Biosecurity Tasmania
Department of Natural Resources and Environment Tasmania

Tasmanian Government

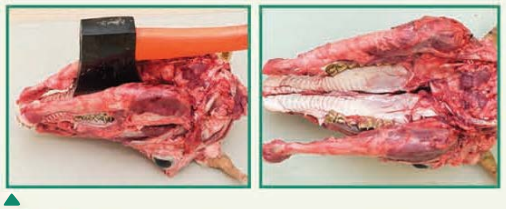
Brain removal techniques

The conventional method for brain removal involves sawing through the bone of the dorsal cranium and removing a cap of bone to expose the brain. Two alternate methods are shown below that, with practice, can be less tiring and faster to perform. The brains removed by these techniques provide a sample suitable for routine diagnostic and transmissible spongiform encephalopathy (TSE) exclusion testing.

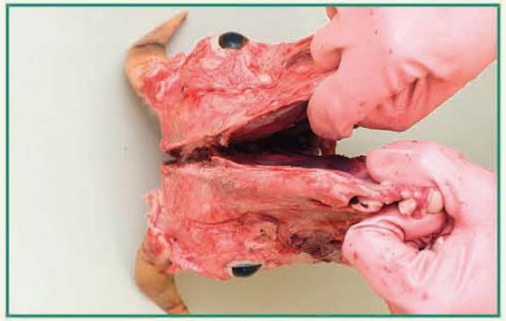
Method 1: Longitudinal craniotomy

Step 1: Remove the head at the atlanto-occipital joint. Remove the tongue and pharynx to expose the hard and soft palate. Split the mandibular symphysis and place the head on a non-slip surface such as a rubber mat or onto the ground, using the separated mandible to improve stability.

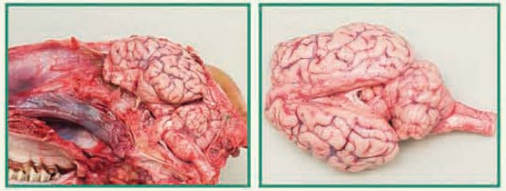
Step 2: Using a hatchet and a mallet, make a dorsal midline cut through the bone from the nose to the foramen magnum. Place hatchet blade at cutting site and use mallet to split the bone. Do not swing the hatchet. The head does not need to be skinned, but making an incision through the skin along the proposed line of cutting helps to stop the hatchet blade from deviating.



Step 3: Turn the head over and cut through the soft and hard palate and ventral cranium.



Step 4: Stand the head upright on the foramen magnum and use a knife to sever any remaining attachments. Lever the nasal bones apart, using the hatchet to increase leverage and split the skull. If nasal bones feel as if they will snap, check all the bone is cut, especially between the occipital condyles.



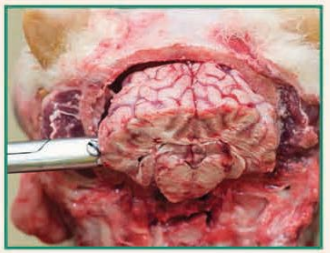
Step 5: Use scissors to cut the remaining meninges and cranial nerve attachments to allow removal of the entire brain. Note: for TSE exclusion testing, remove the dorsal cerebellum (sheep only) and 2-3cm spinal cord and submit these sections **fresh**. Fix remainder of brain in 10% buffered formalin.

Method 2: Transverse craniotomy

When using the transverse craniotomy technique in **cattle**, it is advisable to leave the head attached and immobilise for sawing by tensioning nose grips to a vehicle or other solid object. Alternatively, flex the neck and tie the nose grips to the hock. In **small ruminants**, removing the head is recommended.

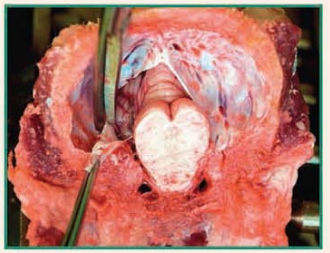
Step 1: Stabilise the head for sawing by placing it on a non-slip surface or mat. The head does not need to be skinned, but a knife cut through the skin at the intended line of sawing is recommended.

