# Journal of Visualized Experiments

## Dissection of the Mouse Pancreas for Histological Analysis and Metabolic Profiling

--Manuscript Draft--

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| **Full Title:** | Dissection of the Mouse Pancreas for Histological Analysis and Metabolic Profiling |
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| **Abstract:** | Our laboratory has been investigating the pancreas specific transcription factor, 1a cre-recombinase; lox-stop-lox- Kristen rat sarcoma, glycine to aspartic acid at the 12 codon (Ptf1acre/+;LSL-KrasG12D/+) mouse strain as a model of human pancreatic cancer. The goal of our current studies is to identify novel metabolic biomarkers of pancreatic cancer progression. We have performed metabolic profiling of urine, feces, blood, and pancreas tissue extracts, as well as histological analyses of the pancreas to stage the cancer progression. The mouse pancreas is not a well-defined solid organ like in humans, but rather is a diffusely distributed soft tissue that is not easily identified by individuals unfamiliar with mouse internal anatomy or by individuals that have little or no experience performing mouse organ dissections. The purpose of this video article is to provide a detailed step-wise visual demonstration to serve as a guide for novices for removal of the mouse pancreas by dissection. The video article should be especially valuable to students and investigators new to research that requires harvesting of the mouse pancreas by dissection for metabolic profiling or histological analyses. |
| **Author Comments:** | NOTE - 2-7-17 - we have revised the video as requested. It should now meet the specifications required by JOVE.<br><br>NOTE - 5-16-17 - we have revised the video to make additional corrections as requested. We have not updated the low resolution video, but have uploaded the revised high quality video for review |

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| | Michael A. Kennedy |
| **Additional Information:** | |
| **Question** | **Response** |
| If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter. | |

May 16, 2017

MIAMI UNIVERSITY
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Editor,
Journal of Visualized Experiments

Dear Editor,

Please consider our revised manuscript entitled "**Dissection of the Mouse Pancreas for Histological Analysis and Metabolic Profiling**" for publication in the *Journal of Visualized Experiments*. Below, you will find our detailed responses to the reviewer's comments.

**Reviewer #1:**
1) Reviewer comment: "Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors."
**Response:** Manuscript was proofread to ensure no spelling errors or grammatical errors were present. If found, they were corrected.

2) Reviewer Comment: "**Introduction:** Paragraph 2 is lacking appropriate references."
**Response:** Additional references were added in paragraph 2 of the Introduction to provide adequate citations.

3) Reviewer Comment: "**Title/abstracts:** As there is no histology or metabolic profiling presented, these must be edited to reflect the manuscript and video content OR results/data showing these should be added."
**Response:** Representative results and figures have been added showing histology and metabolic profiling data.

4) Reviewer Comment: "**Protocol Language:** Please edit the language so that ALL text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Examples NOT in imperative tense: 1.1, 1.1.2, 1.1.3, 1.2, 1.3, 1.4, 1.5, etc.
2) Some long steps were split up."
**Response:** 1) Language was edited to imperative tense or a "Note" was added. 2) Long steps were split up.

5) Reviewer Comment: "**Protocol Detail:** Please add more details to the following protocol steps.
1) 1.6: Mention isofluorane%.
2) 1.8: How do you ensure euthanasia?
3) 1.9: How much isoflurane do you use? What %? It appears that the mouse still alive in 1.9.1. If so, it is confusing to call the glass jar a euthanasia jar, instead perhaps call it anesthesia jar?"
**Response:** 1) Added 99.9%. 2) "Euthanasia" chamber was changed to "anesthesia" chamber. Euthanasia is discussed in a later section 2.3.5. 3) Sentence was changed to say, "Place the head inside the tube lined with a surgical pad soaked with a few drops of isoflurane (99.9%)". Euthanasia was changed to read anesthesia.

6) Reviewer Comment: "**Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol."

**Response:** Discussion was edited to include sections titled: "significance with respect to existing methods, limitations of the technique, critical steps within the protocol, modifications and troubleshooting, and future applications".

7) Reviewer Comment: "**Figures:**:
1) Fig 1-10: Please include scale bars on all images to provide context to the magnification used.
2) Fig 9,10 : Please include arrows to indicate spleen and pancreas."
**Response:** Scale bars have been added to figures 1-10. On figures 9 and 10, arrows have been included to indicate spleen and pancreas.

8) Reviewer Comment: "**Figure/Table Legends:**:
1) Fig 3: It may be best not to call this a "euthanasia chamber as the mouse is euthanized much later in the procedure."
**Response:** The word "Euthanasia" was changed to "Anesthesia" in the title and caption.

9) Reviewer Comment: "**References:**Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]
1) Please abbreviate all journal titles, and also edit the references to match the suggested format."
**Response:** References were edited to match the suggested format.

10) Reviewer Comment: "**Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as mouse strain."
**Response:** Mouse strain information was added to the materials list.

11) Reviewer Comment: "Please define all abbreviations at first use."
**Response:** All abbreviations have been defined at first use.

12) Reviewer Comment: "Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit."
**Response:** Standard abbreviations and symbols have been added/changed.

13) Reviewer Comment: "If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."'
**Response:** Figures and tables have not been previously published and are original- not action was taken for this comment.

**Veterinary Reviewer:**
1) Reviewer Comment: "Isoflurane anesthesia delivered via open drop method is acceptable, however, care should be taken to ensure the mouse does not come into contact with the isoflurane liquid, or the soaked gauze, as this can be irritating and cause discomfort. There are methods designed to ensure the mouse is only exposed to the vapors and not the liquid isoflurane. This should be addressed, see article for an easy way to accomplish

this: Taylor DK, Mook DM. Isoflurane Waste Anesthetic Gas Concentrations Associated with the Open-Drop Method. Journal of the American Association for Laboratory Animal Science: JAALAS. 2009;48(1):61-64."
**Response:** To ensure the animal does not come in contact with the isoflurane, a barrier needs to be placed between the pad soaked with a few drops of isoflurane and the animal. Manuscript was edited to read that a paper towel over the top of the pad will serve as this barrier.

2) Reviewer Comment: "The instructions say to use a "soaked surgical pad" with isoflurane – the use of isoflurane should be minimized and from an occupational health perspective and personnel exposure perspective, the least possible volume that will provide anesthesia should be used. It is unnecessary to "soak" a gauze pad, therefore, with isoflurane. This should be addressed in the text – the video already says (more correctly) "soaked with a few drops of isoflurane"
**Response:** Manuscript was edited to say soaked with a few drops of isoflurane.

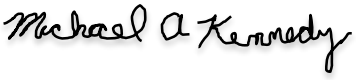
3) Reviewer Comment: "1.6 says isoflurane should be used only in the "vent hood." – scavenging of waste anesthetic gas should be addressed in more detail, as not all "vent hoods" provide enough scavenging. The article above and others are readily available upon searching to address appropriate waste gas scavenging.
**Response:** The scavenging of waste anesthetic gas, regarding isoflurane usage, has been address and referenced.

4) Reviewer Comment: "Text: page 4, section 1.9 states "place the head inside the falcon tube lined with the isoflurane soaked surgical pad and perform a foot pinch to ensure discomfort is not being experienced" – this would better be described as in the video, where the voiceover says "mouse cannot experience discomfort" – an even better description would be "mouse is unresponsive to stimuli."
**Response:** In section 1.9 the words, "discomfort is not being experienced" was change to "the mouse is unresponsive to stimuli."

5) Reviewer Comment: "The video voiceover describes a cervical dislocation after the mouse is anesthetized and removed from the isoflurane jar. If that is done, there is no need to put the mouse's head in a tube with isoflurane during the dissection, as the mouse has been euthanized, and therefore represents an unnecessary occupational exposure to isoflurane."
**Response:** Following transfer from the anesthesia chamber to the dissection table, the mouse is still alive up until the terminal blood draw was performed. After the terminal blood draw, the heart was detached as a secondary method to ensure euthanasia. Therefore, the head of the mouse remained in the tube until euthanasia was performed. At this point the dissection of the pancreas was carried out. This is accurately reflected in the video and article in the revised manuscript.

We hope that our revised manuscript is now considered suitable for publication in the Journal of Visualized Experiments.

Sincerely,



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**KEYWORDS:**

Pancreas, pancreas dissection, mouse pancreas dissection, pancreatic cancer, pancreatic ductal adenocarcinoma, mouse

**SHORT ABSTRACT:**
This video article provides a detailed demonstration of the procedures required to successfully remove the pancreas from a mouse by dissection for histological analysis and metabolic profiling.

**LONG ABSTRACT:**
We have been investigating the pancreas specific transcription factor, 1a cre-recombinase; lox-stop-lox- Kristen rat sarcoma, glycine to aspartic acid at the 12 codon ($Ptf1a^{cre/+}$;$LSL\text{-}Kras^{G12D/+}$) mouse strain as a model of human pancreatic cancer. The goal of our current studies is to identify novel metabolic biomarkers of pancreatic cancer progression. We have performed metabolic profiling of urine, feces, blood, and pancreas tissue extracts, as well as histological analyses of the pancreas to stage the cancer progression. The mouse pancreas is not a well-defined solid organ like in humans, but rather is a diffusely distributed soft tissue that is not easily identified by individuals unfamiliar with mouse internal anatomy or by individuals that have little or no experience performing mouse organ dissections. The purpose of this article is to provide a detailed step-wise visual demonstration to guide novices in the removal of the mouse pancreas by dissection. This article should be especially valuable to students and investigators new to research that requires harvesting of the mouse pancreas by dissection for metabolic profiling or histological analyses.

