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# Collection of human vagal tissue samples for TEM imaging V.2

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This protocol describes the methods used to generate samples of human vagus tissue suitable for TEM imaging.

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Collection of human vagal tissue samples for TEM imaging. **protocols.io**  
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protocol

Leif A. Havton, Natalia P. Biscola, Esther Stern, Plamen V. Mihaylov, Chandrashekar A. Kubal, John M. Wo, Anita Gupta, Elizabeth Baronowsky, Matthew P. Ward, Deborah M. Jaffey, and Terry L. Powley. Human Organ Donor-Derived Vagus Nerve Biopsies Allow for Well-Preserved Ultrastructure and High-Resolution Mapping of Myelinated and Unmyelinated Fibers. Nature Scientific Reports 2021 Dec 13;11(1):23831. doi: 10.1038/s41598-021-03248-1.

vagus nerve, TEM, transmission electron microscopy, human

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Ensure that all appropriate authorizations and permissions for sampling of human tissue are obtained in advance - see protocol for details.

## Sampling

- 1 Vagus nerve samples were obtained intra-operatively from organ donors. Separate consents for organ donation and research were obtained. Informed consent for study participation was obtained from a parent and/or legal guardian. No organ donors were prisoners. A diagnosis of brain death or circulatory death as appropriate had been previously established by independent medical professionals according to local institutional regulations. All procedures related to tissue procurement and research use for tissues were in compliance with and approved by regulatory oversight review committees at University of Indiana School of Medicine, Indiana Donor Network or the local Organ Procurement Organization, and Purdue University. The vagus nerve biopsies were obtained from a continuous series of qualified organ donors meeting the diagnostic and medical criteria for organ donation and with consent for research participation in place. Donors from whom multivisceral transplants (lungs and stomach) were pursued were excluded from the research studies to enable removal of as complete sets of samples as possible. The research team was not involved with the identification and selection of organ donors.
- 2 Age, gender, BMI, race, cause of death, and delay time between death and sample recovery were recorded for all donors/samples. All data was deidentified.
- 3 After the completion of transplant organ harvesting, samples of the vagus nerve were collected. Approximately 10–20 mm long segments of the vagus nerve were removed from the following locations:
  1. Cervical – left / anterior
  2. Cervical – right / posterior
  3. High abdominal trunk above hepatic and celiac branches – ventral / anterior
  4. High abdominal trunk above celiac branches – dorsal / posterior
  5. Ventral /anterior gastric branch at the level of the cardia

## Processing

- 4 All vagus nerve segments were immediately placed in a fixative solution containing 2% paraformaldehyde and 2.5% glutaraldehyde for 5 days at 4 °C.

 [Paraformaldehyde BAKER J.T. Baker Fisher](#)

**Scientific Catalog #02-003-576**

 [Glutaraldehyde 50% EM Grade Aqueous Electron Microscopy](#)

**Sciences Catalog #16320**

The fixative solution was changed daily and the specimens were kept on an oscillating plate

during the immersion fixation process (at 4 °C). The fix solution was always refrigerated and was always less than one week old.

- 5 After completion of the fixation process, the tissue specimens were rinsed (3 × 30 min at 4°C) in PBS, transferred to individual shipping vials under PBS, and shipped overnight on ice to the TEM laboratory for further processing and microscopy - see the protocol below:



Nerve tissue processing for transmission electron microscopy (TEM)

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PREVIEW

RUN