Step 2: Begin sawing vertically in a line 1cm rostral to external ear canal and extend the cut through the bone until the cranium hinges apart. Remove the head at the atlanto-occipital joint if still attached.

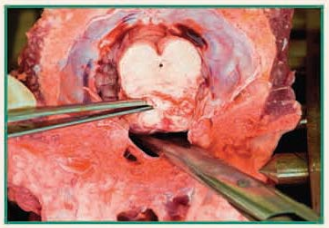


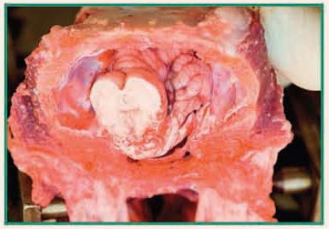
Step 3: Begin with the rostral half of the skull. Using curved scissors, cut the ventral nerve roots and the olfactory bulbs. Remove the rostral brain.

Step 4: Shell out the occipital lobes in the caudal half of the skull to expose the opaque tentorium cerebelli.

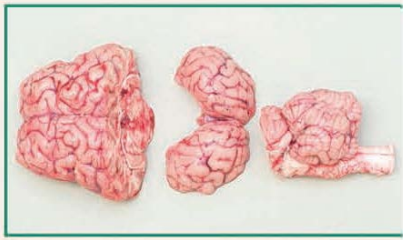


Step 5: Cut the tentorium to expose as much of the cerebellum as possible.





Step 6: Using scissors, cut the cranial nerves on the ventral and lateral surfaces inside the cranium and then around the cut spinal cord at the foramen magnum. Using a finger or plunger from a syringe, gently push the hindbrain and cerebellum rostrally out of the cranium.



Step 7: Fix the three brain segments whole in 10% buffered formalin. Note: for TSE exclusion testing, remove the dorsal cerebellum (sheep only) and 2-3cm spinal cord and submit these sections **fresh**. Fix remainder of brain in 10% buffered formalin.

Updated: Jan 2022

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Brain sampling tips for TSE exclusion testing

Brain samples required for the National TSE Surveillance Program include:

Sheep:

1. fresh dorsal cerebellum
2. fresh spinal cord, 2–3cm in length
3. fix the rest of the brain and brainstem whole.

Cattle:

1. fresh spinal cord, 2–3cm in length
2. fix the rest of the brain and brainstem whole.

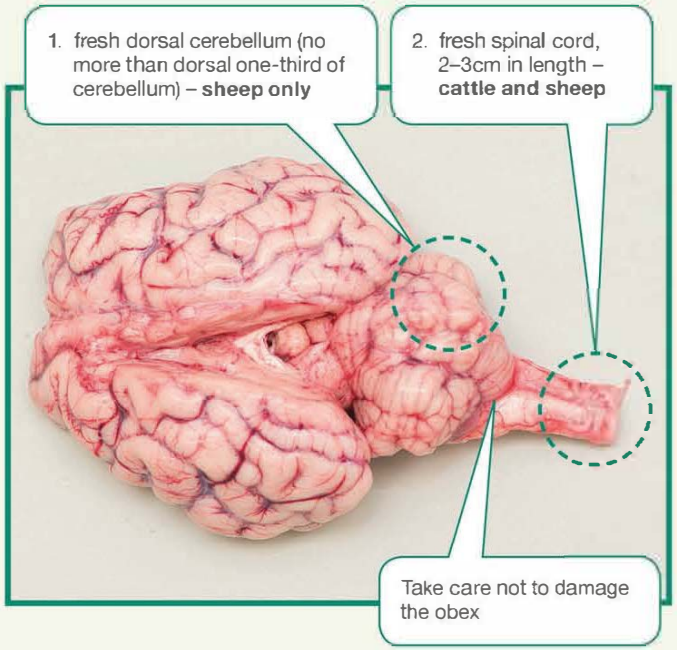


Figure 1 Collection of fresh brain TSE samples

Brain sampling do's:

