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Post-mortem Approach to the Central Nervous and Ophthalmic System

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Central Nervous System

There are three major areas of the CNS which need to be examined, namely the brain, spinal cord and peripheral nerves. Post-mortal changes create a wide array of artefactual change, necessitating early fixation of nervous tissue as soon possible after death.

Brain

Procedure

- In small animals, the skull should ideally be opened with an oscillating plaster cast saw (figure 1). The head is skinned and disinfected, after which the roof of the cranium is opened with the disinfected oscillating saw and lifted off. The brain is then handled very delicately to avoid inducing post-mortal artefacts. By inverting the head, the weight of the brain pulls the organ out of the cranial cavity and allows for severing of nerve roots to release the brain from the cranium.
- This procedure enables the removal of the brain in its entirety and this facilitates the gross examination of the brain as well as collection of samples for microbiology and histopathology.

Figure 1. Oscillating saw ideal for opening the cranium of small animals.

- Another method for removing the brain, which is more practical in large animals/on-farm post mortems, is where the skull is opened by a longitudinal cut through the midline. First, the head is skinned after which it is disinfected. A handsaw or band saw is then used to open the skull longitudinally in the midline. The two halves of the brain are then removed gently, as excessive handling can induce artefactual change.

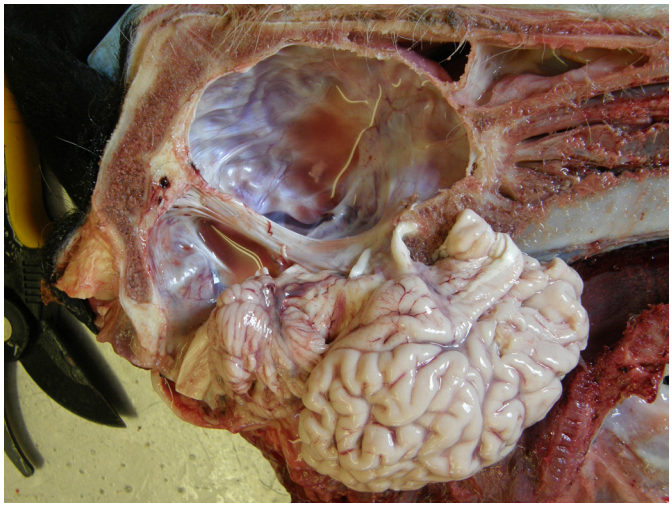

Figure 2. Longitudinal section through the midline of the skull with the removal of the two halves of the brain.

- The removed brain is then examined for any obvious surface pathology.

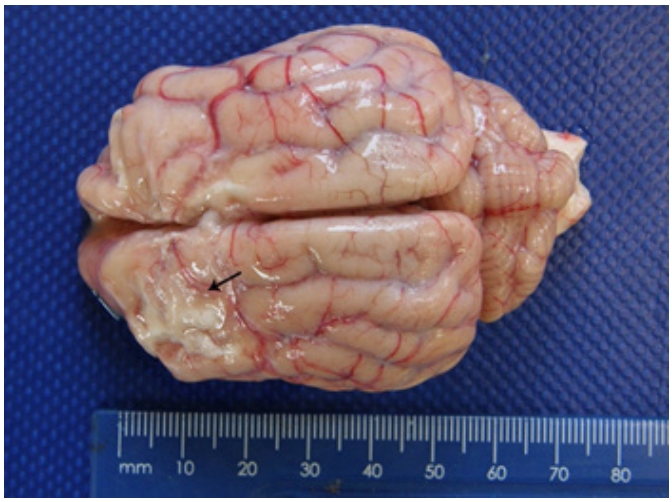

Figure 3. A destructive neoplastic mass is evident in the left anterior lobe (arrow).

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The brain is then bologna sliced (figure 4) to examine for lesions within the brain parenchyma as well as inspection of the ventricular system.

In foetuses and post mortems where decomposition has already set in, the brain tissue can be very difficult to handle, as it becomes a very flimsy and easily fragments and tears on sectioning (figure 5).

