available EasySampler® Stool Collection Kit. For fecal (pancreatic) elastase concentration, stool is placed fresh in a collection tube and is shipped at ambient temperature back to the diagnostic center for testing. JOLI Diagnostics Incorporated was used to measure fecal elastase to maintain uniformity and minimize inter-laboratory variability. For analysis of the stool microbiome, we decided to use the OMNIgene®•GUT kit (OMR-200). The OMNIgeneGut kit stabilizes microbial DNA from stool for fecal microbiome profiling and can be kept at room temperature for 14 days without requiring cold chain transportation.

Pancreatic Fluid

Pancreatic fluid is an ideal specimen for the discovery of biomarkers in pancreatic diseases because it is an abundant proximal body fluid source of proteins shed by the pancreatic ductal cells. The use of stabilization media and collection timing is still under investigation by Consortium basic scientists, and degradation experiments are underway to account for the high enzymatic activity of pancreas fluid that interferes with the quality of nucleic acids. Currently, we are collecting pancreas fluid via the endoscopic method during EUS or EGD for 0 – 20 minutes after IV secretin (ChiRhoStim®) stimulation followed by centrifugation, aliquoting and fresh frozen storage at −80ºC. See Figure 4 for more details.

Pancreatic Tissue

Human pancreatic tissue is needed to understand the biologic changes that precede, or are a consequence of, chronic pancreatitis, diabetes, and pancreatic cancer. Pancreas tissue can be collected by surgical excision, fine-needle aspiration biopsy, and endoscopic retrograde cholangiopancreatography with brushings/biopsy of the pancreatic duct. After excision of tissue from the patient, time until immersion into preservative or snap freezing should ideally be kept under 45 minutes, to minimize ischemia and degradation, especially of RNA. See Figure 5 for more details.
LONG-TERM STORAGE/PRESERVATION

Storage and preservation issues were also addressed. Two ml cryovials with conical bottoms are recommended. This facilitates storage and rapid retrieval of any specimen in the biobank from a uniform type of box on freezer shelves with standardized labeling. A freezer-durable method of permanently labeling the cryovial is paramount, and we prefer a barcoding system to facilitate accurate data entry and retrieval.

All freezers should have autonomous continuous temperature monitoring and an alarm system with 24-hour telephone notification of a responsible and available party if there is a freezer malfunction. Freezers should be on a stable power grid with back-up generators and should be positioned well above ground level in an effort to prevent damage by flooding. We also recommend that the biobank be housed in several different freezers in different participating consortium sites to minimize the chance of complete loss due to freezer malfunction or a natural disaster.

BIOINFORMATICS DATA MANAGEMENT SYSTEM

Recording the biospecimen information, the bioinformatics data, is also an important component. Bioinformatics data is comprised of two parts: specimen operational data and analytical data. Specimen operational data refers to sample logistic collection status; analytical data refers to sample clinical characters. Specimen operational data includes sample data collection, processing, storage, shipping, receiving and restoring. Sample SOPs are electronically configured. Specimen operational data stored in the data management system is the foundation of the bioinformatics data analysis of either genetics or analytics. Analytical data is collected and stored in the Integrated Information Management System, which is the Web based electronic data management system that the coordinating center developed.

PEDiatric CONSIDERATIONS

The above protocols were adapted for biospecimen collection from children, and when necessary modified to account for their unique differences from adults. Unlike adult subjects, the amount of blood that can be drawn from pediatric patients varies based on the size of the child. As such, guidelines were developed for maximum amount of blood volume per single draw and per month. Additionally, while collection of urine and saliva from children is non-invasive and theoretically desirable, there are practical issues to consider. Young children are not often able to urinate on demand and may provide a lower volume due to smaller bladder capacity and more frequent needs for urination. Likewise, it is difficult to obtain saliva of adequate quantity and quality from children due to age-dependent abilities to both comprehend and follow instructions. It is more likely to obtain higher yields of DNA from blood samples than saliva, which makes the blood sample a preferred method for DNA collection in most children.
DISCUSSION

A major collaborative effort within the CPDPC has been the establishment of an annotated repository of biospecimens (blood, saliva, urine, stool, pancreatic and duodenal juice, stools and when feasible pancreatic tissue) to facilitate the identification and validation of biomarkers for risk stratification and/or early detection of the diseases study by the Consortium. We have developed SOPs for a biorepository established by the NCI and the NIDDK funded CPDPC. We believe that the timing and handling, storage, and preservation methods used during specimen collection can improve the quality of biospecimens and future biomarker discovery research. It is our goal that the CPDPC SOPs will serve as a guide for investigators developing biorepositories in pancreas research. The rigorous adherence to standardized biospecimen guidelines will provide pancreas researchers with a rich source of well-annotated and characterized human biospecimens, facilitating investigations to better understand the pathophysiology and biologic mechanisms of chronic pancreatitis, diabetes, and pancreatic cancer. The impact of this effort transcends the CPDPC initiative since the biospecimens will be available to the scientific community, through ancillary study collaborations during the life of the CPDPC or through the NCI and NIDDK central repositories, at the end of the study.

