

Appendix 1A Brain Sampling Procedures

Entire heads may be submitted fresh or frozen to an approved laboratory

Obex harvesting technique

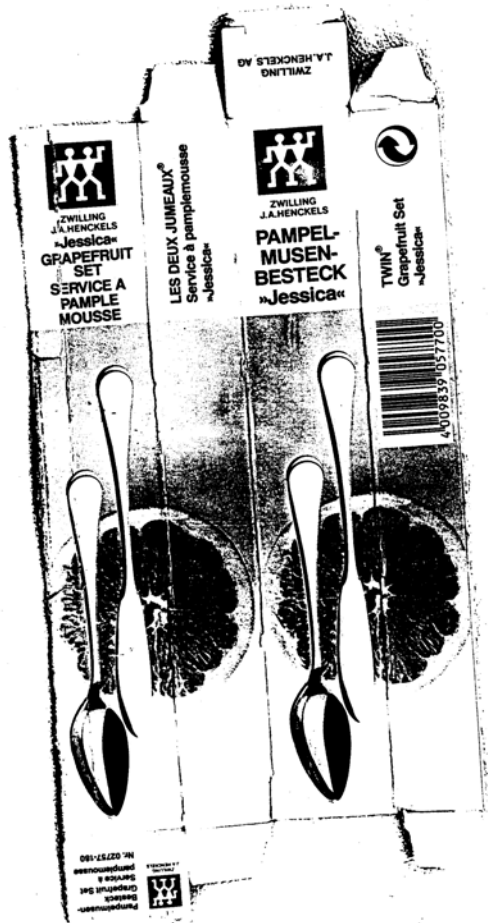
Recommended tools:

- 1) obex removal spoon and
- 2) rat tooth forceps

Additional tools that you may choose to use,

- 3) scalpel
- 4) scissors

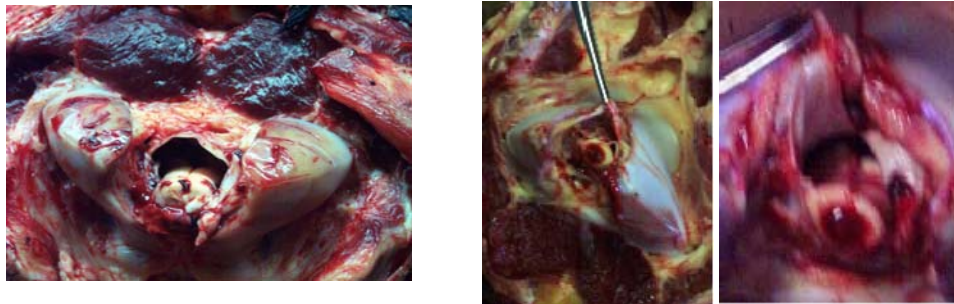
Obex removal spoon and knife may be fashioned from the “Zwilling J.A. Henkle - Jessica Grapefruit spoon and knife”. Alternatively, any butter knife or suitably long and concave spoon (6mm concavity) can be modified. The edges of the



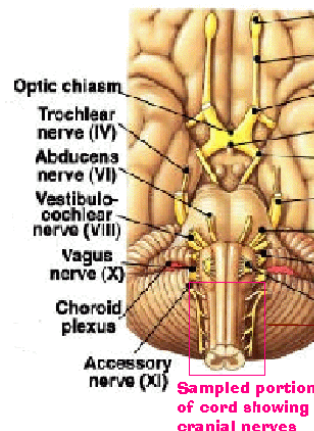
knife must be sharpened. The edges of the spoon should be cut down to measure 22 - 26 mm at the widest point and sharpened to a blade.

With the aid of an autopsy knife, disarticulate the head at the level of the atlanto-occipital articulation. Place the head dorsal side down on a table with the foramen magnum (the opening of the spinal canal) facing you.

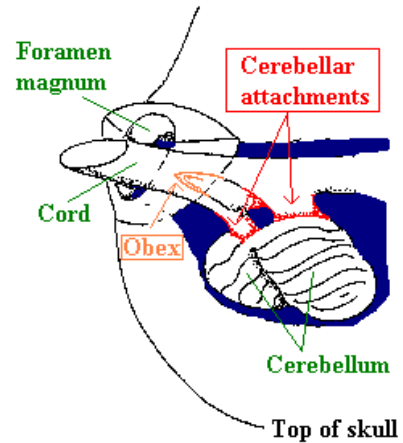
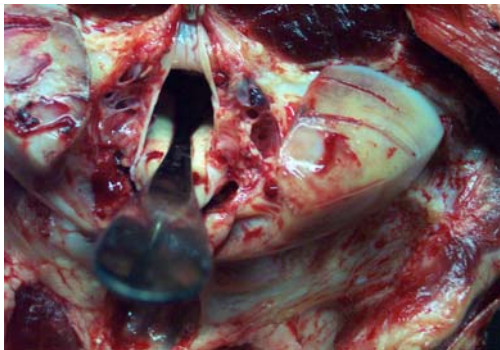
Using the forceps with your left hand, grasp the dura mater (the thick lining around the spinal cord) and with the scissors in your right hand make a single cut down the center line to form two flaps (this step is not mandatory). Remove the congealed blood from around the cord.