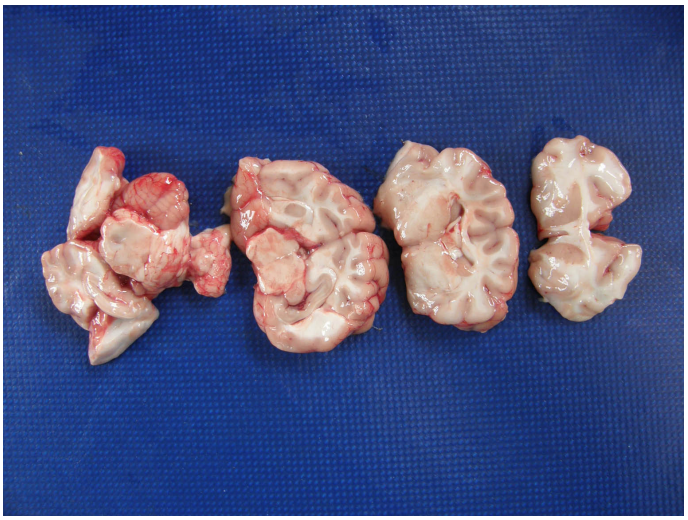


Figure 4. Bread loaf slicing of the brain into 1 cm slices for examination.

To avoid these complications one fixes the entire brain in a large container filled with 10% buffered formalin overnight.

This overnight formalin fixation firms up the tissue and makes it easy to handle and therefore, high quality slices can be obtained which significantly improves the gross examination of the tissue.

Figure 5. Bologna slicing of slightly autolysed brain which has become more liquefied resulting in sticking and tearing of the brain tissue.

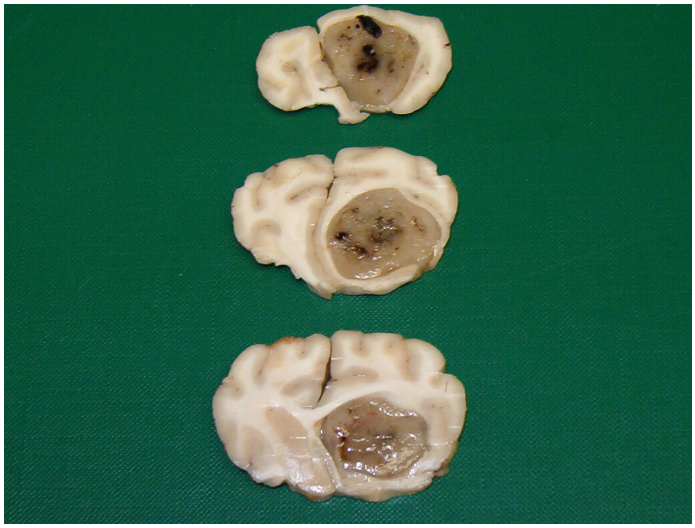


Figure 6. Brain tissue fixed overnight to facilitate bologna slicing and gross examination of the brain tissue.

Ancillary Laboratory Tests

Brain Smears.

Smears prepared from the hippocampus, where there is a high concentration of capillary vessels, provides the best chance of demonstrating parasites (*Ehrlichia*, *Babesia*, *Anaplasma* and *Theileria*). Preparing the smears from the pulverised grey matter of the hippocampus provides the highest quality smears that facilitates easy examination of extended capillaries.

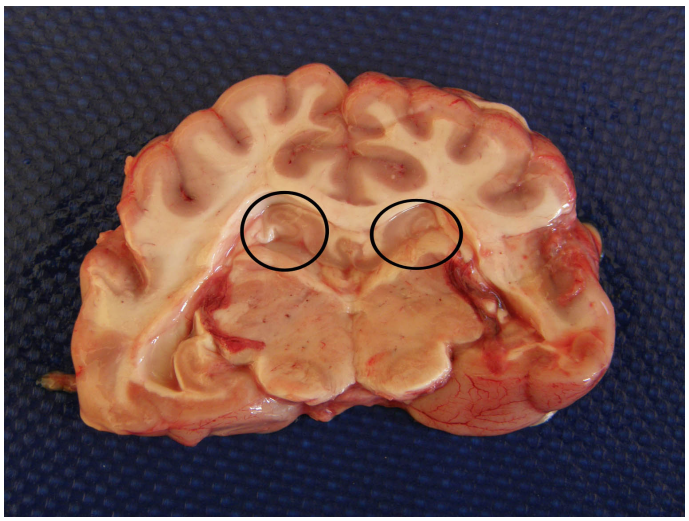


Figure 7. Hippocampus (circles) is the target site preparation of brain smears.