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REFERENCES


Pancreas. Author manuscript; available in PMC 2019 November 01.
FIGURE 1.
Blood product standard operating procedure workflow
FIGURE 2.
Urine standard operating procedure workflow
FIGURE 3.
Saliva standard operating procedure workflow
FIGURE 4.
Endoscopic pancreatic function tests collection and pancreas fluid standard operating procedure workflow
FIGURE 5.
Pancreas tissue standard operating procedure workflow

- Add RNase Inhibitor
- Fresh Frozen
- Add Proteinase Inhibitor
- 10% Neutral Buffered Formalin
- Paraffin Blocks / Permanent Sections
- Freeze -80°C
- 5 mm²
- 10 mm²
**Table 1.**

**Essential Data Elements**

<table>
<thead>
<tr>
<th>Data Elements</th>
<th>Examples</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol Adherence</td>
<td>Yes/no</td>
<td>Were all steps of the standard operating procedure followed</td>
</tr>
<tr>
<td>Biospecimen type</td>
<td>Serum, urine</td>
<td>Solid tissue, whole blood, or another product derived from a person</td>
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<tr>
<td>Time of collection</td>
<td>06:32, 15:03</td>
<td>Time of day the biospecimen was obtained</td>
</tr>
<tr>
<td>Collection mechanism</td>
<td>Blood draw, surgical biopsy</td>
<td>How the biospecimens were obtained</td>
</tr>
<tr>
<td>Short term stabilization</td>
<td>On ice</td>
<td>The initial process by which biospecimens were stabilized during collection</td>
</tr>
<tr>
<td>Processing time</td>
<td>3 hours</td>
<td>The time between biospecimen acquisition and being placed in long-term storage.</td>
</tr>
<tr>
<td>Type of preservative</td>
<td>PAXgene, SUPERase- IN RNase Inhibitor</td>
<td>The media used to maintain the biospecimens</td>
</tr>
<tr>
<td>Long term storage temperature</td>
<td>−80°C, liquid nitrogen</td>
<td>The temperature at which the biospecimens were kept until distribution or analysis</td>
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<tr>
<td>Long term storage duration</td>
<td>3–8 y</td>
<td>The time between biospecimen acquisition and distribution or analysis</td>
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<tr>
<td>Shipping temperature</td>
<td>Dry ice</td>
<td>The temperature or range at which biospecimens were kept during shipment or relocation</td>
</tr>
<tr>
<td>Freeze/Thaw Cycles</td>
<td>2</td>
<td>Number of times samples were frozen and thawed before distribution or analysis</td>
</tr>
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</table>
### Standard Operating Procedures Highlights

