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Standard Operating Procedures for Biospecimen Collection, Processing, and Storage: From the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC)

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Abstract

Differences in methods for biospecimen collection, processing, and storage can yield considerable variability and error. Therefore, best practices for standard operating procedures are critical for successful discovery, development, and validation of disease biomarkers. Here, we describe standard operating procedures developed for biospecimen collection during the DREAM (*D*iabetes *RE*lated to *A*cute pancreatitis and its *M*echanisms) Study within the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC). Notably these protocols were developed using an integrative process based on prior consortium experience and with input from working groups with expertise in immunology, pancreatitis and diabetes. Publication and adoption consistent biospecimen protocols will inform future studies and allow for better comparisons across different metabolic research efforts.

Keywords

laboratory protocol; pancreas; immunology; type 1 diabetes; biosamples; biorepository

INTRODUCTION

Scientific breakthrough research would not be possible without the participation of individuals who selflessly donate biological specimens, which are used as a *research bridge* between basic and translational research. Best practices for specimen collection, processing and storage are key for consistency and validity of research findings emanating from biospecimen testing.[1–3] Thus, the careful curation and storage of biospecimens is also part of both ethical and scientific importance.[1,3,4]

The type 1 diabetes in acute pancreatitis consortium (T1DAPC), supported by the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), has established a biospecimen committee to develop standard operating procedures (SOP) for biospecimen collection during the DREAM (*D*iabetes *RE*lated to *A*cute pancreatitis and its *M*echanisms) Study to improve our understanding of the mechanistic underpinnings resulting in the development of diabetes after acute pancreatitis (AP). This SOP builds upon the document developed for the Chronic Pancreatitis Diabetes Pancreas Cancer Consortium (CPDPC), supported by the National Cancer Institute (NCI) and the NIDDK.[2]

There are several unique components of the T1DAPC biospecimen committee structure worth reporting. Notably several working groups were queried (the immunology, the pancreatitis and the diabetes working groups), and by an integrative process their efforts (reported elsewhere in this collection) were incorporated into the biorepository design as detailed below. The T1DAPC SOPs will serve as a valuable resource for investigators wishing to engage in performing research within and outside of the consortium with an interest in pancreatitis related diabetes.

OBJECTIVES

In addition to the previously outlined objectives of the DREAM Study, a major collaborative effort within the consortium will be the establishment of an annotated repository of biospecimens (serum, plasma, peripheral blood mononuclear monocytes [PBMC], DNA, RNA, and stool). The T1DAPC biorepository will allow the identification of biomarkers to achieve the following objectives: 1) risk stratification of pancreatitis participants, 2) early detection of type 1 diabetes, 3) narrowing knowledge gaps of interrelationships between the endocrine and exocrine pancreas, and 4) informing the development of future strategies to prevent or reverse diabetes after AP.

MEETINGS

The T1DAPC formed a Biospecimen Committee, including 2 Co-Chairs (C.W. and D.L.C.) and a data coordinating center (DCC) Biostatistician (A.M.D.), which met monthly for 18 months. The Biospecimen Committee was composed of representatives of all the clinical centers, members of the T1DAPC Working Groups (pancreatitis, diabetes, immunology) and the NIDDK. The committee co-chairs and DCC Biostatistician met at a minimum weekly to organize the committee and provided monthly reports to the T1DAPC Steering Committee as requested.

RESOURCES UTILIZED

CPDPC SOPs were used as a starting point along with T1DAPC clinical center SOPs. Blood and stool (as detailed in Table 1 and 2) were the initial T1DAPC SOPs to be developed for the DREAM Study. It was decided that if ancillary studies were developed that needed additional biospecimens, the CPDPC SOP would be consulted for initial guidance (pancreas juice, urine, saliva, tissue and other).