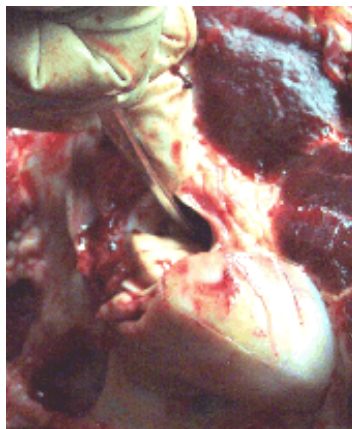
With the forceps in your left hand holding the dura, sever the cranial nerves from the cord. This can be done with scissors or with the obex knife by passing the knife around the cord, keeping the flat side of the knife against the cord (see next picture for position of obex knife with relationship to the cord). This is the most important step in freeing up the cord. The cord must be completely free from attachments (by cranial nerves) in all directions. If you do not sufficiently free the cord up, you will not be able to sample anteriorly enough to get the obex, or one side will be missing.



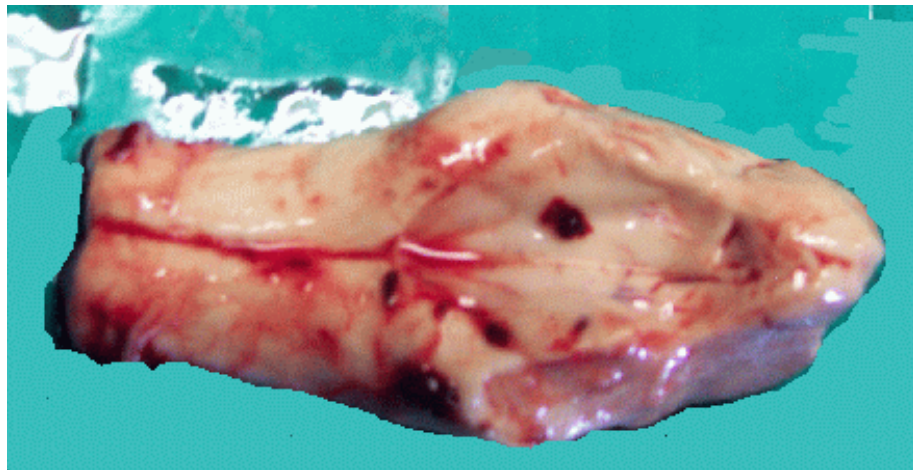
Insert the obex spoon face down into the vertebral canal and move it forward lodging the tip as far craniially as it will go. This should position the tip of the knife over the part of the cord just anterior to the obex and just behind the cerebellar attachments.



Pick up the head and (if right handed) hold in left hand, dorsal side down. While pushing down with index finger, maneuver the tip of the obex knife back and forth over the cord to sever it from the rest of the brain just rostral to the obex and caudal to the cerebellar attachments. Rotate the skull with your other hand in the opposite direction to the knife, to facilitate the cutting of the cord.



Use the obex knife to gently pull the severed portion of the brain from the canal. The obex is recognizable as a V-shaped depression. This portion is the single most important site for diagnostic testing.



Contact the laboratory to which you will be submitting the samples and request information on the sample preparation for submission (fresh frozen or formalized).

Freeze samples and batch ship in frozen state to an approved laboratory for ELISA testing.

Alternatively, place the brain stem and cerebellum in a 500 ml. glass jar containing 10% buffered formalin fixative. The ratio of the volume of fixative to tissue should be 10:1. Fix the specimen for 24 hours - 5 days depending on the size of the sample (penetration of formalin is at least 5 mm of tissue thickness / day) and then ship to an approved laboratory. These tissues will be subject to histopathology and immunohistochemistry.

Tissues for scrapie surveillance testing may be packaged and shipped as non-regulated substances in accordance with the TDG regulations. The basic four parts packaging system may be used. Following fixation the specimen must be

packaged in primary watertight receptacle (whirl pak bag) with labeling. Enclosed in second durable watertight packaging (whirl pak bag) with adequate absorbent material to absorb all fluid in case of breakage. Outer packaging, such as a fibreboard box, protects the contents from external physical damage. The description of the contents on the shippers way bill should include the notation "non hazardous, unregulated, sample".