A. **Day 1:**
1. Label specimen cups and cassettes with animal ID and relevant information before beginning.
2. Anesthetize and euthanize the mouse according to IACUC protocol of your institution.
3. Optional: weigh the mouse for total body weight; record the weight in grams.
4. It is helpful to always **orient animals in the same direction**, e.g. head up or head right, so that the side of any lesions can be recalled accurately. Tape or pin down limbs and wipe down fur with 70% ethanol.
5. Open the abdomen; start at the pubis, and continue through the sternum to open the thorax; continue past the upper thorax with skin incision to the chin.
6. Identify the pancreas by **gently lifting** the spleen. One can either dissect each separately or use the spleen to help pull the pancreas away from the upper GI tract during dissection. Recover the entire pancreas including left (near spleen) and right lobes (near GI). Pancreas and pancreatic lymph nodes are pale and soft tissues are difficult to distinguish grossly, but present very distinct histology. Place all together.
7. **Weigh the pancreas for subsequent beta cell mass calculations.** Average pancreatic wet weight is about 20 grams.
8. Place the pancreas on a dry sponge in a cassette. Spread it out gently to flatten it in as compact an area as possible. One should not see any major “mounds” of pancreatic tissue, nor should there be excessive stretching. Use only one sponge. Close the cassette lid securely and place in fixative (formalin).
9. One can combine other organs, such as the spleen, into the same cassette as the pancreas; however, the submandibular salivary glands and lymph nodes are best put into a separate cassette.
10. It is suggested, for best practice, to include a piece of the duodenum (small intestine) with the right lobe of the pancreas for orientation and identification purposes.

**Reagents:**
1. General Laboratory Source
   - 10% Bleach
   - 70% Ethanol
   - Phosphate buffered saline (PBS)
   - Anesthetic, as described in Institutional IACUC protocol
   - 10% neutral buffered formalin (NFB)
2. Thermo Fisher
   - 10% neutral buffered formalin (NBF), cat # 23-245-685

**Equipment:**
- Ventilated work station to protect prosector from formalin fumes
- Gloves and laboratory attire (e.g. lab coat, apron, etc.)
- Cutting board
- Serrated, blunt-ended forceps
- Fine, blunt-ended scissor
- Tape or pins to immobilize mouse on cutting board (optional)
- Specimen containers
- Cassettes with lids
- Biopsy sponges
- Weighing scale
11. Trim the spleen by taking a center cross section or longitudinal sample. Avoid submitting areas of the spleen that have been crushed by the forceps. Place into cassette, close the lid securely, and place into fixative.

12. Reflect the skin of the neck laterally to expose the salivary glands (submandibular, sublingual, and parotid) and associated mandibular lymph nodes. These glands and nodes are removed bilaterally “en-bloc” and placed in the cassette and fix.

13. The thymus gland is in the thoracic cavity where the esophagus enters from the neck region. Harvest as one piece and place in cassette and fix.

14. Thyroid glands are immediately caudal to the larynx on either side of the trachea. They may be difficult to see without magnification, but if a 2mm section of trachea immediately caudal to the larynx is removed, thyroid glands usually will be included. This section of trachea can be placed in a cassette and fixed.

15. When fixing specimens, fix for no longer than 12 hours, if possible, to minimize over fixation. The preferred fixation time for 10% neutral buffered formalin (NBF) is 4-8 hours, depending on mouse sample thickness (i.e., penetration rate is ~1mm/hour). 4% fresh paraformaldehyde is an excellent fixative and may be preferred over formalin. Fixation time is generally overnight at 4°C.

16. For samples intended for immunohistochemistry, it is imperative to standardize the time in fixative so that animals are handled consistently between treatment groups.

17. End fixation by transferring cassettes to either PBS or 70% ethanol. The samples are now ready for processing and embedding. This is often done by the institution’s histology core or by a participating Laboratory Member. Suggestions and tips for this part of the process are given in the next section.

18. Used formalin should be discarded as hazardous waste.

**B. Suggestions on Tissue Section Selection**

1. Collect unstained slides from two levels within the tissue. A 0.0 um level at full case and a second level at 150um are recommended. Levels can be collected on one slide.