- ✓ Take care not to damage key TSE brain sites when removing the brain and taking the fresh samples (see Figure 2).
- ✓ Use enough 10% buffered formalin and a sufficiently large histology pot so the brain does not fix in a distorted position:
  - sheep brain – use a 1L pot filled to the top with formalin
  - cattle brain – use a 2L pot filled to the top with formalin.
- ✓ Allow the brain to fix in the formalin pot at room temperature.
- ✓ Check the case meets the TSE eligibility criteria (listed on the TSE lab submission form). **Cows nine years of age or older are ineligible.**

Brain sampling don'ts:

- ✗ Don't split the brain in half lengthways (longitudinally) as this damages TSE sites.
- ✗ Don't submit a half fixed/ half fresh brain. To culture, use a swabbing technique that will keep the brain intact (see below).
- ✗ Don't remove the fresh spinal cord sample from too close to the cerebellum – imagine a perpendicular line behind the cerebellum and avoid sampling on the cranial side of the line.
- ✗ Don't remove more than one third of the dorsal cerebellum when removing the fresh cerebellum sample in sheep.
- ✗ Don't put pots containing tissue and formalin in the fridge or freezer.

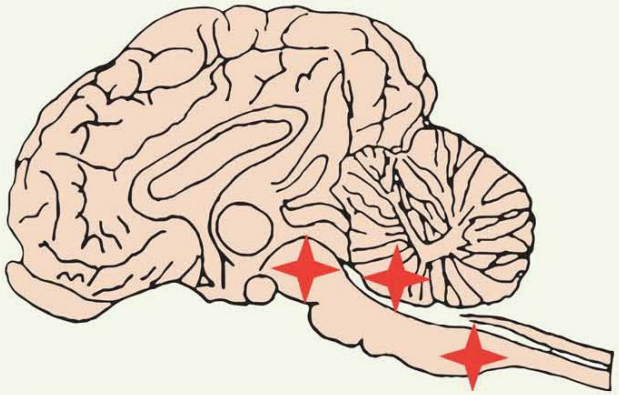
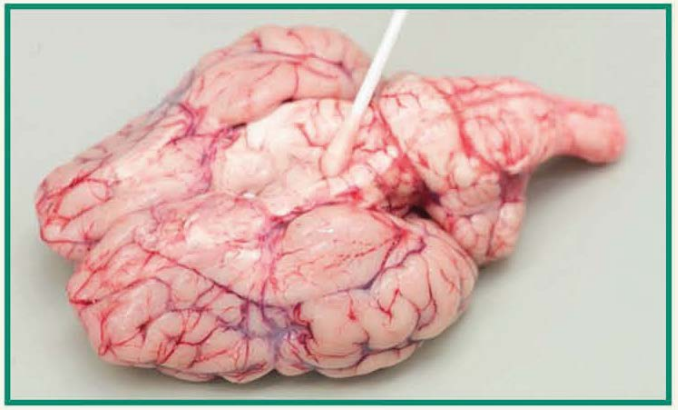


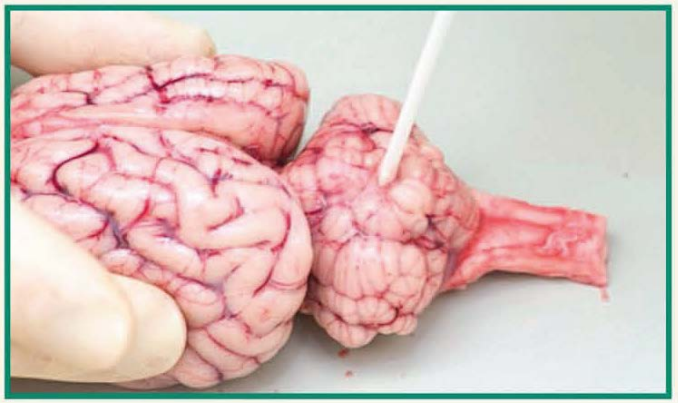
Figure 2 Key brain sites for TSE exclusion

Brain swabbing methods that keep the brain intact

Method 1: For most meningitis cases it is suitable to swab the base of the brain.



Method 2: *Listeria* can be recovered by stabbing a swab through the dorsal cerebellum into the brainstem. The swab must be angled in a cranial direction so it does not damage the obex.



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