**INTRODUCTION:**
The mouse has emerged as an important animal model of human pancreatic cancer[1,2]. In the $Ptf1a^{cre/+}$;$LSL\text{-}Kras^{G12D/+}$ mouse model, the Kristen rat sarcoma (K-Ras) oncogene is activated exclusively in the pancreas, resulting in initiation of precancerous lesions in the pancreas, known as pancreatic intraepithelial neoplasias (PanINs), that progress to pancreatic ductal adenocarcinomas, commonly referred to as PDACs[3]. This mouse model system provides one of the best available animal models for human pancreatic cancer[4,5], with the additional advantage that the PanINs emerge within the first five months of life and frequently progress to PDAC within a single year[4,5], whereas pancreatic cancer most frequently occurs in humans 60-70 years of age.

Extraction of the pancreas by dissection from the $Ptf1a^{cre/+}$;$LSL\text{-}Kras^{G12D/+}$ mice at various ages allows for detailed longitudinal histological examination of cancer development in the pancreas, ranging from the earliest PanIN stages through the progression to PDAC[3,4,5]. Harvesting the pancreas at ages ranging from five to fifteen months can also be used to prepare tissue extracts to characterize global changes in pancreas[4] metabolism that occur during the transition from healthy to diseased tissue[6,7].

This article presents a complete visual guide of the steps required to perform a mouse pancreas extraction and provides guidelines for storage of a pancreas for further analysis. This guide will be equally valuable for individuals conducting research on other pancreatic diseases, including

type I diabetes, and should be especially useful to students and investigators new to research involving harvesting of the mouse pancreas using dissection for metabolic profiling or histological analyses.

**PROTOCOL:**

The procedures carried out in the video and described below have been approved by the Institutional Animal Care and Use Committee (IACUC) at Miami University.

**1. Preparation and Stimulus Test**

1.1) Establish two distinct areas for the surgical procedure, the operating table and the post-operation table. Stage both areas with all materials and utensils necessary.

1.1.2) Stage the operating table under a vented hood. Arrange the table with the equipment in a manner that allows the continuous and unimpeded performance of the procedure.

1.1.3) Establish a postoperative table in the same room and near the main table of operation. Maintain both tables as sterile environments throughout the procedure.

1.2) Place the following supplies on the operating table: one glass jar with lid, one 15 mL tube, one pair of surgical scissors, one squeeze bottle of 70% ethanol, two foam boards, two forceps, two 1 mL 21 gauge syringes, two 50 mL tubes, one centrifuge tube, one cryogenic vial, four surgical pads, ten pins, a dispenser of sterilizing wipes, and a sharps container.

1.3) Place the following supplies on the post-operation table: one analytical balance, one 4 L dewar of liquid nitrogen, a shallow wide mouth dewar, a floating microtube rack, one pair of surgical scissors, two forceps, four cryogenic vials, and a dispenser of sterile wipes.

1.4) Fill one 50 mL and one 15 mL tube to 75% volume with formalin.

1.5) Using a pin in each corner, affix one surgical pad to an approximate 30 cm x 30 cm foam board to serve as the dissection board. Use the remaining four pins during the operation. Prepare a smaller foam board with a surgical pad to transfer the organs to the post-op table.

CAUTION: Isoflurane (99.9%) is a toxic chemical, and should be used in a vent hood to ensure the maximum level of safety from the scavenging of waste anesthetic gas[8]. Additional information regarding the risks to researchers associated with the use of the open-drop method using isoflurane can be found in an article by Taylor and Mook[8].

1.6) Place one surgical pad into the glass jar and soak with a few drops of isoflurane (99.9%) and place a paper towel over the top to prevent direct contact of the mouse with the isoflurane. Similarly, use a surgical pad to line the remaining tube and soak with a few drops of isoflurane and place an additional pad over the top to prevent direct contact between the mouse and the isoflurane.

1.7) Pour liquid nitrogen into the shallow wide mouth dewar until the maximum fill line is reached.

1.8) Place the mouse selected for dissection into the anesthesia chamber, *i.e.* the glass jar with a pad soaked with a few drops of isoflurane (99.9%) covered with the lid, for ~1 min.

Note: This time varies from mouse to mouse. Once the mouse is unconscious, remove it from the chamber and place it onto the operating board.

1.9) Orient the mouse so that it is lying ventral side up and with its head pointed away from the scientist. Place the head inside the tube lined with a surgical pad soaked with a few drops of isoflurane (99.9%), and perform a stimulus test by a foot pinch to ensure that the mouse is unresponsive to stimuli.

1.9.1) If this test fails and the mouse responds to the foot pinch test, repeat step 1.8.

**2. Initial Incision, Heart Puncture, and Euthanasia**

2.1) Pin the limbs of the mouse to the surgical foam board and wet the ventral side of the mouse with 70% ethanol.

2.2) Pinch the fur/skin near the urethral opening with forceps and pull slightly upwards. Make an incision with the surgical scissors through the abdominal cavity starting from the urethral opening, up the midline and ending at the chin.

2.2.1) Near the starting point of the initial incision, grab one side of the fur/skin with the forceps and make another incision with the scissors downward and diagonally towards the back paw.

2.2.2) Repeat this in the same manner on the opposite side.

Note: The fur/skin may be pinned down to create a wider opening, but is not necessary.

2.3) Locate the heart and remove the pericardium, which is the sac around the heart, to avoid clogging of the syringe needle.

2.3.1) Grasp the pericardium with the forceps and cut it with the scissors. Perform the heart puncture by carefully inserting the syringe needle into the beating heart and slowly start to retract the plunger.

2.3.2) For optimal blood collection, use the plunger of the needle to mimic the pumping action of the heart and avoid drawing too quickly.

Note: Typically about 1 mL of blood can be collected.

2.3.3) After completing the blood collection, dispel the blood into the centrifuge tube and dispose of the syringe into the sharps container.

2.3.4) After the heart puncture is performed, carry out euthanasia by removing the attachments connecting the heart.

Note: Heparin, an anti-coagulant, was not added to the syringe in this procedure prior to the heart puncture to allow the blood to coagulate for serum collection in this specific study. However, if the researcher wanted to prevent blood coagulation to collect plasma, heparin could be added to the syringe prior to the heart puncture.

2.4) If the study involves genotyping of the mouse, snip a portion of the ear with the scissors and place into a centrifuge tube for a genotype verification.

**3) Pancreas Extraction**

3.1) Locate the stomach on the left side of the mouse. Begin gently (so as to avoid tearing) separating the pancreas from the stomach and duodenum by using two forceps.

Note: When detaching the pancreas from the stomach and intestines, it is very important that the forceps are used gently to guide the pancreas tissue away from the organs and to not crush or tear the pancreas with the forceps.

3.1.1) Continue to separate the pancreas from the small intestine jejunum and ileum sections, and lastly from the caecum of the large intestine.

3.2) At the caecum, reposition the forceps and continue separation of the pancreas along the remaining colon towards the rectum.

Note: At this point, it is convenient to cut and remove the portion from the stomach to the region of the colon immediately preceding the rectum.

3.3) Locate the pancreas and attached spleen. Slide the pancreas towards the right side of the mouse. Separate the remaining connections between the pancreas and thoracic cavity with the forceps to fully detach the pancreas and adjoined spleen.

3.4) Remove the pancreas and spread it out for examination. Leave the spleen attached to the pancreas for identification purposes.

3.4.1) Remove all connective tissue, fat and mesenteric tissue from the pancreas.

Note: This tissue is whiter in color and thus can be easily distinguished from the pancreas tissue that is pinker in color. This is particularly important if the whole pancreas needs to be removed.

For example, if the pancreas needs to be weighed and compared to body weight or between groups of animals. In the Ptf1a$^{cre/+}$;LSL-Kras$^{G12D/+}$ mouse model, specifically in the older months, hard fibrous pancreatic tissue could be present. In this case, careful removal of the pancreas must be conducted as the intestines could be interlaced in tumor tissue. In advanced cases, abnormal spleen and liver tissue may also be present.

3.5) If desired, remove other organs at this point.

**4) Data Collection and Storage**

4.1) After extraction of the organs, move the samples to the postoperative area for preservation.

4.2) Weigh each organ and place them into their respective cryogenic vial.

Note: Along with the mass of each sample, any irregularities should be recorded for future reference.

4.3) Once the organs are weighed, place them into the liquid nitrogen for snap freezing.

4.4) After snap freezing, store the organs at -80 °C for long-term storage.

4.5) Place the formalin stored samples on the bench top overnight, and the next morning change their solution from formalin to 70% ethanol.

Note: These samples should be stored at 4 °C for long-term storage.

4.6) For long term storage, freeze the blood and ear punch at -80 °C.. For serum collection from the blood, allow the blood to coagulate for 30 min then centrifuge it. Remove the serum portion using a pipette and then store at -80 °C.

**5) Clean Up**

5.1) Sanitize all the dissection tools with the sterilizing wipes. Cap the tube lined with the isoflurane soaked surgical pad. Replace the surgical pad on the foam board with a fresh surgical pad. Dispose the portions of the mouse that were not collected per the facility's animal disposal policy.

**REPRESENTATIVE RESULTS:**

**Figure 1** shows an overview of the operating environment area and **Figure 2** shows the post operation area. While this setting provides the minimal amount of equipment and staging, individuals may choose to alter this to best suit individual needs. The protocol should be optimized according to the specific needs of the experiment. This procedure is conducted in a manner that terminates the life of a mouse, requiring proper euthanization[9]. When the

researcher is ready, the mouse is placed into the anesthesia chamber with the isoflurane-soaked pads (**Figure 3**).