2. The number of sections obtained from each level depends on the number of stains required for the experiment (e.g. how many different primary antibodies are to be used).

3. One slide containing both levels should be stained with H&E as well.

**Additional information:**

This protocol is adapted from the Standard Operating Procedure (SOP - attached) for the Univ. of Florida College of Medicine Department of Pathology, Immunology and Laboratory Medicine Molecular Pathology Core. The purpose of this SOP is to outline proper procedures for correct harvesting, fixing and submitting of Type 1 diabetic (T1D) mouse pancreas for histological services and analyses. This SOP is applied to all Type 1 diabetic studies that require histological services and analyses on mouse pancreas. It is being presented here as a resource for those interested in doing immunohistochemical studies on mouse pancreas and diabetes.

It is recommended that the tissue sections be visualized using the Aperio system. Image analysis can be conducted on scanned slides to determine fractional insulin area (FIA). If pancreatic weight was obtained at necropsy, multiply FIA by the pancreatic wet weight to obtain beta cell mass. Insulitis can be scored on H&E stained slides using the following scale:

- 0 = no infiltrates
- 1 = peri-islet infiltrates
- 2 = intra-islet infiltrates to <or= 50%

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*Last Edited May 27, 2010 by the BCBC Coordinating Center*
3 = intra-islet infiltrates >50%
4 = non-islet infiltrates (optional)
The average scoring per animal can be expressed as percentage of 100 or by number of islets with each score.
T1D MOUSE NECROPSY FOR PANCREAS AND RELATED ORGANS

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline proper procedures for correct harvesting, fixing, and submitting of Type 1 diabetic mouse pancreas for histological services and analyses.

2 SCOPE

This SOP shall be applied to all Type 1 diabetic studies that require histological services and analyses on mouse pancreas.

3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment is appropriately cleaned, maintained in good working order, and available as requested.

3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

4 EQUIPMENT and MATERIALS

4.1 Ventilated work station to protect prosector from formalin fumes

5 SAFETY

5.1 Formalin: Avoid inhalation, ingestion, and skin contact. If skin contact occurs, flush affected area with water. Label all containers with a formalin warning label. Use formaldehyde spill kit for spills up to 1 liter. Call EHS for larger spills.

5.2 Work in well-ventilated area with appropriate personal protective equipment (e.g., gloves, etc.)

6 PROCEDURE

6.1 Label specimen cups and cassettes with animal ID and relevant information before beginning. Printed cassettes can be requested from the Molecular Pathology Core (MPC).

6.2 Anesthetize and euthanize the mouse according to IACUC protocol.

6.3 Optional: weigh mouse for total body weight. Record weight in grams.

6.4 It is helpful to always orient animals in the same direction, e.g., head up or head right, so that the side of any lesions can be recalled accurately. Tape or pin down limbs and wipe down fur with 70% ethanol.

6.5 Open the abdomen, starting at the pubis, and continue through the sternum to open the thorax. Continue past the upper thorax with skin incision to the chin.

6.6 Identify the pancreas by gently lifting the spleen. One can either dissect each separately or use the spleen to help pull the pancreas away from the upper GI tract during dissection. Recover the entire pancreas including left (near spleen) and right lobes (near GI). Pancreas and pancreatic lymph nodes are pale and soft tissues and are difficult to distinguish grossly, but are very distinctive histologically.

6.6.1 Weigh the pancreas for subsequent beta cell mass calculations. Average pancreatic wet weight is about 20 grams.

6.6.2 Place the pancreas on a dry sponge in a cassette. Spread it out gently to flatten it in as compact an area as possible. One should not see any major “mounds” of pancreatic tissue, nor should there be excessive stretching. Use only one sponge. Close the cassette lid securely and place in fixative.

6.6.3 One can combine other organs, such as the spleen, into the same cassette as the pancreas; however, the submandibular salivary glands and lymph nodes are best put into a separate cassette.

6.6.4 It is suggested best practice to include a piece of the duodenum (small intestine) with the right lobe of the pancreas for orientation and identification purposes.