- Hippocampal grey matter is pulverised between two glass slides. The pulverised material is then collected at the one end of the slide. With the second slide held

at an angle and with pressure applied, the pulverised material is spread over length of ½ to 1 cm, after which the pressure is released. Pressure is then re-applied, and the material spread over a further ½ to 1 cm. This is then repeated multiple times across the slide to create the smear (figure 8). This procedure spreads out the blood capillaries within the brain parenchyma into parallel arrays, which facilitates easy examination for parasites within blood vessels under the microscope.

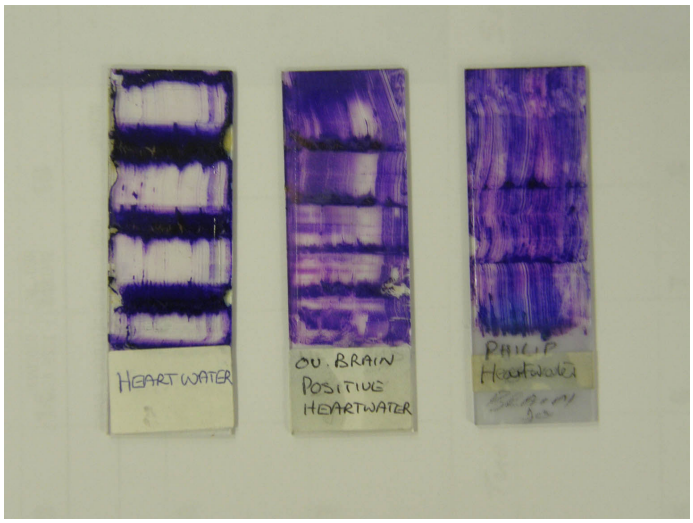


Figure 8. Stained brain smears to reveal the extended parallel arrays of pulverised brain parenchyma, which contain the brain capillaries.

- Inclusion of the white matter of the hippocampus complicates the pulverising procedure and results in poor quality smears where extended capillaries are difficult to find.