PROCESS

Similarly to the aforementioned CPDPC effort, consensus was obtained for best practices and SOP developed and added to the manual of operations (MOP). These were relatively straightforward for standard serum, plasma and stool collections since these have been well characterized and utilized across many consortia.[2,5,6] The consortium currently has several clinical centers, some with satellite sites, from which study participants are enrolled[7] (Fig. 1). Most samples collected from these participants are processed according to the SOPs and aliquots stored locally until shipment to the study biorepository, Penn State University Institute for Personalized Medicine (PSU-IPM) and the NIDDK Central Repository (DKCR) (Figs. 1 and 2; Tables 1 and 2). However, the working groups specific to the T1DAPC brought unique insight to the biorepository. The rationale and mechanisms of obtaining these samples are described in detail elsewhere.[7,8] Specifically, the immunology working group strongly supported the use of centralized and standardized processing of PBMC[9] to minimize the variability introduced by having multiple laboratories process these samples and to standardize processing and storage. The heparinized (green top) blood samples have an aliquot taken with blood cell stabilizers added, frozen, stored and remaining green top samples sent same day with overnight shipping to a centralized PBMC processing facility. Cryopreserved PBMC are then periodically shipped to the central repository on liquid nitrogen until needed for functional assays (Fig. 1).

The pancreatitis working group finalized and advised the biospecimen committee on collection of stool samples as outlined Table 1 and Figure 1. The clinical centers will send an aliquot of stool samples to JOLI Diagnostic, Inc. (Williamsviille, NY, http://jolidiagnostic.com) for fecal elastase measurement with data being sent back to the clinical site. The remainder of stool samples are initially collected in OMNIgene-GUT and OMNImet-GUT tubes (DNA Genotek, Ottawa, Canada, https://www.dnagenotek.com), and then aliquoted, for future microbiome and metabolome studies respectively (Table 1). These samples will be sent to the central repository periodically for storage and distribution as indicated (Fig. 1).

The diabetes working group focused on aspects related to monitoring progression to diabetes as described in the diabetes working group manuscript.[10] A unique aspect of this is timed sampling of blood following a glucose or standardized meal challenge and the use of P800 plasma collection tubes (Becton Dickinson, Franklin Lakes, NJ) containing a cocktail of protease inhibitors specifically used to stabilize labile hormones (Fig. 2 and Table 2). The clinical sites are responsible for aliquoting and storing these samples and then periodically sending batched samples to the study biorepository (Fig. 1).

The biorepository is responsible for sending samples periodically to the designated endocrine laboratory (Indiana University Center for Diabetes and Metabolic Diseases Translation Core) for analysis of the samples from the glucose or meal challenge procedure. Also, periodically a serum aliquot from each participant visit will be sent to the autoantibody laboratory (Barbara Davis Center for Diabetes - University of Colorado) for analysis of traditional T1D associated autoantibodies.[11] Finally, the Tempus tube (Applied Biosystems, Waltham, Mass) samples will be distributed for RNA transcriptome analysis as described in

the immunology working group manuscript.[8] A PAXgene tube (Qiagen, Germantown, Md) will be collected once from each participant in the study for future genomic studies (Fig. 1).

The biorepository will keep in reserve 20–30% of samples for eventual long-term storage at the DKCR. Ancillary studies are anticipated throughout the duration of the study and upon approval samples will be sent to investigators.

PREPARATION OF WRITTEN SOPs

Representatives from each clinical center with expertise in immunology, diabetes, pancreatitis and stool testing were solicited for their opinion on the initial versions of the SOPs and a final version was approved by the Steering Committee and included in the MOP.

TRAINING

Because sample processing was relatively straightforward, biospecimen collection and processing was incorporated into DREAM Coordinator training. Coordinators and lab technicians were required to undergo a separate Data Management System (DMS) training specific to sample management.

BIOINFORMATICS DATA MANAGEMENT SYSTEM

The Sample Tracking (ST) module of the DCC DMS[12] serves as the bioinformatics data management system in T1DAPC. In ST, users can generate sample specific barcoded labels. After labeling samples, users will use ST to associate participant information (ID, visit, collection date) with the sample barcode, as well as sample volume. Sample tracking is also used to ship samples from the sites to labs and the biorepository, and for the labs and biorepository to mark samples received.