Once the mouse is unconscious, remove the mouse and place it dorsal side on the board. A toe-pinch procedure should be performed to ensure that the mouse is unresponsive to pain (**Figure 4**). Apply 70% ethanol to sterilize the initial incision area. The terminal blood draw must be conducted first, prior to the pancreas removal, to ensure adequate blood retrieval. Prior to blood removal, the pericardium should be removed to prevent clogging of the 21 G needle opening. After completing the terminal blood draw, the heart is detached as a secondary method of euthanasia and the pancreas is then removed.

Begin by locating the stomach, which provides a good starting point for pancreas removal (**Figure 5**). Note: Extreme care should be exercised during removal of the pancreas, which is a delicate and fragile tissue, and therefore all operations should be performed with gentle force. Using forceps, begin the dissection by starting to gently pull the pancreas away from the stomach and continue to separate the pancreatic tissue from the outer lining of the gastrointestinal (GI) tract working from the stomach to the duodenum, jejunum and ileum (**Figure 6**). Once the caecum is reached, easier removal of the pancreas is achieved by repositioning the forceps so that one forceps is holding the caecum and the other forceps is used to continue to separate the pancreas from the large intestine (**Figure 7**). After removal from the large intestine, the pancreas is placed on the right side of the mouse and any remaining attachments are severed (**Figure 8**).

The pancreas should be fanned out for inspection and any abnormalities should be recorded (**Figure 9**). In the $Ptf1a^{cre/+}$;LSL-$Kras^{G12D/+}$ mouse strain, the pancreas could potentially contain a hardened tumor (**Figure 10**). Other organs should also be examined for potential metastasis. Once the pancreas has been removed, it should be weighed and the weight recorded. A portion of the pancreas should be snap-frozen in liquid nitrogen for future metabolic profiling analysis or other testing and a portion of the pancreas should be placed in formalin for future histological analysis. **Figure 11** shows the initial storage of the various organs collected from the dissection for use in later analysis. The organs collected by dissection and stored for further study will depend on the goals of the individual researcher.

Tissue and blood samples can be used for histological analyses and for metabolic profiling. An example of the histological analysis of the pancreas tissue is shown in **Figure 12**. Metabolic profiling can be conducted on the snap-frozen tissue samples and blood sample. Representative nuclear magnetic resonance spectroscopy (NMR) spectra of the hydrophilic and hydrophobic components of pancreas tissue extracts are shown in **Figure 13A** and **13B**, respectively. A representative NMR spectrum collected on a serum sample prepared from blood collected at the time of a terminal blood draw procedure is shown in **Figure 14**.

**FIGURE LEGENDS:**

**Figure 1: Staging of Operating Area.** General layout of correct tools and operating conditions for the dissection.

**Figure 2: Staging of Post-Operation Area.** General layout of correct tools and operating conditions for the postoperative procedures.

**Figure 3: Anesthesia Chamber.** Proper environment for anesthesia via isoflurane.

**Figure 4: Stimulus Examination.** The stimulus test conducted on the mouse prior to the initial incision to ensure any pain or discomfort is not being endured.

**Figure 5: Beginning Removal of Pancreas.** The orientation of the mouse indicating the initial extraction of the pancreas, location indicated by the forceps.

**Figure 6: Pancreas Extraction Along the Intestines.** Process of isolating the pancreas from the gastrointestinal tract.

**Figure 7: Pancreas Removal at the Caecum.** Repositioning of the forceps once the caecum is reached.

**Figure 8: Pancreas Removal.** Place the pancreas on the right side of the mouse. Any remaining attachments should be cut to full remove the pancreas.

**Figure 9: Pancreas Examination.** The pancreas with attached spleen being examined after removal from the mouse. Spleen is indicated by the vertical arrow, and the pancreas is indicated by the horizontal arrow.

**Figure 10: Pancreas Examination.** The pancreas with attached spleen displaying a pancreatic tumor being examined after removal from the mouse. Spleen is indicated by the vertical arrow, and the pancreas is indicated by the horizontal arrow.

**Figure 11: Storage of Organs Removed.** Appropriate storage of organs and samples collected, prepared for long-term storage and future analysis.

**Figure 12: Histological Analysis of Pancreas Tissue.** Hematoxylin and eosin stained images from pancreas tissue. A) Normal pancreas tissue from a Ptf1a$^{cre/-}$;LSL-Kras$^{G12D/-}$ control mouse. B) PanIN tissue from the pancreas of a Ptf1a$^{cre/+}$;LSL-Kras$^{G12D/+}$ study mouse.

**Figure 13: Metabolic Profiling Analysis.** One-dimensional proton nuclear magnetic resonance spectroscopy (NMR) spectra of A) hydrophilic and B) hydrophobic phase components of pancreas tissue extracts following tissue homogenization and subjected to chloroform/methanol extraction. The NMR spectra were acquired at 850 MHz and are suitable for use in metabolic profiling analyses.

**Figure 14: Representative NMR Spectrum of Serum.** The blood collected by the terminal blood draw procedure can be used for metabolic profiling analysis. This spectrum shows a typical one-dimensional proton 850 MHz NMR spectrum collected on the serum obtained from a terminal blood draw sample.

**DISCUSSION:**

**Significance with Respect to Existing Methods**

While other informal videos of mouse dissections exist, this video article provides the first professional quality, peer reviewed, visual demonstration of all of the detailed steps required for extraction and harvesting of the mouse pancreas by dissection[10]. With the pancreas being a main organ for metabolic activity and insulin production, dissection and harvesting of the pancreas allows for the preservation of the physiological characteristics[11]. By isolating the pancreas, future analysis may be conducted on the sample. This procedure allows for the comparison and study of interactions from other tissues within the same organism within the same time frame.

**Limitations of the Technique**

The greatest limitation of this procedure is termination of the mouse's life, thereby preventing longitudinal collection and sampling of multiple tissue samples from the same mouse. In order to analyze trends related to age, sex, or other quantifiers, a cross-sectional population must be implemented, as we have done for our study of metabolic biomarkers of pancreatic cancer. Another limitation of this protocol is the inability to pause the procedure. Once euthanization is initiated, the procedure must be carried out in its entirety.

**Critical Steps within the Protocol**

Execution of the stimulus test by pinching the hind paw of the mouse is critical to ensure that the mouse receives humane treatment. If the mouse does not react to this stimulus, then the procedure may be carried out as planned. However, should the mouse display a distressed response as a result of the stimulus test, the mouse should be returned to the anesthesia chamber for an additional period of time and the test repeated until a reaction to the stimulus test is not observed[12].

Similarly, the terminal heart puncture followed by the removal of the connections to the heart immediately after the terminal blood sample is collected as a secondary method of euthanasia ensures the humane sacrifice of the mouse. To ensure an effective blood draw, the scientist should use a pumping motion with the syringe that is similar to the heartbeat of the mouse, allowing for maximum collection of blood for analysis.

**Modifications and Troubleshooting**

Switching the organs from formalin to 70% ethanol solutions prepares the organs for the embedding process required for histological analysis. Different storage solutions may be required should the scientist choose to perform other experiments with the organs. Before

analysis, it is important to limit any potential thawing of the organs stored in the -80 °C freezer to preserve the organ's integrity.

Use of the Ptf1a$^{cre/+}$;LSL-Kras$^{G12D/+}$ mouse model minimizes the occurrence of non-pancreatic primary tumors and diseases[13]. Thus, it is important to note any irregularities that are apparent to the pancreas or other organs during dissection and collection of the tissue samples for analysis.

**Future Applications**

Harvesting of the mouse pancreas by dissection allows for multiple types of analysis to be conducted on the same sample. The most popular of these include, but are not limited to, fluorescence microscopy, hematoxylin and eosin histology, immunohistochemistry, mass spectrometry, and nuclear magnetic resonance spectroscopy[6,7,14,15]. Diseases like diabetes, pancreatitis, and pancreatic cancer can be studied using the techniques mentioned above[16].

**ACKNOWLEDGMENTS:**

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**DISCLOSURES:**

The authors have nothing to disclose.