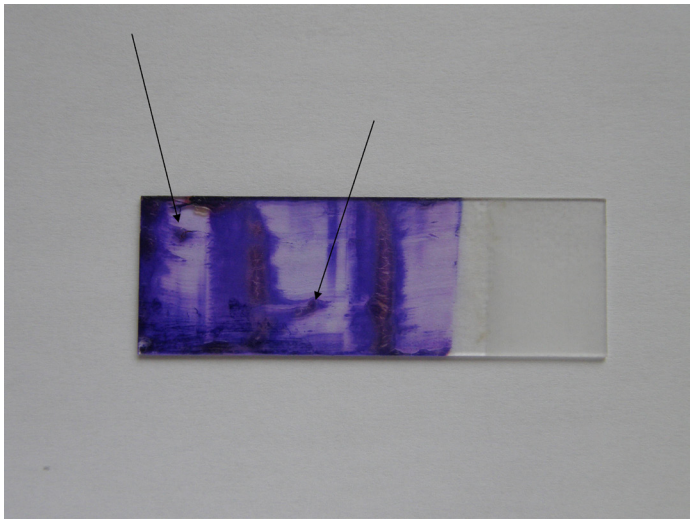
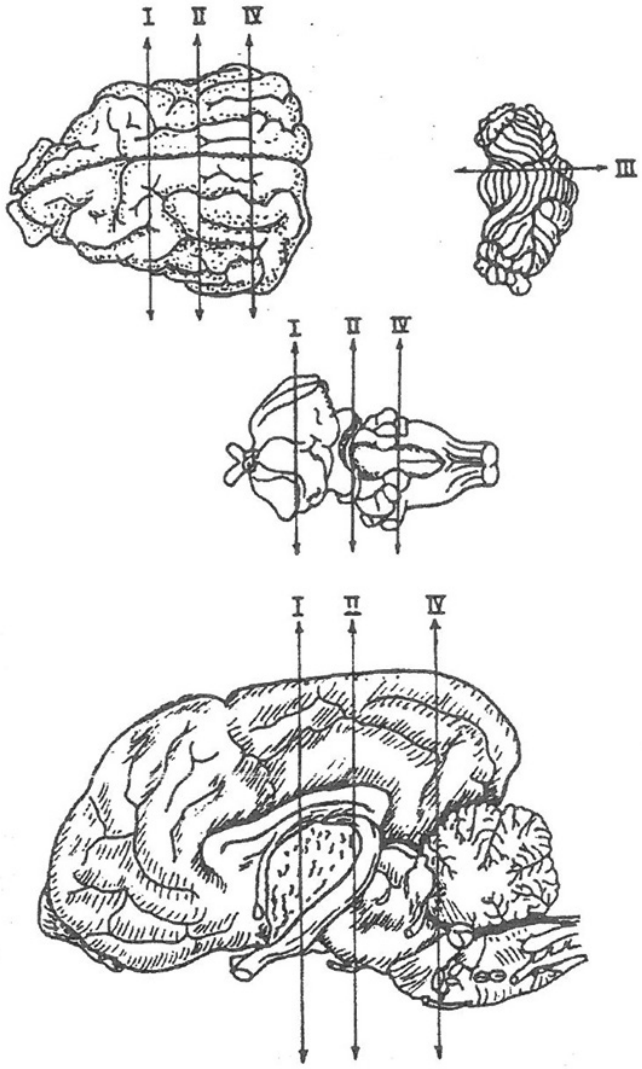


Figure 9. White matter fragments (arrows) negatively affect the quality of brain smears.

Histopathology



The schematic drawing above indicates which brain slice sites should be selected for placing into 10% buffered formalin for histopathology.

* I = transverse section through thalamus
* II = transverse section through the hippocampus
* III = median section through the cerebellum
* IV = transverse section through the brainstem

Spinal Cord

Procedure

- The vertebral column should be opened by a para-median longitudinal section through the vertebrae exposing the cord.

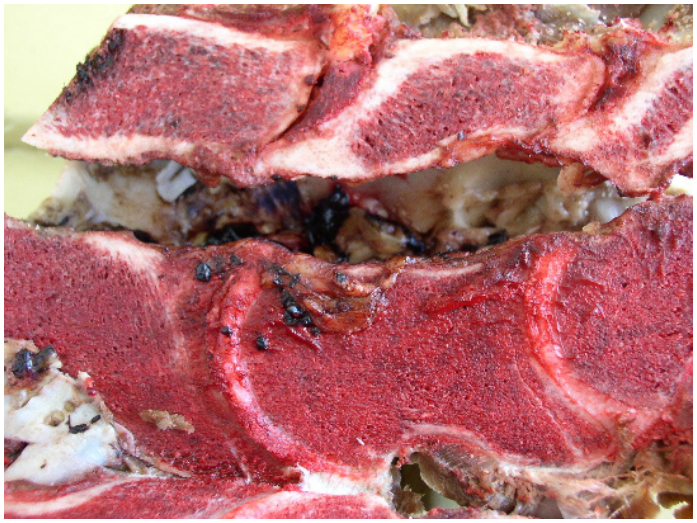


Figure 10. Image above shows an example of a longitudinal vertebral column section with spinal cord removed.

- Cord is then gently removed by careful dissection of the spinal nerve roots from the outside of the dura mata. The dura mata is then opened and removed from the spinal cord.
- The cord is then bologna sliced to evaluate for any gross pathology. Areas of obvious pathology plus sections from the cervical, thoracic and lumbar protuberance zones (no thicker than 1 cm), are collected into 10% buffered formalin.

Figure 11. Vertebral synovial cyst in a horse, which has compressed the dura and underlying spinal cord.