LONG-TERM STORAGE/PRESERVATION

The Penn State Institute for Personalized Medicine was selected to serve as the T1DAPC biorepository. Aliquoted samples are stored in 2 mL cryovials suitable for long-term storage. These will be stored in −80°C freezers at the clinical sites until shipment to the biorepository. Upon shipment to the biorepository, samples will be maintained in −80°C freezers, or liquid nitrogen (LN) tanks for PBMCs, located at two separate sites within the Penn State College of Medicine and supported by separate electric grids and backup generators. All −80°C freezers have direct CO2 backup systems, while the LN freezer is connected directly to an LN storage tank that provides automated backup. All −80°C and LN freezers are monitored continuously (24 h/d; 7 d/wk) by remote monitoring with output providing continuous temperature records of each unit as well as notification systems in case of temperature deviation.

Location and status of each sample are maintained in a FreezerPro database (version 7.5.1 Azenta Life Sciences, Chelmsford, Mass). Accuracy of the location information in FreezerPro is routinely verified by monthly spot checks and immediately following the upload of any large data sets.

DISCUSSION

We have successfully developed an SOP within the T1DAPC for the DREAM Study to facilitate the identification and validation of biomarkers for risk stratification, early detection and to improve our understanding of pancreatitis related diabetes. It has been well described in the literature that 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.[1–3] This trend is particularly true for biospecimens collected for pancreas research in which prior biomarker development is lacking. 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.

The DREAM biospecimens will be a rich source for biomarker investigations that will improve our understanding of pancreatitis related diabetes and will have far reaching impact for the present and future. Biospecimens will be available to the scientific community through ancillary study collaboration with a T1DAPC clinical center during the lifetime of the T1DAPC. In the future, approximately 20–30% of the DREAM biospecimens along with matching essential data elements will be stored at the DKCR and will be available for the wider scientific community at the end of the DREAM Study.

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Abbreviations

AP	Acute Pancreatitis
CPDPC	Chronic Pancreatitis Diabetes Pancreas Cancer Consortium
DCC	Data Coordinating Center
DMS	Data Management System
DKCR	NIDDK Central Repository
DREAM	Diabetes RElated to Acute pancreatitis and its Mechanisms
LN	Liquid Nitrogen
MOP	Manual of Operations
NCI	National Cancer Institute
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
PBMC	Peripheral Blood Mononuclear Cells
PSU-IPM	Penn State University Institute for Personalized Medicine
SOP	Standard Operating Procedures
ST	Sample Tracking Module
T1DAPC	Type 1 Diabetes in Acute Pancreatitis Consortium

REFERENCES

1. Feng Z, Kagan J, Pepe M, et al. The Early Detection Research Network's Specimen reference sets: paving the way for rapid evaluation of potential biomarkers. Clin Chem. 2013;59:68–74. [PubMed: 23193062]
2. Fisher WE, Cruz-Monserrate Z, McElhany AL, et al. Standard operating procedures for biospecimen collection, processing, and storage: From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer. Pancreas. 2018;47:1213–1221. [PubMed: 30325860]
3. Moore HM, Kelly AB, Jewell SD, et al. Biospecimen reporting for improved study quality (BRISQ). Cancer Cytopathol. 2011;119:92–101. [PubMed: 21433001]
4. Biorepositories and Biospecimen Research Branch. NCI Best Practices for Biospecimen Resources. Bethesda, MD; National Cancer Institute, National Institutes of Health: 2016. Available at: https://biospecimens.cancer.gov/bestpractices/. Accessed April 2022.
5. Sanderson-November M, Silver S, Hooker V, et al. Biorepository best practices for research and clinical investigations. Contemp Clin Trials. 2021;116:106572. [PubMed: 34583056]