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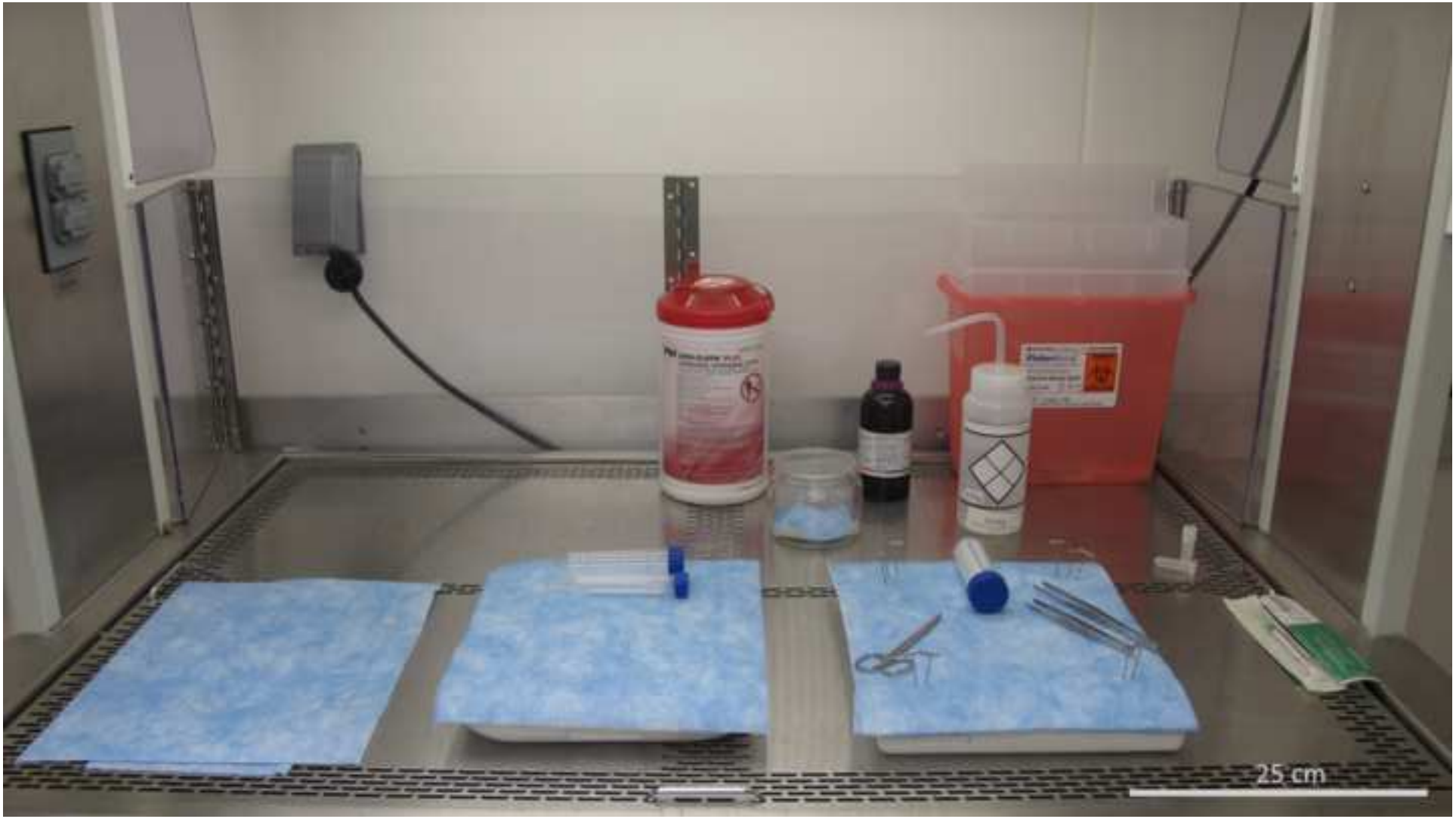

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Figure 4

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Figure 5

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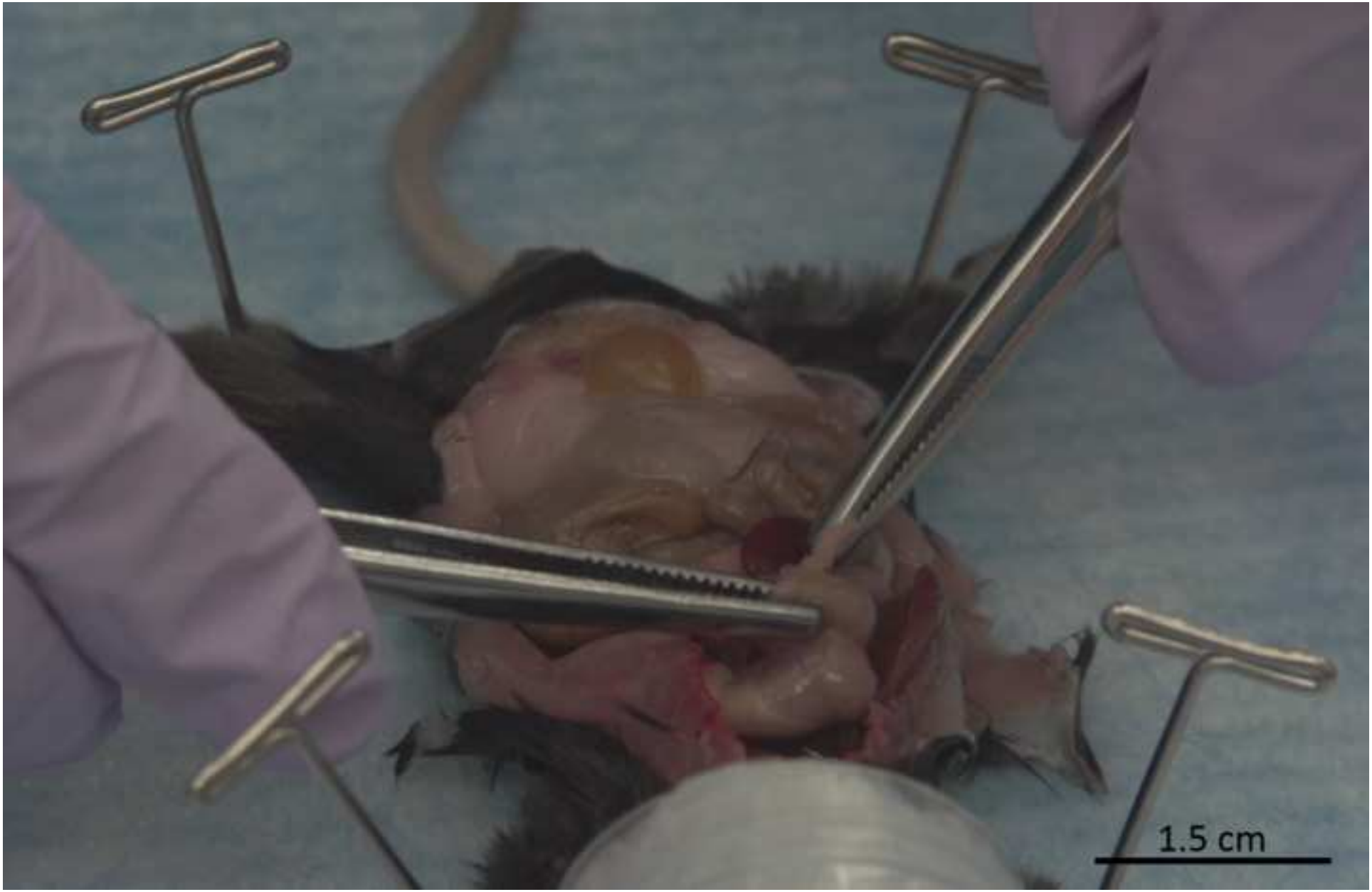

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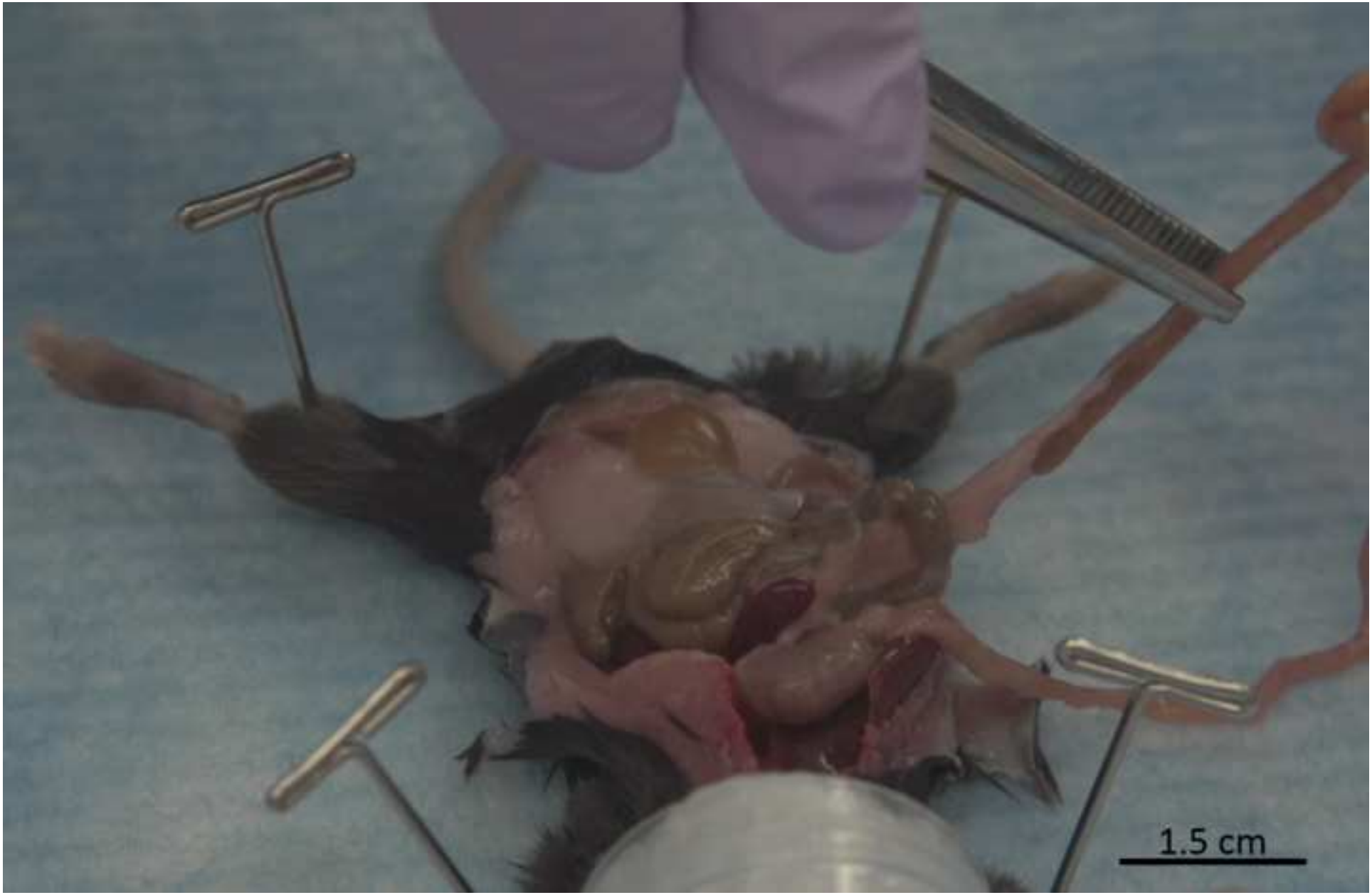

