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We use this protocol and it's working

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Abstract

Protocol for collection, histological processing, and imaging of human vagus nerves.

Materials

- 4% paraformaldehyde
- Mordant & tissue dye
- Ethanol
- Clearite
- Paraffin
- Xylene
- Bouin's fixative (mordant)
- Weigert's iron hematoxylin solution
- Biebrich scarlet-acid fuchsin solution
- Phosphomolybdic-phosphotungstic acid solution
- Aniline blue solution
- Acetic acid
- Microscope with color camera

Collect human vagus nerve samples.

1 We collected vagus nerve samples from embalmed human cadavers. The study was deemed exempt by the Duke University Institutional Review Board. The bodies were donated to the Duke Anatomical Gifts Program and we accessed them after they were used for medical training courses. The cadavers were embalmed with DUMC Embalming Fluid from the Carolina Biological Supply Company (33.3% ethanol, 13.2% phenol, 3.7% formaldehyde, 1% methyl isobutyl ketone, and 1% methanol).

2 We collected cervical vagus nerve samples bilaterally. We collected 2 cm samples approximately where a line from the rostral end of the sternum to the earlobe intersects with the vagus nerve, also corresponding to the midlevel of the thyroid cartilage. We measured from the "valley" of the common carotid bifurcation to the center of each sample.

3 We collected samples of the anterior and posterior subdiaphragmatic vagus nerve trunks along the esophagus between the diaphragmatic esophageal hiatus and the gastroesophageal junction.

4 We dyed the rostral end of each sample green to maintain orientation during processing.

5 We placed each sample between two histology sponges in a mega-sized histology cassette. We placed the cassettes in a tub with 4% paraformaldehyde in a 4^{o}C refrigerator.

Perform histological processing.

6 We rinsed each sample with deionized water.

7 We processed each sample on the long cycle in the Leica ASP300S Tissue Processor for ~10 hours: 70, 80, 95, 95, 100, 100,100% ethanol for 30, 35, 40, 40, 40, 40, 40 min, respectively; Clearite for 50min, three times; paraffin wax for 50 min, three times.

8 We cut each sample in half transversely and embedded the halves together cut side down in paraffin in order to obtain transverse sections starting from the center of the nerve and moving outward.

9 We collected 5 µm sections, placing two serial sections per microscope slide for fifteen slides.

10 The slides were air dried overnight at room temperature, then baked at 37^{o}C overnight.

11 Of the 15 slides per sample, we stained slides 2 and 14 with Masson's trichrome as follows.

12 The slides were baked at 60^{O}C for 1.5 hours and then cooled overnight.

13 We deparaffinized the slides and hydrated them to distilled water: xylene (2× 6 min), 100% ethanol (5 min), 95% ethanol (4 min), 70% ethanol (3 min), dH2O (2× 1 min).

14 We placed the slides in Bouin's fixative (mordant) at room temperature overnight.

15 We washed the slides in running tap water until the yellow color disappeared (~10 minutes).

16 We rinsed the slides in distilled water.

17 We placed the slides in Weigert's Iron Hematoxylin solution for 10 min.

18 We washed the slides in running tap water for 10min.

19 We placed the slides in Biebrich Scarlet-Acid Fuchsin solution for 5 min.

20 We washed the slides in running tap water for 2 min.

21 We placed the slides in Phosphomolybdic-Phosphotungstic Acid solution for 10 min.

22 We transferred the slides directly to Aniline Blue solution for 3 min.

23 We differentiated the counterstain (A. blue) in 1% Acetic Acid solution for 1 min.

24 We dehydrated, cleared, and coverslipped the slides: 95% ethanol (1 min), 100% ethanol (4 min), xylene (5 min).

Perform microscopy.

25 Each sample was imaged at 10x using a Nikon Ti2 microscope with a Photometrics Prime 95B-25MM camera (Nikon Instruments Inc.). We selected the best of four slices for each sample based on the quality of the slice (no tearing or fraying).