Figure 12. Bologna sliced cord from a horse with a vertebral synovial cyst, which has resulted in acute haemorrhagic malacic lesions in the cord.

- Each section of spinal cord selected for histopathology must be placed in a separate formalin container, which is clearly marked as to which anatomical cord location the specimen is from e.g. C5/C6 or T1/T2 etc.
- In the "wobbler syndrome" of horses and dogs' resection of complete segments of the spinal column is indicated. Following removal of the musculature, transverse sections are made through the centre of adjacent cervical vertebrae (black lines) and each entire segment is placed in a separate, labelled bucket of 10% buffered formalin. White arrow demonstrates the site of narrowing of the vertebral canal

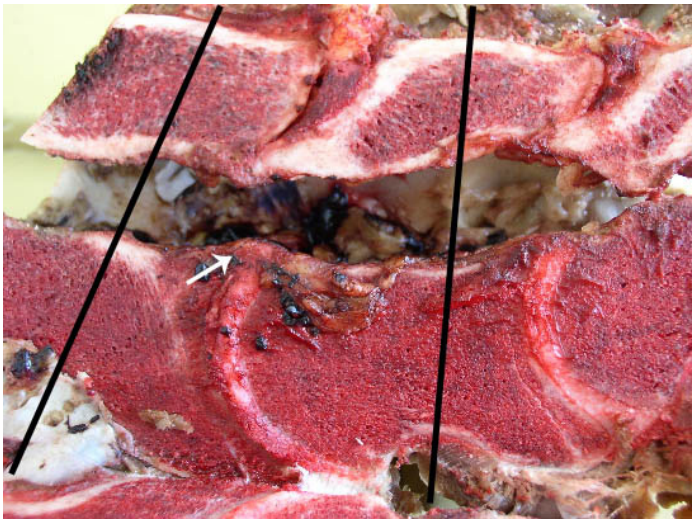


Figure 13. Schematic guide will sample collection in the "wobbler syndrome".

- These segments are then left to fix in formalin for at least 24 hours.
- Following fixation, the cord is then removed by grasping the dura mata with forceps and dissecting the lateral aspect, on the outside of the dura with a pair of straight scissors, cutting the spinal nerve roots and releasing the cord.

- Each segment of fixed cord is then placed in another marked formalin container and submitted to the laboratory.

Figure 14. Arrows in the image above demonstrate roughed proliferative reactive bone on the vertebral body and dorsal spinous process as a consequence of friction caused by a vertebral synovial cyst.

- The bones are placed in a pressure cooker and boiled to remove all soft tissue and allow for examination of the vertebrae and their articular facets.

Peripheral nerves

Procedure

- Where possible a minimum length of 3-4cm of peripheral nerve should be dissected out.
- Nerve tissue is extremely delicate and should not be grasped with rat-tooth forceps or other crushing instruments.
- Remove all fat surrounding the nerves to allow good fixation.
- Segments of peripheral nerve are placed in an extended state on a piece of cardboard and allowed to dry for 5-10 minutes.
- The cardboard with attached peripheral nerve is then inverted and submerged in 10% buffered formalin. This ensures fixation in the extended state facilitating the preparation of high-quality histological section.

Ophthalmic System (Eye)

Ocular pathology can be most rewarding for both the clinician and pathologist. Remember the eye is an extension of the central nervous system and therefore many disease processes that produce brain pathology induce characteristic ocular pathology. This can prove useful when necropsy is being performed in the field on a large animal where opening of the skull may be difficult. However, freshness of the material and use of rapidly acting fixatives form the cornerstone of ocular histopathology. Eyes collected > 8 hours after death, especially under conditions of high ambient temperature, often produce poor quality sections, making lesion interpretation exceedingly difficult.

Sample Collection

The skin and eyelids are removed from the head to reveal the globe in the orbital cavity. The eye is then removed as rapidly and as gently as possible.