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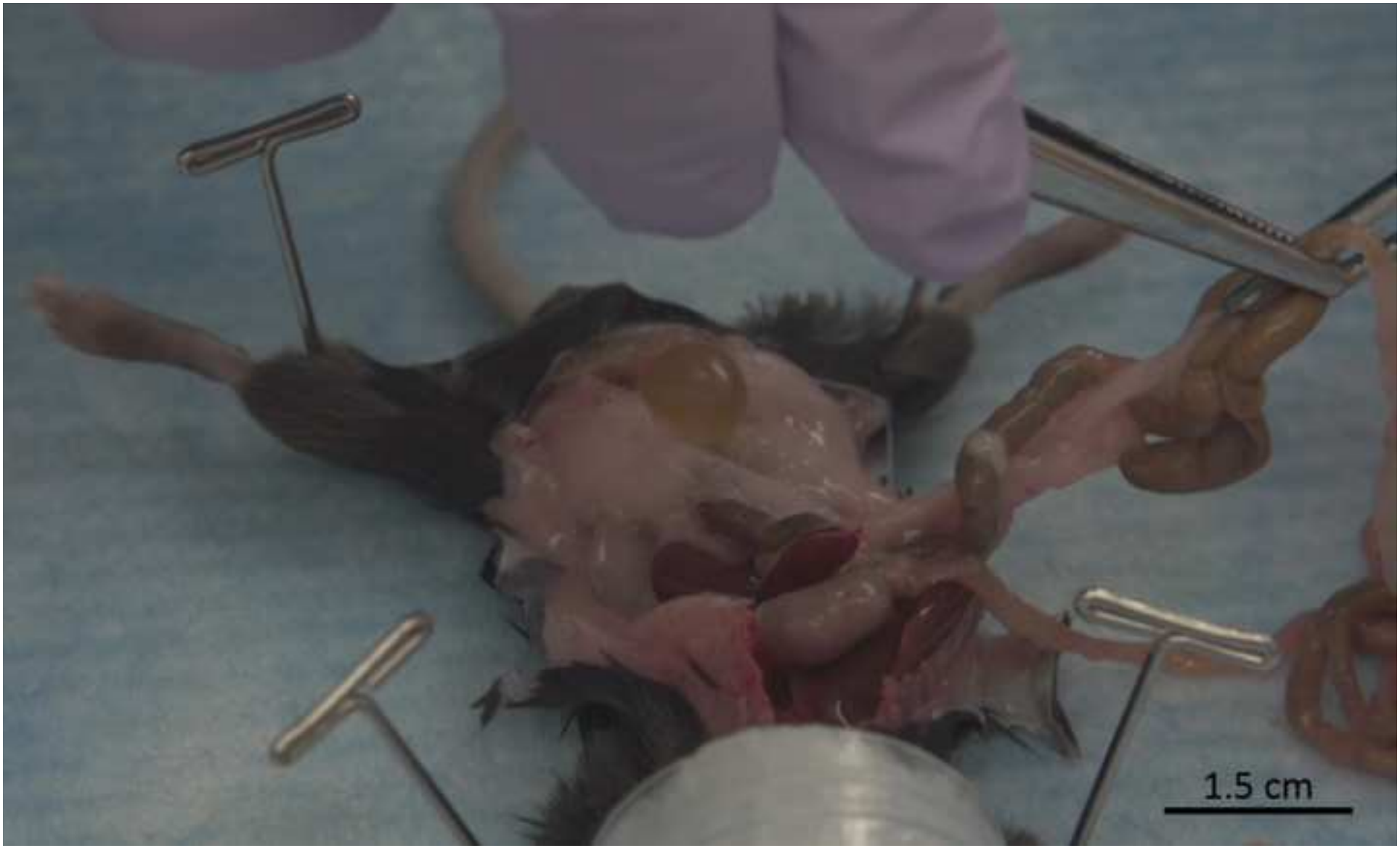

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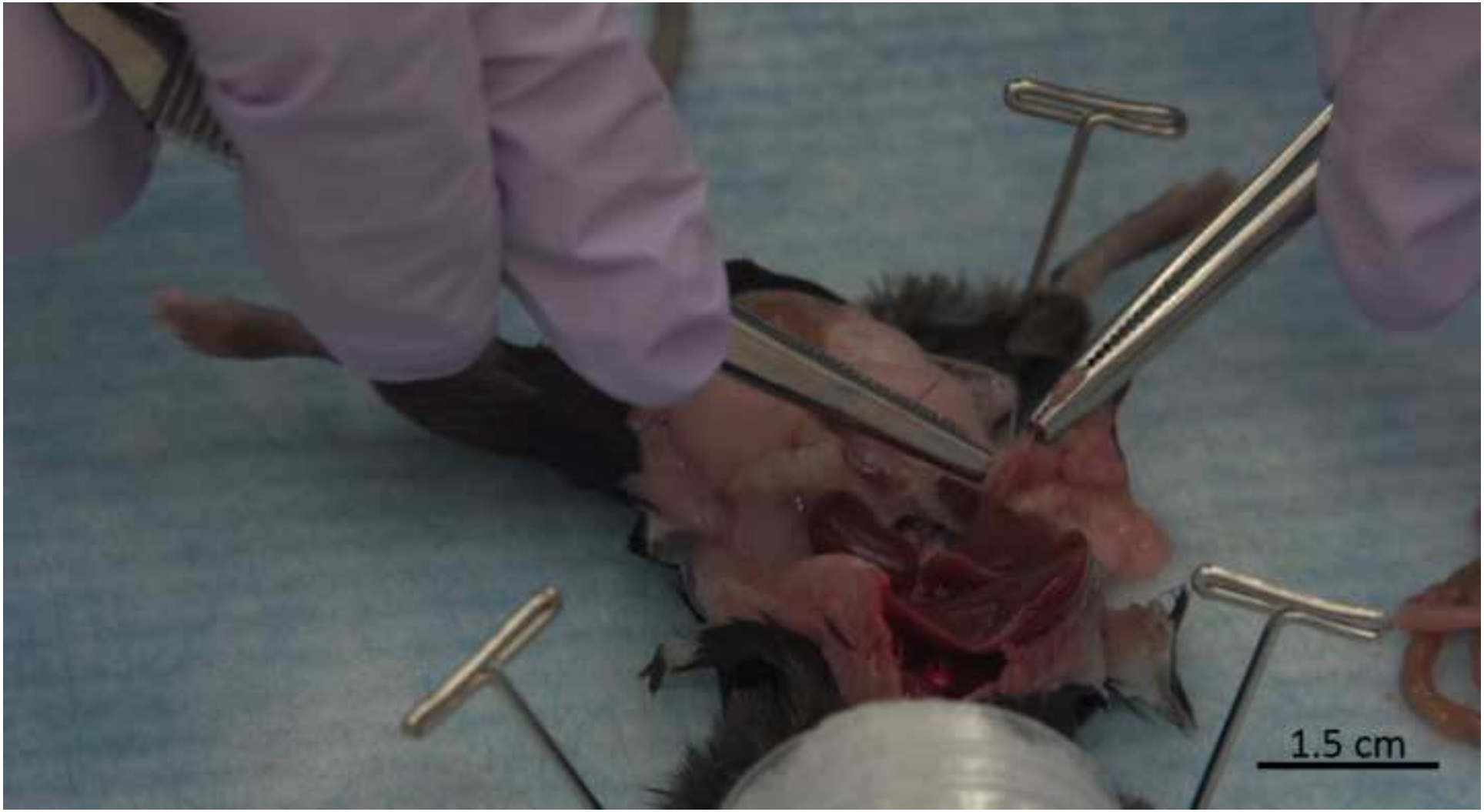