6. Hullsiek KH, George M, Brown SK. Designing and managing a flexible and dynamic biorepository system: a 15-year perspective from the CPCRA, ESPRIT, and INSIGHT clinical trial networks. Curr Opin HIV AIDS. 2010;5:538–544. [PubMed: 20978398]
7. Hart PA, Papachristou GI, Park WG, et al. Rationale and design for the *D*iabetes *RE*lated to Acute pancreatitis and its *M*echanisms (DREAM) study: A prospective cohort study from the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC). Pancreas. 2022;51:XXX–XXX.
8. Casu A, Grippo PJ, Wasserfall C, et al. Evaluating the Immunopathogenesis of Diabetes Following Acute Pancreatitis in the *D*iabetes *RE*lated to *A*cute Pancreatitis and Its *M*echanisms (DREAM) Study: From the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC). Pancreas. 2022;51:XXX–XXX.
9. Ahmed S, Cerosaletti K, James E, et al. Standardizing T-Cell biomarkers in type 1 diabetes: challenges and recent advances. Diabetes. 2019;68:1366–1379. [PubMed: 31221801]
10. Dungan KM, Hart PA, Andersen DK, et al. Assessing the pathophysiology of hyperglycemia in the *D*iabetes *RE*lated to *A*cute Pancreatitis and Its *M*echanisms Study (DREAM): From the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC). Pancreas. 2022;51:XXX–XXX.
11. So M, O'Rourke C, Ylescupidez A, et al. Characterising the age-dependent effects of risk factors on type 1 diabetes progression. Diabetologia. 2022;65:684–694. [PubMed: 35041021]
12. Dyer AM, Baab K, Merchlinski A, et al. Rationale and development of a data coordinating center to support the Type 1 Diabetes in Acute Pancreatitis Consortium (T1DAPC). Pancreas. 2022;51:XXX–XXX.

