Collection Protocol for Human Pancreas

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Abstract

This dissection and sampling procedure was developed for the Network for Pancreatic Organ Donors with Diabetes (nPOD) program to standardize preparation of pancreas recovered from cadaveric organ donors. The pancreas is divided into 3 main regions (head, body, tail) followed by serial transverse sections throughout the medial to lateral axis. Alternating sections are used for fixed paraffin and fresh frozen blocks and remnant samples are minced for snap frozen sample preparations, either with or without RNAase inhibitors, for DNA, RNA, or protein isolation. The overall goal of the pancreas dissection procedure is to sample the entire pancreas while maintaining anatomical orientation.

Endocrine cell heterogeneity in terms of islet composition, size, and numbers is reported for human islets compared to rodent islets. The majority of human islets from the pancreas head, body and tail regions are composed of insulin-containing β-cells followed by lower proportions of glucagon-containing α-cells and somatostatin-containing δ-cells. Pancreatic polypeptide-containing PP cells and ghrelin-containing epsilon cells are also present but in small numbers. In contrast, the uncinate region contains islets that are primarily composed of pancreatic polypeptide-containing PP cells. These regional islet variations arise from developmental differences. The pancreas develops from the ventral and dorsal pancreatic buds in the foregut and after rotation of the stomach and duodenum, the ventral lobe moves and fuses with the dorsal. The ventral lobe forms the posterior portion of the head including the uncinate process while the dorsal lobe gives rise to the rest of the organ. Regional pancreatic variation is also reported with the tail region having higher islet density compared to other regions and the dorsal lobe-derived components undergoing selective atrophy in type 1 diabetes.

Additional organs and tissues are often recovered from the organ donors and include pancreatic lymph nodes, spleen and non-pancreatic lymph nodes. These samples are recovered with similar formats as for the pancreas with the addition of isolation of cryopreserved cells. When the proximal duodenum is included with the pancreas, duodenal mucosa may be collected for paraffin and frozen blocks and minced snap frozen preparations.

Video Link

The video component of this article can be found at http://www.jove.com/video/4039/

Protocol

1. Procedure is optimally accomplished with three staff members however the procedure can be conducted with one well trained individual. Two individuals can generally complete the procedure in 90 minutes. Actual duration is dependent on numbers of tissues received and ease of

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1. Fix tissues for 16 hours (range 20 ± 4 hours) using an automatic paraffin processor or using manual timing. Process to paraffin blocks using an automatic processor (Appendix 2).

2. Keep vials with RNALater at room temperature for 30 minutes to allow for equilibration, remix and rapidly freeze in liquid nitrogen or in the dry ice-isopentane slurry then transfer to a -80 °C freezer.

3. Pancreas Dissection

4. Spleen, Lymph Node Dissections and Duodenal Mucosa Dissections

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6. Submit Fixed Samples for Paraffin Processing

7. Clean dissection areas and disinfect. Wash dissection instruments and re-sterilize. Place remnant human tissues in formalin fixative for storage as biomedical waste. Discard biomedical waste according to local regulations within one month.

8. Update the Sample Inventory System
9. Representative Results

This procedure will process an intact human pancreas with representative sampling throughout the organ including demarcation of the three major regions, namely, head, body and tail within 2 hours. The uncinate region, found in the posterior head region, is included in the head dissection. The median pancreatic weight in organ donors without diabetes was 82.4 grams (52.7 - 139.0 grams). Pancreas regional weights were approximately equal (Figure 3).

This procedure can also allow for reconstruction of the entire pancreas using stained slides from sequential paraffin and OCT blocks. Multiple formats are feasible allowing for maximum utilization for current and future technologies. The current nPOD case worksheet is provided (Appendix 1) that is used to document recovered samples and subsequent processing by aliquot types and quantities.

Figure 1. Overall scheme of the procedure. The entire pancreas is processed while maintaining anatomical orientations. The head region is longer in the superior-inferior axis due to the presence of the ventral pancreatic lobe in the posterior region that includes the uncinate region. Hatched area at junctions denote regions used for minced samples in cryovials. P, Paraffin; O, OCT.
Figure 2. Lettering scheme for pancreas sections. Transverse pancreas slices can be further divided into halves or quarters and lettered in a clockwise manner.

Figure 3. Pancreas weights from organ donors without diabetes. The intact pancreas from adult donors (>17 years old) was weighed then divided into regions and regional weights obtained. Data are means ± SEM (N=15).

Discussion

The goal of this procedure is to define a standard pancreas processing that can be harmonized across multiple collection sites and thus allow comparisons of results obtained by different groups. The dissection method is based on standard surgical pathology practices with additional steps for annotation of major pancreas regions followed by intra-regional subdivisions. Standardization of biospecimen processing methods is required to facilitate collection of high quality samples exemplified by the publication of best practices for biospecimen collection from national biobanking organizations such as the National Cancer Institute. The biobanking of human pancreas samples from donors representing various stages of diabetes and suitable control populations has been reported. However, exact details for those samples collected from the pancreatic head region have been lacking. Considerable inter-individual islet heterogeneity is well known with up to 5-fold variations in islet size distribution.
so that annotation of pancreatic region is needed when analyzing inherent heterogeneity\(^\text{(1)}\). Processing pancreatic samples in standardized formats will assist with resolving individual patient factors so that other underlying key disease factors can be better resolved.

**Disclosures**

No conflicts of interest declared.

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**References**