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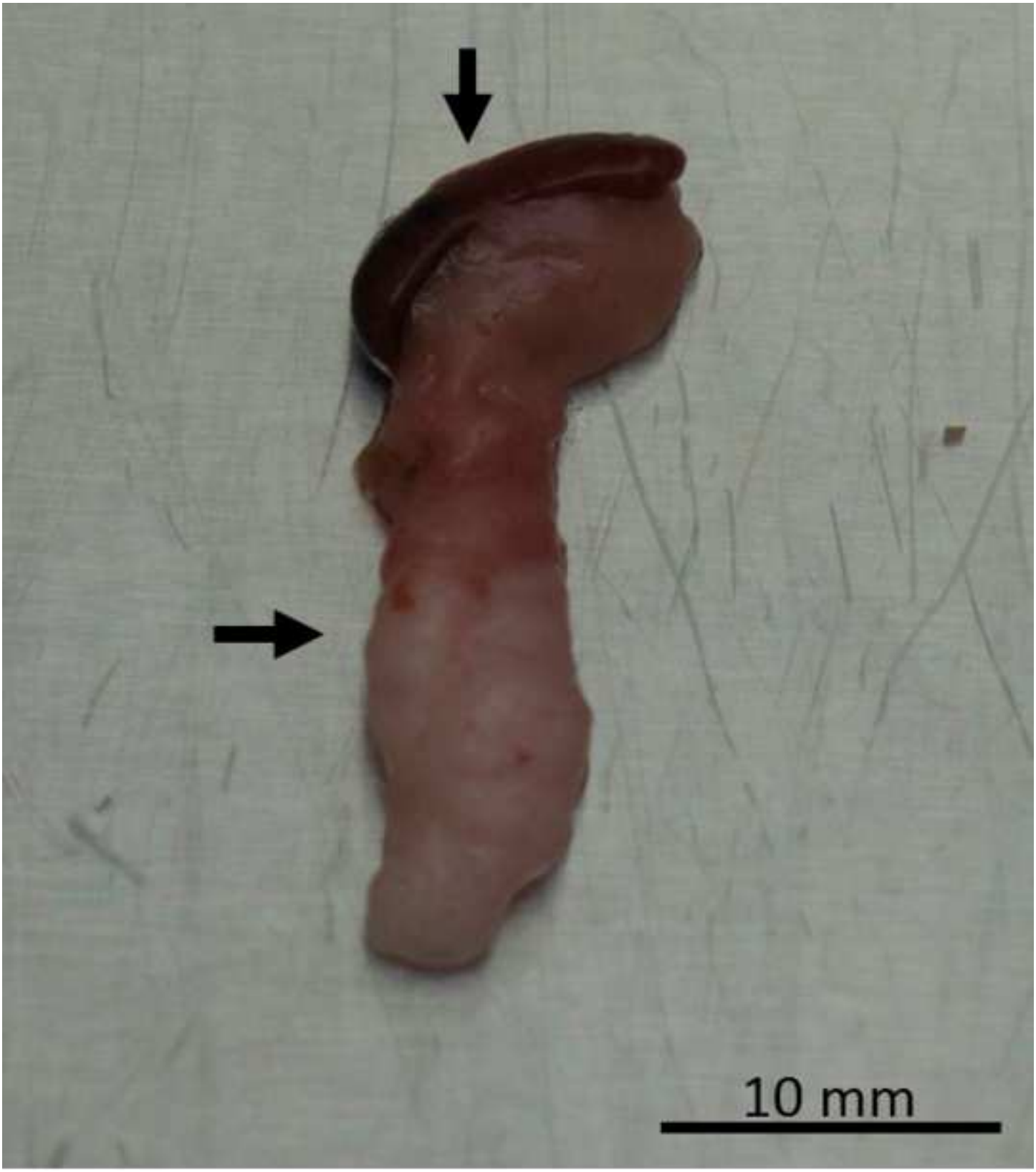

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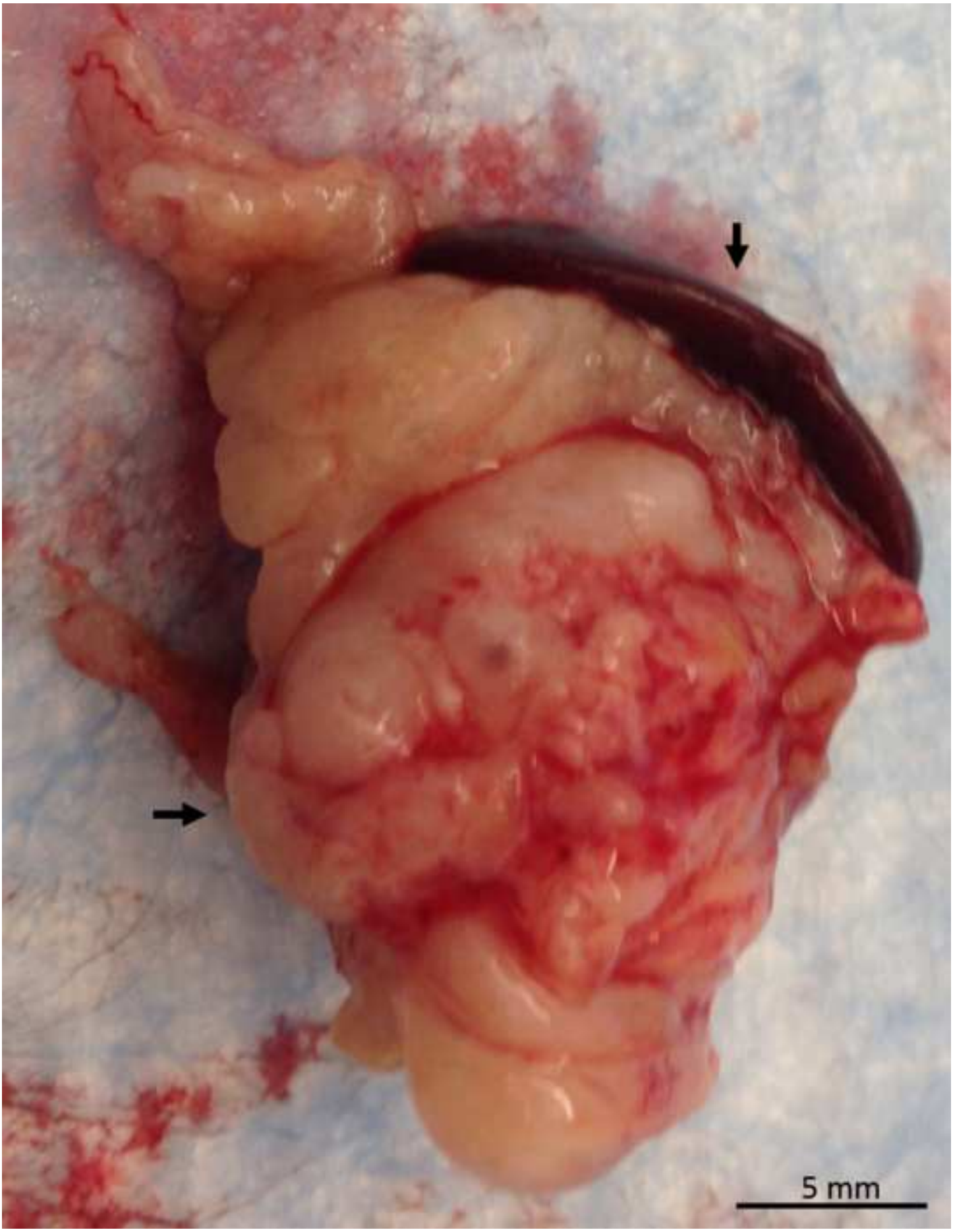

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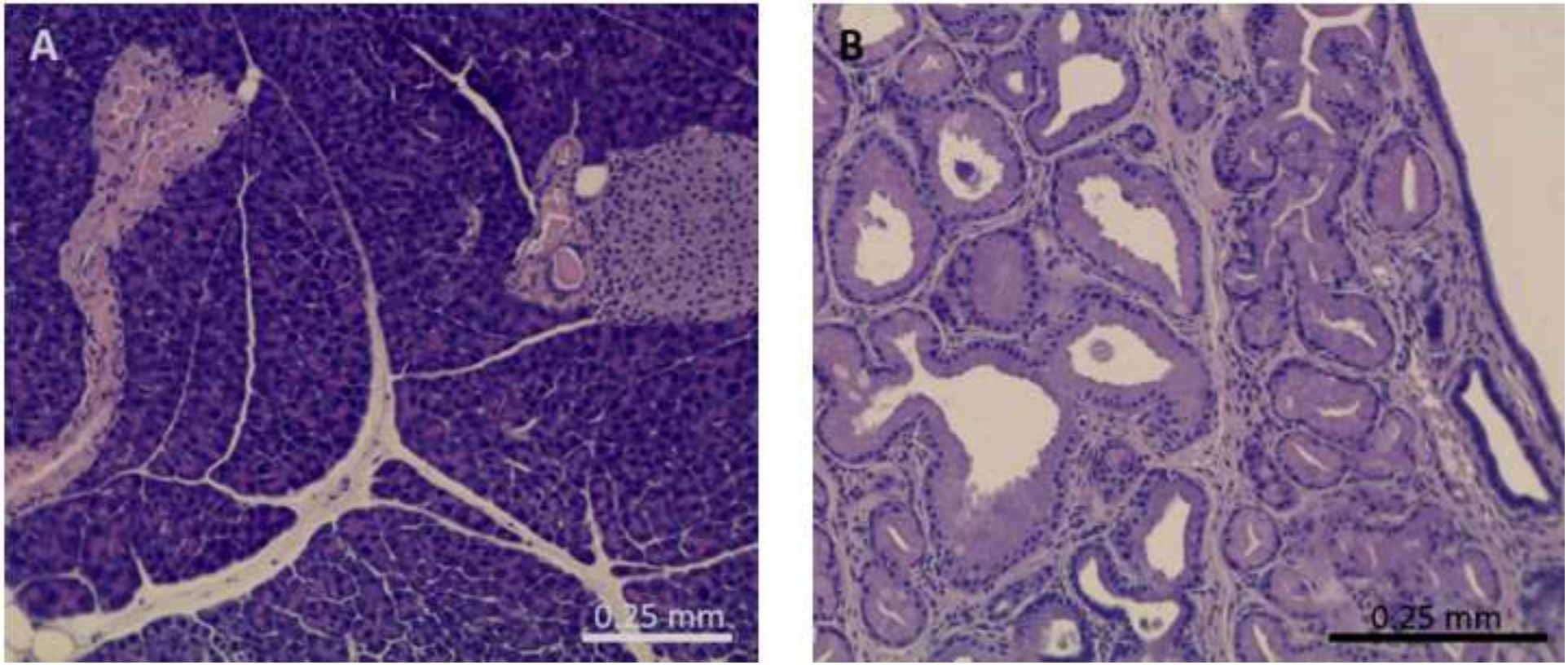