6.7 Trim the spleen by taking a center cross section or longitudinal sample. Avoid submitting areas of the spleen that have been crushed by the forceps. Place into cassette, close the lid securely, and place into fixative.
6.8 Reflect the skin of the neck laterally to expose the **salivary glands (submandibular, sublingual, and parotid) and associated mandibular lymph nodes**. These glands and nodes are removed bilaterally “en-bloc” and placed in the cassette and fixed.

6.9 The **thymus** gland is in the thoracic cavity where the esophagus enters from the neck region. Harvest as one piece and place in cassette and fix.

6.10 **Thyroid glands** are immediately caudal to the larynx on either side of the trachea. They may be difficult to see without magnification, but if a 2mm section of trachea immediately caudal to the larynx is removed, thyroid glands usually will be included. This section of trachea can be placed in cassette and fixed.

6.11 When fixing specimens, fix for no longer than 12 hours, if possible, to minimize over-fixation. The preferred fixation time for 10% neutral buffered formalin (NBF) is 4-8 hours, depending on mouse sample thickness (i.e., penetration rate is ~1mm/hour). 4% freshly prepared paraformaldehyde is an excellent fixative and may be preferred over formalin for immunohistochemistry depending on the antigen. Fixation time is generally overnight at 4ºC.

6.12 For samples intended for immunohistochemistry, it is imperative to standardize the time in fixative so that animals are handled consistently between treatment groups.

6.13 End fixation by transferring cassettes to either PBS or 70% ethanol. Submit to the MPC for processing and embedding.

6.14 Used fixative should be discarded as a hazardous waste.

6.15 Request the following services on the MPC Submission Form:
   6.15.1 Process and embed
   6.15.2 Unstained slides containing two levels – full face at 0 um and second level at 150um, with three unstained slides each level with both levels on each slide
   6.15.3 Staining – H&E or AF/H&E (special stain)
   6.15.4 IHC Staining – Ki67+Insulin, others as needed, request additional unstained slides for each

6.16 Slide labels will be printed as described below:
   6.16.1 Animal ID
   6.16.2 Tissue
   6.16.3 Stain
   6.16.4 Chromagen (if for IHC)
   6.16.5 Date (if for IHC)
   6.16.6 Core Reference Number (as determined by MPC)

6.17 Stained slides will be scanned using the Aperio system.

6.18 Image analysis will be conducted on scanned slides to determine fractional insulin area (FIA). If pancreatic weight was obtained at necropsy, multiply FIA by the pancreatic wet weight to obtain beta cell mass.

6.19 Insulitis will be scored based on the following scale:
   6.19.1 0 = no infiltrates
   6.19.2 1 = peri-islet infiltrates
   6.19.3 2 = intra-islet infiltrates to ≤ 50%
   6.19.4 3 = intra-islet infiltrates > 50%
   6.19.5 4 = non-islet infiltrates (optional, does not refer to islet-associated inflammation)

6.19.6 The scores from different levels can be averaged for each animal and expressed as percentage of 100 or by number of islets with each score.

6.20 RENI recommendations for histopathology only (insulitis scoring and image analysis requires submitting both lobes):
   6.20.1 Localization: left lobe
   6.20.2 Direction: longitudinal/horizontal
   6.20.3 Remarks: a cut surface as large as possible
6.21 The left lobe of the pancreas represents a major part of pancreatic tissue being located close to the spleen in the greater omentum. For trimming, the whole left lobe is removed and fixed in order to achieve a cut surface as large as possible. The right lobe is removed together with the adjacent small intestine (duodenum).

7 REFERENCES

7.1 RENI Tissue trimming guide  
http://reni.item.fraunhofer.de/reni/trimming/index.php

8 RELATED DOCUMENTS

Note: All references to SOPs and/or forms in this SOP refer to the current approved version.

8.1 SOP 412 Specimen Trimming  
8.2 SOP 414 Specimen Fixation

9 REVISION HISTORY

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<th>Version</th>
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Preparer | Martha Campbell-Thompson, DVM PhD | Date |
Regulatory Review By | | Date |
Approved By | Martha Campbell-Thompson, DVM PhD | Date |
Review Cycle | Two years |