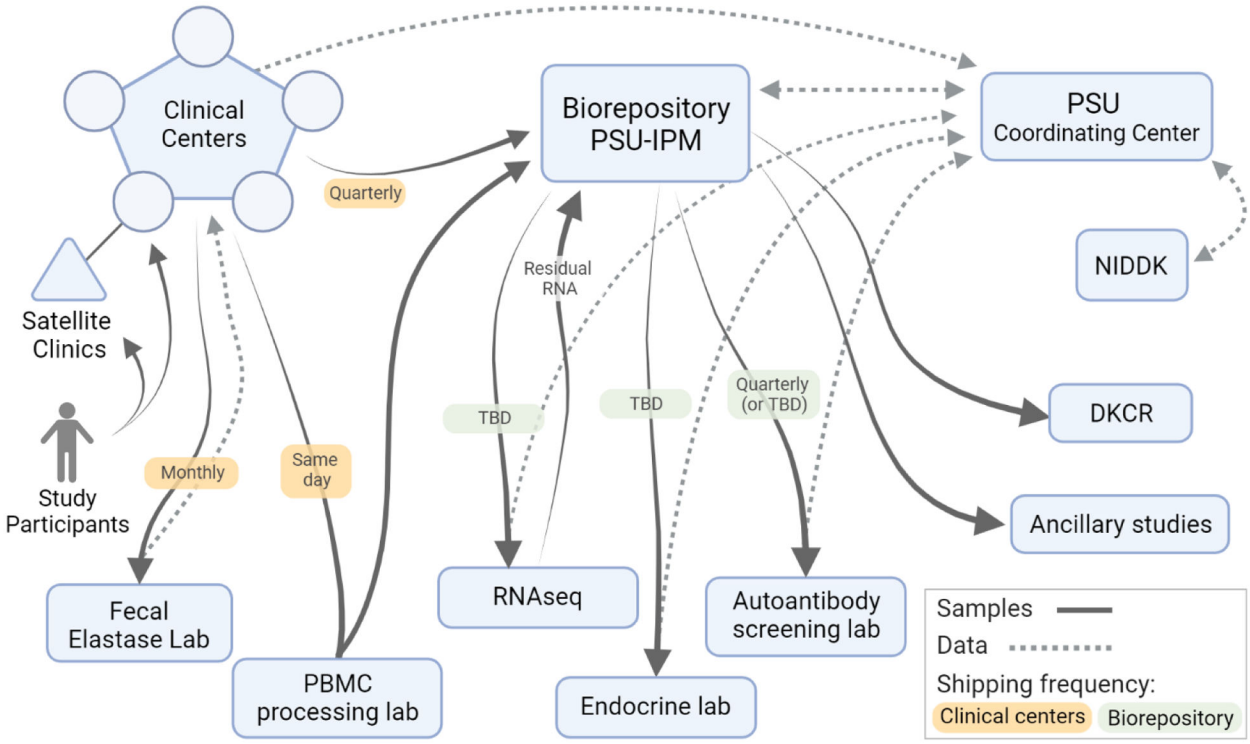

FIGURE 1.

The overall flow of biospecimens and data within the T1DAPC. DKCR, NIDDK Central Repository; PBMC, peripheral blood mononuclear cells; PSU-IPM, Penn State University Institute for Personalized Medicine; TBD, to be determined.