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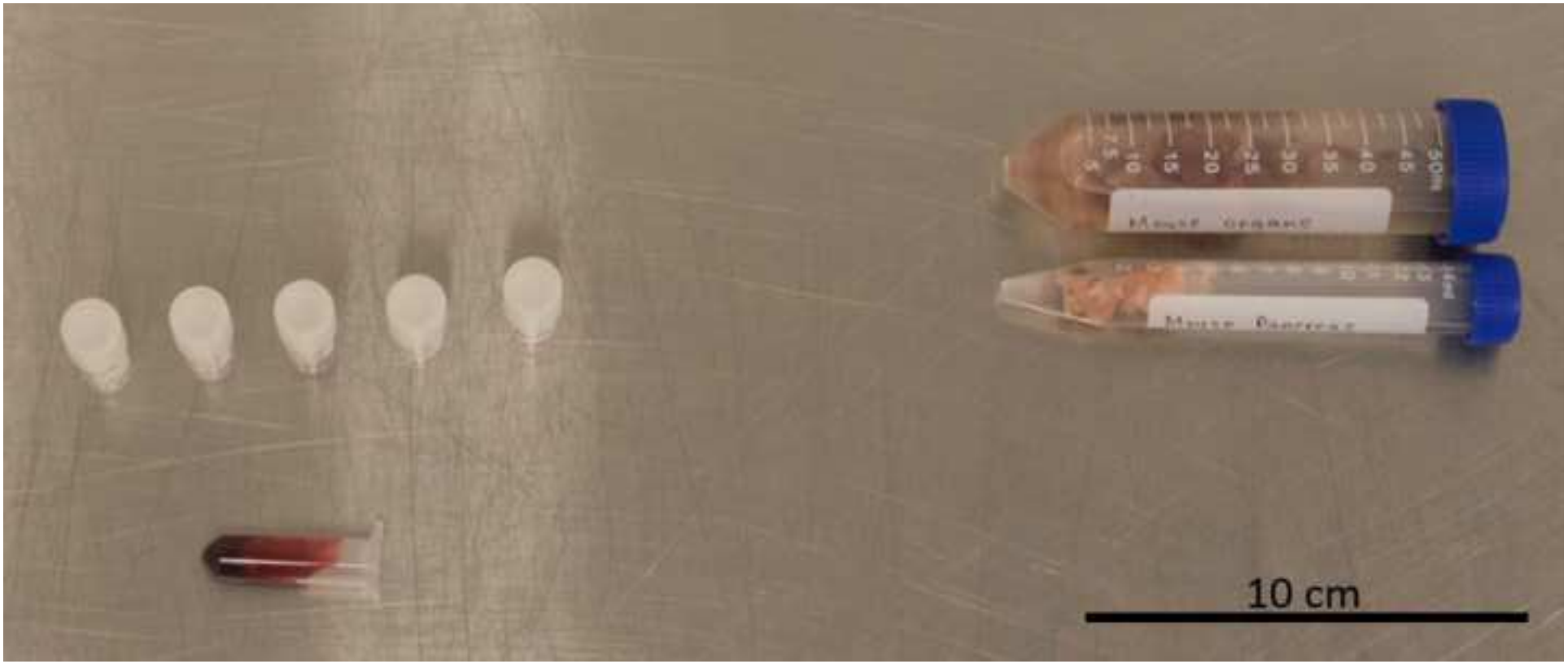

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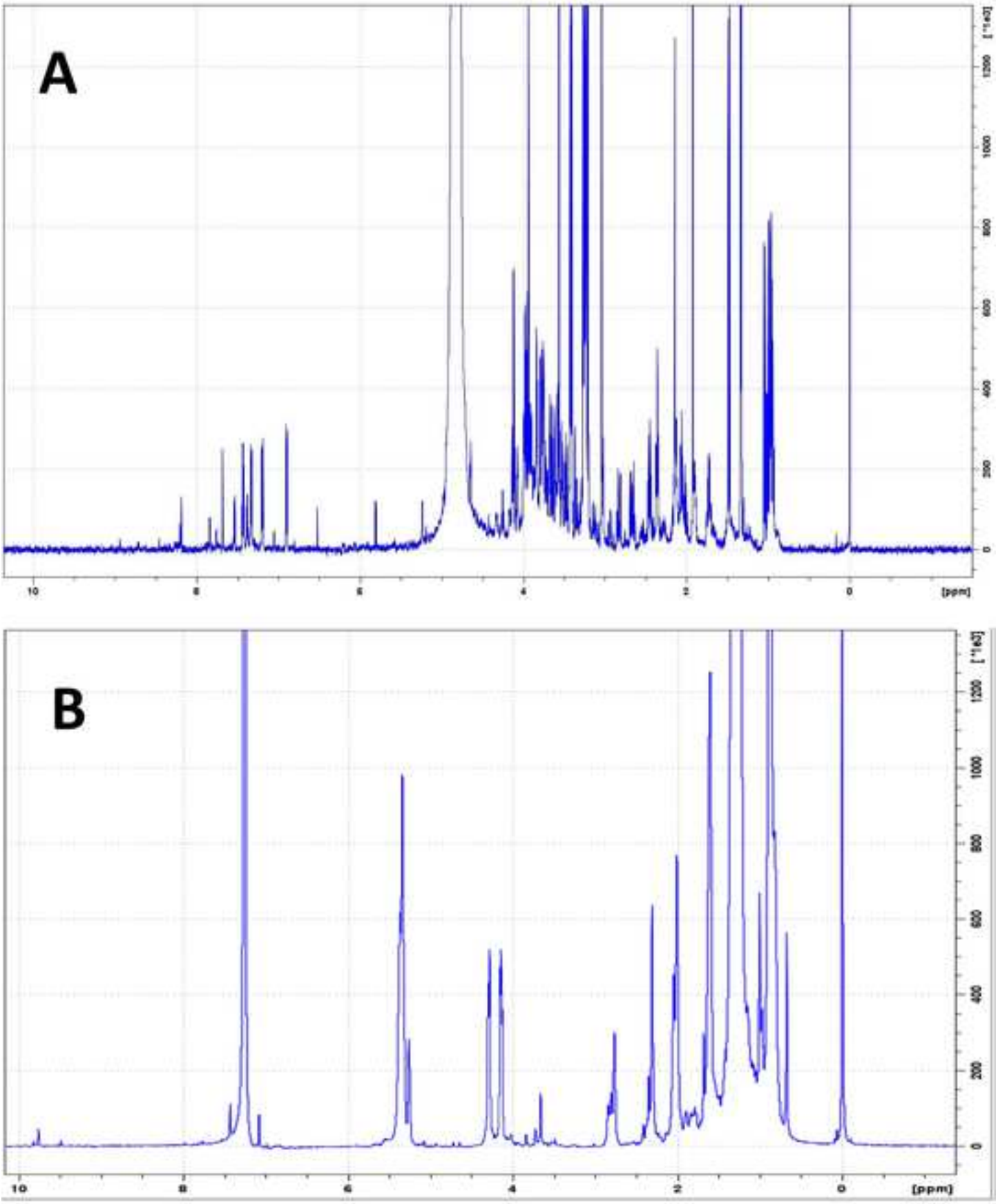

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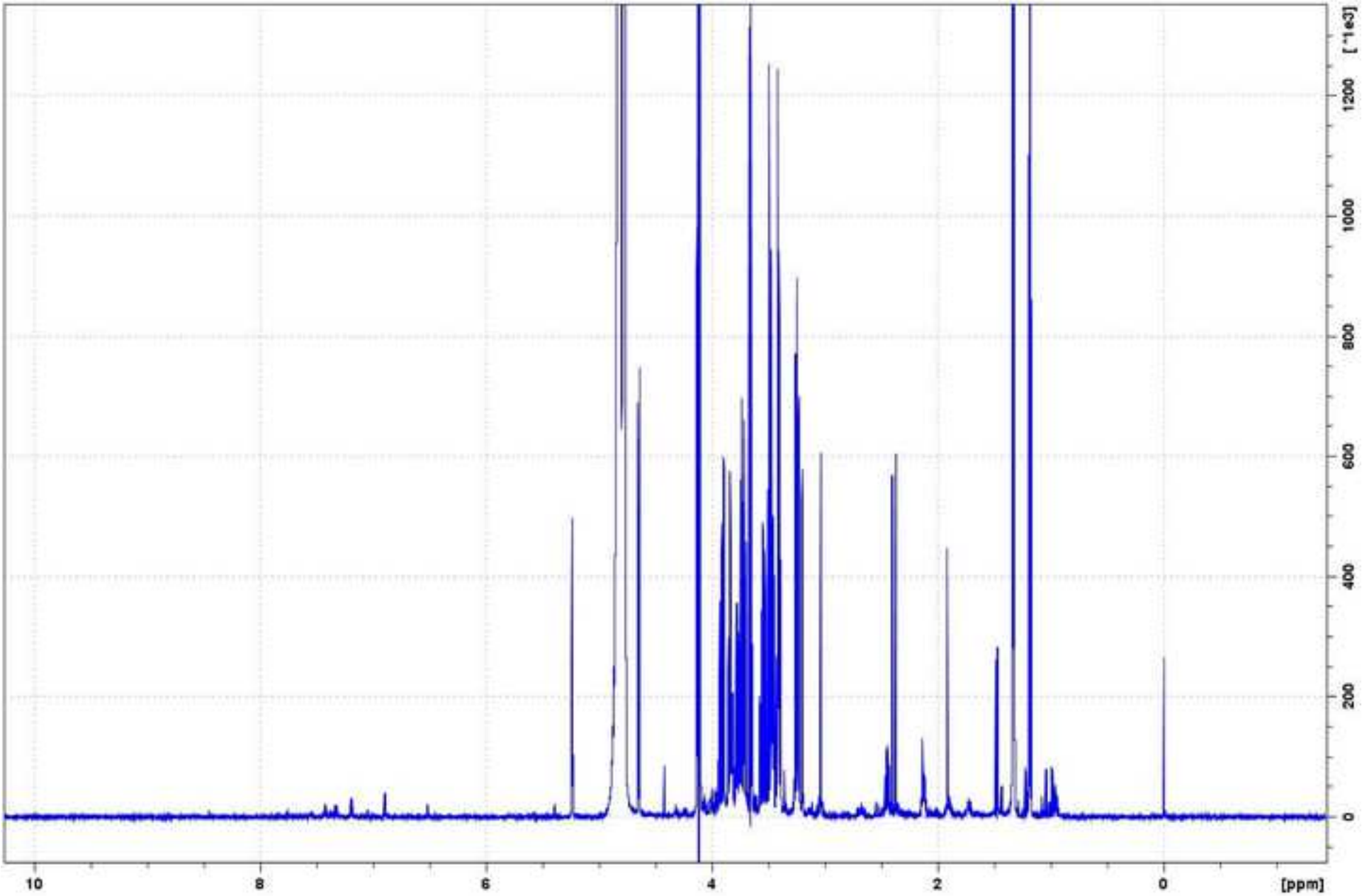