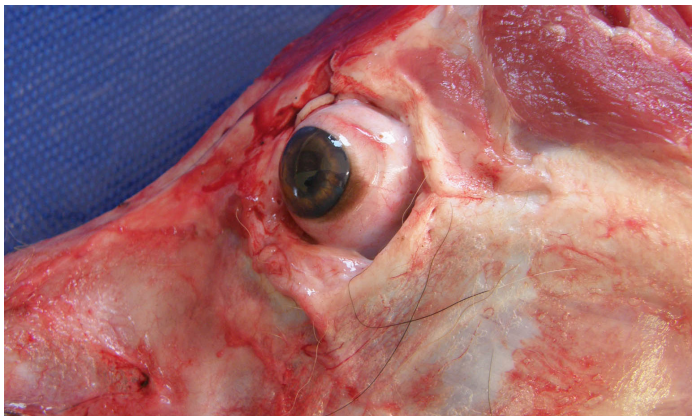


Figure 15

By grasping the outer sclera, the globe can be placed under traction while circumferential blunt dissection is made around the fornix. Extra ocular muscles are then severed and the eye is removed from the orbit.

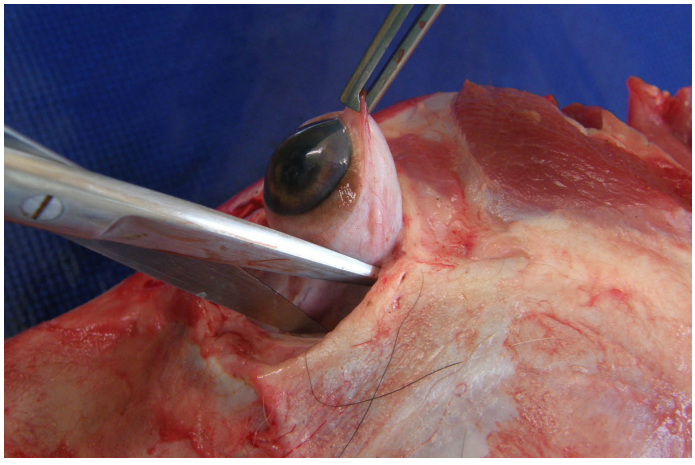


Figure 16

All peri-orbital fat and muscle remnants are then carefully dissected away from the sclera, by suspending the eyeball by its own weight and grasping the peri-orbital fat with forceps, facilitates dissection along scleral junction and the eyeball literally unravels from the peri-ocular fat and muscle.

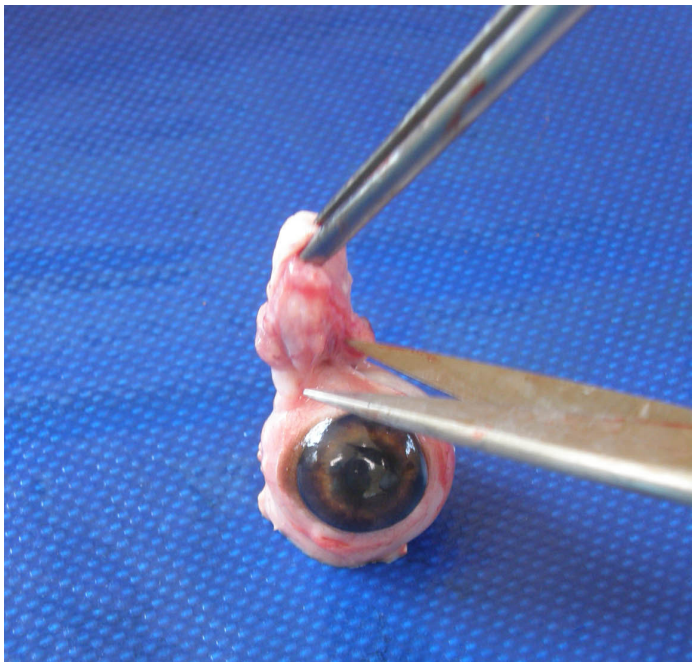


Figure 17

A small volume of 10% buffered formalin is then drawn up into a syringe. Using a new, narrow gauge needle, formalin is then injected into the globe by puncturing through the sclera immediately adjacent to the optic nerve. The formalin is injected until the globe until it is visibly distended and there is increased resistance.