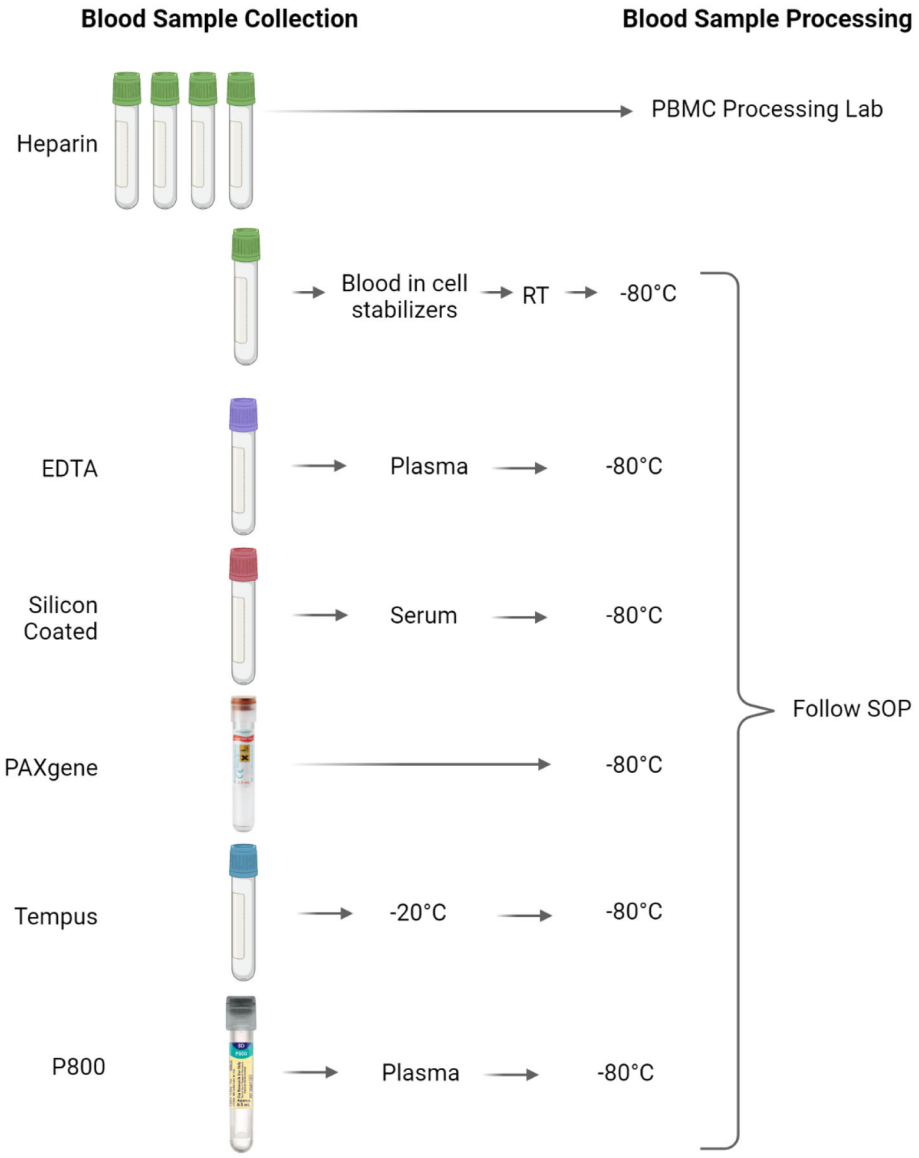

FIGURE 2.
Blood sample collection.

TABLE 1.

Detailed Specimens Collected in T1DAPC

Specimen Type	Collection	Type of Preservative	Short-term Stabilization	Centrifuge	Processing Time	Aliquot	Long-term Storage
Plasma	Blood draw	EDTA tube * (purple top)	4°C (refrigerated or on wet ice)	1200 *g* at room temperature for 10 min with brake on	<4 h	250 μL × 20	−80°C
Serum	Blood draw	Silicone-coated (red top)	Room temperature for 30–60 min, then 4°C until centrifugation	1200 *g* at room temperature for 10 min with brake on	<4 h	250 μL × 20	−80°C
DNA	Blood draw	PAXgene tube *	Room temperature	NA	NA	NA	−80°C
RNA	Blood draw	Tempus tube †	Room temperature, then −20°C for 24 h	NA	NA	NA	−80°C
Blood in cell stabilizers	Blood draw	Sodium heparin tube (green top), PROT1 proteomic stabilizer	Room temperature	NA	<12 h	600 μL × 6 (250 μL blood added to 350 μL PROT1)	−80°C
Stool for fecal elastase	Home collection	NA	Room temperature	NA	NA	NA	−80°C
Stool for metabolome	Home collection	OMNImet-GUT ‡	Room temperature	Vortex each tube at medium setting for 60 s	NA	~ 0.8 mL × 3	−80°C
Stool for microbiome	Home collection	OMNIgene-GUT ‡	Room temperature	Vortex each tube at medium setting for 60 s	NA	~ 0.8 mL × 3	−80°C

* Mix gently by inverting 2 to 3 times

† Shake vigorously or vortex for 10 seconds

‡ For a minimum of 30 seconds, shake the sealed tube as hard and fast as possible in a back and forth motion.

NA indicates not applicable.

TABLE 2.

Detailed Metabolic Procedure Specimens Collected in T1DAPC

Specimen Type	Collection	Metabolic Test	Type of Preservative	Short-term Stabilization	Centrifuge	Processing Time	Aliquot	Long-term Storage
Plasma	Blood draw	OGTT (6 time points)	2 ml P800 tube*	4°C (refrigerated or on wet ice)	1200 *g* at room temperature for 10 min with brake on	<4 h	250 μL × 4 per time point	−80°C
		MMTT (8 time points)	8.5 ml P800 tube*		1200 *g* at room temperature for 20 min with brake on		250 μL × 4 and 500 μL × 6 per time point	
Serum	Blood draw	OGTT (6 time points)	6 ml Silicone-coated (red top)	Room temperature for 30–60 min, then 4°C until centrifugation	1200g at room temperature for 10 minutes with brake on	<4 h	250 μL × 12 per time point	−80°C
		MMTT (8 time points)	4 ml Silicone-coated (red top)				250 μL × 8 per time point	
		FSIGTT (21 time points)	3 or 5 ml Silicone-coated (red top)				500 μL × 3 or 500 μL × 5 per time point	

*Mix gently by slowly inverting 8 to 10 times

OGTT indicates oral glucose tolerance test; MMTT, mixed meal tolerance test; FSIGTT, frequently sampled intravenous glucose test.