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| Name of Material/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| Glass Jar | Corning | 3140-150 | The glass jar used in the video has be |
| Lid of Glass Jar | Corning | 9985-150 | The glass jar lid used in the video has |
| 15 mL Falcon Tubes | Fisher Scientific | 339650 | |
| Surgical Scissors | Fisher Scientific | 9201 | |
| Squeeze bottle | Fisher Scientific | 03-409-10DD | |
| 100% Ethanol | Fisher Scientific | 22-032-103 | |
| Formalin | Fisher Scientific | 245-684 | |
| Foam Boards | Therapak | 562908 | |
| Forceps | Fisher Scientific | 200205SHN | |
| 1 mL 21G Syringes | BD Biosciences | 309624 | |
| 50 mL Falcon Tubes | Fisher Scientific | 339652 | |
| 2.0 mL Microcentrifuge Tubes | Fisher Scientific | 02-681-258 | |
| Surgical Pads | Fisher Scientific | S67011 | |
| T-Pins Length:  2" | Advance Store Products | X32T-05 | |
| | | | |
| Sterilizing Wipes | Professional Disposables International Inc. | Q85084 | |
| Sharps Container | Fisher Scientific | 14-827-122 | |
| Analytical Balance | Marshall Scientific | ME-AE200 | |
| 4L Dewar | Taylor-Wharton | 4LD | |
| Shallow Wide Mouth Dewar | Fisher Scientific | F3087-V | |
| Floating Microtube Rack | VWR | 60986-100 | |
| Cryogenic Vial 1.2 mL, Sterile | Fisher Scientific | 10-500-25 | |
| Isothesia (Isoflurane) | Henry Schein Animal Health | 050033 | |
| Liquid Nitrogen | Wright Brothers | NIT-60-XX | |
| Mouse Kras Strain | The Jackson Laboratory | OO8179 | |
| Mouse Cre Strain | MMRRC | OOO435-UNC | |

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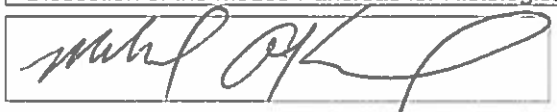
| Signature: |  Date: 10/23/2016 |

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May 16, 2017

Editor,

Journal of Visualized Experiments

Dear Editor,

Please consider our revised manuscript entitled "**Dissection of the Mouse Pancreas for Histological Analysis and Metabolic Profiling**" for publication in the *Journal of Visualized Experiments*. Below, you will find our detailed responses to the reviewer's comments.

**Reviewer #1:**

1) Reviewer comment: "Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors."

**Response:** Manuscript was proofread to ensure no spelling errors or grammatical errors were present. If found, they were corrected.

2) Reviewer Comment: "**Introduction:** Paragraph 2 is lacking appropriate references."

**Response:** Additional references were added in paragraph 2 of the Introduction to provide adequate citations.

3) Reviewer Comment: "**Title/abstracts:** As there is no histology or metabolic profiling presented, these must be edited to reflect the manuscript and video content OR results/data showing these should be added."

**Response:** Representative results and figures have been added showing histology and metabolic profiling data.

4) Reviewer Comment: "**Protocol Language:** Please edit the language so that ALL text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Examples NOT in imperative tense: 1.1, 1.1.2, 1.1.3, 1.2, 1.3, 1.4, 1.5, etc.
2) Some long steps were split up."

**Response:** 1) Language was edited to imperative tense or a "Note" was added. 2) Long steps were split up.

5) Reviewer Comment: "**Protocol Detail:** Please add more details to the following protocol steps.
1) 1.6: Mention isofluorane%.
2) 1.8: How do you ensure euthanasia?
3) 1.9: How much isoflurane do you use? What %? It appears that the mouse still alive in 1.9.1. If so, it is confusing to call the glass jar a euthanasia jar, instead perhaps call it anesthesia jar?"

**Response:** 1) Added 99.9%. 2) "Euthanasia" chamber was changed to "anesthesia" chamber. Euthanasia is discussed in a later section 2.3.4. 3) Sentence was changed to say, "Place the head inside the tube lined with a surgical pad soaked with a few drops of isoflurane (99.9%)". Euthanasia was changed to read anesthesia.

6) Reviewer Comment: "**Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol."

**Response:** Discussion was edited to include sections titled: "significance with respect to existing methods, limitations of the technique, critical steps within the protocol, modifications and troubleshooting, and future applications".

7) Reviewer Comment: "**Figures:**:
1) Fig 1-10: Please include scale bars on all images to provide context to the magnification used.
2) Fig 9,10 : Please include arrows to indicate spleen and pancreas."

**Response:** Scale bars have been added to figures 1-10. On figures 9 and 10, arrows have been included to indicate spleen and pancreas.

8) Reviewer Comment: "**Figure/Table Legends:**:
1) Fig 3: It may be best not to call this a "euthanasia chamber as the mouse is euthanized much later in the procedure."

**Response:** The word "Euthanasia" was changed to "Anesthesia" in the title and caption.

9) Reviewer Comment: "**References:**Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list

all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]
1) Please abbreviate all journal titles, and also edit the references to match the suggested format."

**Response:** References were edited to match the suggested format.

10) Reviewer Comment: "**Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as mouse strain."

**Response:** Mouse strain information was added to the materials list.

11) Reviewer Comment: "Please define all abbreviations at first use."

**Response:** All abbreviations have been defined at first use.

12) Reviewer Comment: "Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit."

**Response:** Standard abbreviations and symbols have been added/changed.

13) Reviewer Comment: "If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**Response:** Figures and tables have not been previously published and are original- comment is ignored.

**Veterinary Reviewer:**

1) Reviewer Comment: "Isoflurane anesthesia delivered via open drop method is acceptable, however, care should be taken to ensure the mouse does not come into contact with the isoflurane liquid, or the soaked gauze, as this can be irritating and cause discomfort. There are methods designed to ensure the mouse is only exposed to the vapors and not the liquid isoflurane. This should be addressed, see article for an easy way to accomplish this: Taylor DK, Mook DM. Isoflurane Waste Anesthetic Gas Concentrations Associated with the Open-Drop Method. Journal of the American Association for Laboratory Animal Science: JAALAS. 2009;48(1):61-64."

**Response:** To ensure the animal does not come in contact with the isoflurane, a barrier needs to be placed between the pad soaked with a few drops of isoflurane and the animal. Manuscript was edited to read that a paper towel over the top of the pad will serve as this barrier.

2) Reviewer Comment: "The instructions say to use a "soaked surgical pad" with isoflurane – the use of isoflurane should be minimized and from an occupational health perspective and personnel exposure perspective, the least possible volume that will provide anesthesia should be used. It is unnecessary to "soak" a gauze pad, therefore, with isoflurane. This should be addressed in the text – the video already says (more correctly) "soaked with a few drops of isoflurane"

**Response:** Manuscript was edited to say soaked with a few drops of isoflurane.

3) Reviewer Comment: "1.6 says isoflurane should be used only in the "vent hood." – scavenging of waste anesthetic gas should be addressed in more detail, as not all "vent hoods" provide enough scavenging. The article above and others are readily available upon searching to address appropriate waste gas scavenging.

**Response:** The scavenging of waste anesthetic gas, regarding isoflurane usage, has been address and referenced.

4) Reviewer Comment: "Text: page 4, section 1.9 states "place the head inside the falcon tube lined with the isoflurane soaked surgical pad and perform a foot pinch to ensure discomfort is not being experienced" – this would better be described as in the video, where the voiceover says "mouse cannot experience discomfort" – an even better description would be "mouse is unresponsive to stimuli."

**Response:** In section 1.9 the words, "discomfort is not being experienced" was change to "the mouse is unresponsive to stimuli."

5) Reviewer Comment: "The video voiceover describes a cervical dislocation after the mouse is anesthetized and removed from the isoflurane jar. If that is done, there is no need to put the

mouse's head in a tube with isoflurane during the dissection, as the mouse has been euthanized, and therefore represents an unnecessary occupational exposure to isoflurane."

**Response:** Following transfer from the anesthesia chamber to the dissection table, the mouse is still alive up until the terminal blood draw was performed. After the terminal blood draw, the heart was detached as a secondary method to ensure euthanasia. Therefore, the head of the mouse remained in the tube until euthanasia was performed. At this point the dissection of the pancreas was carried out. This is accurately reflected in the video and article in the revised manuscript.

We hope that our revised manuscript is now considered suitable for publication in the Journal of Visualized Experiments.

Sincerely,

Michael A. Kennedy, PhD

Eminent Scholar and Professor

Department of Chemistry and Biochemistry

Miami University

Oxford, OH 45056

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