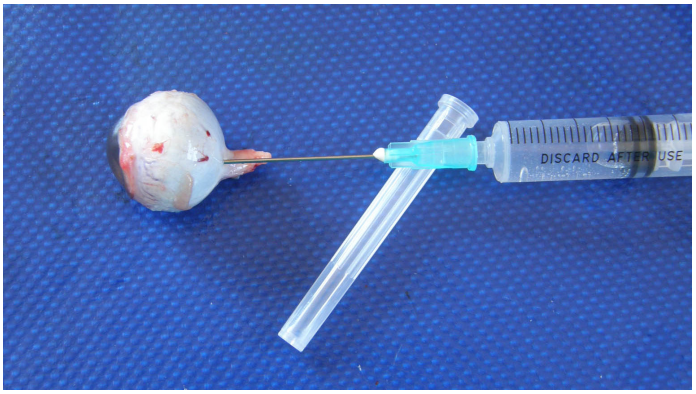


Figure 18

The needle is then removed and the formalin-distended globe placed in a jar of 10% buffered formalin.

Figure 19

On reception at the laboratory, the eye is left in formalin to fix for 24 hours. They are then passed through a series of increasing concentrations of ethanol (75%-95%) to harden the vitreous prior to trimming for histological processing. In **birds and reptiles**, which have intra-ocular bone an additional decalcification step is required following the ethanol treatment.

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QUESTION 1

Under which of the following situations would you consider PREFIXING brain tissue prior to Bologna slicing for gross examination?

a. Foetal brain
b. Brain from a geriatric animal
c. Brain from a fresh post mortem (less than 1 hour after death)
d. Brain of a large ruminant
e. Equine brain

QUESTION 2

Which of the following is the PREFERRED site or preparation of brain smears?

a. Frontal lobe
b. Cerebral cortex
c. Pons
d. Cerebellum
e. Hippocampus

QUESTION 3

Which of the following parasites would you NOT expect to find in the brain smear?

a. Babesia
b. Ehrlichia
c. Dirofilaria
d. Anaplasma
e. Theileria

QUESTION 4

Which of the following areas of brain are NOT routinely collected for histopathology?

a. Transverse section of the frontal lobe
b. Transverse section of the thalamus
c. Transverse section of the hippocampus
d. Medial section of the cerebellum
e. Transverse section of the brainstem

QUESTION 5

Which of the following zones of the spinal cord are NOT routinely collected for histopathology?

a. Any obvious lesion
b. Cervical protuberance
c. Thoracic protuberance
d. Lumbar protuberance
e. Cauda equina

QUESTION 6

What is the PREFERRED sample for the diagnosis of wobbler syndrome in dogs and horses?

a. Atlanta axial site
b. Section through two consecutive cervical vertebra bodies including the spinal cord
c. Mid-thoracic spinal cord
d. Lumbosacral site
e. Coccygeal vertebral site

QUESTION 7

Which of the following steps would be considered the MOST IMPORTANT procedure for peripheral nerve formalin fixation to ensure high-quality sections?

a. Remove all surrounding perineural fat
b. Retrieve spinal cord samples with rack tooth forceps
c. Maximum length of 0.5 cm
d. Fix the peripheral nerve in the extended state
e. Use 5% buffered formalin for fixation

QUESTION 8

What time frame post-mortally can one STILL EXPECT to obtain quality diagnostic samples of the globe with formalin fixation?

a. 10 hours
b. 15 hours
c. 5 hours
d. 19 hours
e. 24 hours

QUESTION 9

Which of the following procedures would be considered the MOST CRITICAL step in obtaining good fixation of the retina?

a. Removal of periorbital fat prior to fixation
b. Removal of the periorbital muscle prior to fixation
c. Removal of the eyelid skin prior to fixation
d. Injecting 10% buffered formalin into the globe
e. Making an incision through the sclera prior to fixation

QUESTION 10

Eyes from which of the following species must be DECALCIFIED after formalin fixation prior to processing?

a. Canine
b. Avian
c. Feline
d. Bovine
e. Equine

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