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Collection of human nasal cavity tissue at the time of autopsy and in preparation for routine microscopy.

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ABSTRACT

This protocol describes the collection of human nasal cavity tissue, including cribriform plate and olfactory and respiratory epithelium at autopsy, as well as the preparation of the tissue for paraffin-embedding (fixation and decalcification).

Note, the extended decalcification period with EDTA can lead to changes in epitopes. Antibody conditions may require re-optimization when compared to non-EDTA-treated tissue.

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1 The body of the deceased has been transferred to the morgue at the Department of Pathology at participating hospitals and kept at 4ºC.

2 Within an ideal post mortem interval of 4 and 48 hrs, and following the completion of consent forms that address the collection of the deceased person's brain and/or other specified tissues for the purpose of a restricted or an unrestricted autopsy, the body of the deceased is transferred into one of the autopsy / dissection rooms and placed into the supine position.

3 The technician under the supervision of a pathologist, who is physically present, opens the skull in a traditional, axial plane with a circular blade saw to then open the dura and to remove the brain, beginning rostrally by lifting the frontal lobes. During that maneuver, the olfactory bulbs are either retained sitting on the cribriform plates underneath them, or are removed together still connected to the olfactory tract (that is slightly attached to the inferior portions of the frontal lobes).

4 Following the removal of the brain from the skull, having been disconnected from dura folds, cranial nerves, vasculature and the upper portion of the cervical cord, the technician removes any fluid remnants from the anterior cranial fossa to visualize the area of the crista gallei that anchors the (now dissected) falx leaf of the dura. By using the same (or a smaller) circular blade saw, the technician begins to cut an axially positioned rectangle into the bone around the cribriform plates and crista gallei with the front being parallel to the inner boundary of the frontal sinuses of the forehead, with the rear in proximity to the optic nerves (covered by dura) and anterior to the sella, and with the lateral sides flanking the remaining olfactory bulbs or the exposed cribriform plates. The desired size of said rectangle measures 25-35 mm in width (anterior and posterior), 40-55 mm in length, and importantly, it should measure 30-50 mm in depth. Note, the size of the to-be-removed piece of bone containing the upper roof of the nasal cavity with its olfactory and respiratory epithelia will vary based on the deceased person's height, sex, age and bone thickness.

5 Using a bone chisel (with a blade width of ~20-25 mm), the technician / pathologist will insert the tip of the blade at the cranial end of the demarcated rectangle, with the axis of the blade aiming in the direction of the mouth of the deceased. He/she/they will then gradually advance the chisel by using a hammer. This, to effectively dislodge upward the complete upper portion of the nasal cavity in one piece with the anatomy grossly intact.

6 Once dislodged, the tissue block will be removed and placed into a 100 cc cup of 10% formalin to begin the process of fixation, followed by decalcification.

7 Fixation and decalcification: The bone piece and underlying, attached epithelia are fixed in 10% formalin for 7 days at 4ºC. The tissue is then switched to Tris/EDTA solution pH 7.2-7.4 (68g Tris, 220g EDTA, 4L of dH_20) for decalcification for a minimum of 4 weeks, and until the bone is soft enough for cutting. The Tris/EDTA buffer is changed every 2-3 days during this time.

8 The bone piece is then cut with a sharp scalpel blade into 3 sections: an anterior, middle and posterior piece. Each of these pieces are then processed for paraffin embedding.

9 Two sections from each block are stained with H&E, and Alcian Blue pH 2.5 respectively to determine which block(s) contain predominant olfactory epithelium (as the blocks with contain either respiratory epithelium, or respiratory epithelium plus olfactory epithelium).