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Collection Mechanism</th>
<th>Type of Preservative</th>
<th>Short-term Stabilization</th>
<th>Centrifuge</th>
<th>Processing Time</th>
<th>Aliquot</th>
<th>Long-Term Storage</th>
<th>Sample Specific Data Elements</th>
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<tbody>
<tr>
<td>Blood DNA</td>
<td>Blood draw</td>
<td>PAXgene Blood RNA Tube</td>
<td>Room temperature</td>
<td>---</td>
<td>&lt;4 h</td>
<td>1.7 ml</td>
<td>−80ºC</td>
<td>• Participant fasting yes/no • Date and time participant last ate or drank anything besides plain water</td>
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<tr>
<td>Blood RNA</td>
<td>Blood draw</td>
<td>PAXgene Blood RNA Tube</td>
<td>Room temperature for 2 hours, then −20ºC freezer for at least 24 hours</td>
<td>---</td>
<td>---</td>
<td>2.5 ml</td>
<td>−80ºC</td>
<td>• Participant fasting yes/no • Date and time participant last ate or drank anything besides plain water</td>
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<tr>
<td>Plasma</td>
<td>Blood draw</td>
<td>EDTA tube (purple top)</td>
<td>4ºC (refrigerated or on wet ice)</td>
<td>1200g at room temperature for 10 minutes with brake on</td>
<td>&lt;4 h</td>
<td>250 μl × 10 cryovials, then 1.8 ml for the remainder of the sample</td>
<td>−80ºC</td>
<td>• Participant fasting yes/no • Date and time participant last ate or drank anything besides plain water</td>
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<tr>
<td>Serum</td>
<td>Blood draw</td>
<td>Red top (serum) tubes (silicon-coated) with no additives</td>
<td>Room temperature</td>
<td>1200g at room temperature for 10 minutes with brake on</td>
<td>&lt;4 h</td>
<td>250 μl × 10 cryovials, then 1.8 ml for the remainder of the sample</td>
<td>−80ºC</td>
<td>• Participant fasting yes/no • Date and time participant last ate or drank anything besides plain water</td>
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<td>Specimen Type</td>
<td>Collection Mechanism</td>
<td>Type of Preservative</td>
<td>Short-term Stabilization</td>
<td>Centrifuge</td>
<td>Processing Time</td>
<td>Aliquot</td>
<td>Long-Term Storage</td>
<td>Sample Specific Data Elements</td>
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<tr>
<td>PBMCs from Buffy Coat Layer</td>
<td>Blood draw</td>
<td>EDTA tube (purple top)</td>
<td>Room temperature</td>
<td>Multiple spins</td>
<td>&lt;4 h</td>
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<td>Liquid nitrogen</td>
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<td>• Date and time participant last used chewing gum</td>
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<tr>
<td>Saliva</td>
<td>Passive drool</td>
<td>Fresh-frozen and SUPERase-IN RNase Inhibitor</td>
<td>4°C (refrigerated or on wet ice)</td>
<td>Unspun</td>
<td>&lt;4 h</td>
<td>250 μl</td>
<td>−80°C</td>
<td>• Date and time participant last brushed teeth</td>
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<td>• Date and time participant last used mouth wash</td>
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<td>• Date and time participant last used mouth wash</td>
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<tr>
<td>Urine</td>
<td>Midstream “clean catch”</td>
<td>Fresh frozen, SUPERase-IN RNase Inhibitor, and Complete Protease Inhibitor</td>
<td>4°C (refrigerated or on wet ice)</td>
<td>1200 g at 4°C for 10 minutes with brake on</td>
<td>&lt;4 h</td>
<td>1.8 mL with additional cryovial for pellet</td>
<td>−80°C</td>
<td>• Urine dipstick results</td>
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<td>• Urine dipstick results</td>
</tr>
<tr>
<td>Stool for fecal elastase</td>
<td>By participant at home</td>
<td>Fresh no preservative</td>
<td>Room temperature</td>
<td>---</td>
<td>&lt;48 h</td>
<td>---</td>
<td>−20 to −70°C</td>
<td>---</td>
</tr>
<tr>
<td>Stool for microbiome</td>
<td>By participant at home</td>
<td>OMNIgene•GUT kit</td>
<td>Room temperature</td>
<td>---</td>
<td>&lt;14 d</td>
<td>100 mg</td>
<td>−80°C</td>
<td>• Total parenteral nutrition and enteral</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Collection Mechanism</td>
<td>Type of Preservative</td>
<td>Short-term Stabilization</td>
<td>Centrifuge</td>
<td>Processing Time</td>
<td>Aliquot</td>
<td>Long-Term Storage</td>
<td>Sample Specific Data Elements</td>
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<tr>
<td>Pancreatic fluid</td>
<td>Upper endoscopy with secretin stimulation</td>
<td>Fresh frozen *</td>
<td>4°C (refrigerated or on wet ice)</td>
<td>1200 g at 4°C for 10 minutes with brake on</td>
<td>&lt;4 h</td>
<td>250 μl × 12 cryovials, then 1.8 ml for the remainder of the sample, with additional 2 cryovials for pellets</td>
<td>−80°C</td>
<td>feeds within last year</td>
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<tr>
<td>Tissue</td>
<td>Biopsy, surgery, or ERCP</td>
<td>Fresh frozen, SUPERase-IN RNase Inhibitor, and cOmplete Protease Inhibitor</td>
<td>4°C (refrigerated or on wet ice)</td>
<td>---</td>
<td>&lt;45 min</td>
<td>∼100 mg</td>
<td>−80°C</td>
<td>• Warm ischemia time • Sample weight</td>
</tr>
</tbody>
</table>

* Use of SUPERase-IN RNase Inhibitor, and cOmplete Protease Inhibitor will be determined pending an additional study.