Department of
Primary Industries and
Regional Development

GOVERNMENT OF
WESTERN AUSTRALIA

Protect
Grow
Innovate

Disease investigation guide: Ruminants

Current at August 2025

Day to day routine: On farm biosecurity

Based on *Footrot Standard Operating Procedure: Decontamination of Boots and Equipment*.

Purpose

This document guides you in decontaminating boots and equipment used in day-to-day disease investigation. Use this guide when there is a negligible to low index of suspicion for a reportable or notifiable animal disease.

If you have a concern about a reportable or notifiable disease, call the Emergency Animal Disease Hotline on 1800 675 888 prior to entering the property

Requirements

You will need:

- appropriate personal protective equipment e.g. safety glasses, clean boots, gloves and coveralls
- a scrubbing brush
- a tub for a footbath
- a bucket
- appropriate surface disinfectant like Chlorhexidine
- a hand brush
- a spray bottle
- access to water
- a hoof pick.

Follow the Material Safety Data Sheet (MSDS) for correct use of disinfectant. All personal protective equipment described in this document must be on hand and used. **Take care when disposing of used disinfectant.**

Procedures and actions

Boots

- If visiting a property, wear clean boots before entering the paddocks and yards.
- When you exit the property, use the tub to create a disinfectant solution as per MSDS and package insert.
- Remove mud, faeces, dirt and other debris from the boots with a hoof pick or similar tool and scrub thoroughly to ensure all organic material is removed before rinsing.

Equipment

- If you are required to bring equipment to the property visit, ensure the equipment is clean.
- When exiting the property, where possible clean the equipment from the top to bottom. Thoroughly sweep contaminated material such as dust, dirt, mud, and faeces off the equipment.
- Wash down the equipment thoroughly with disinfectant, water and a broom. **Be aware of water around any electrical components of equipment.**

Clothes

- If visiting any property, wear clean overalls before entering the paddocks and yards. As part of your “clean-on-clean-off” protocol, this includes clean boots (multiple sets are advised when visiting multiple properties).
- Use a clean set of overalls if travelling from one property to another on the same day.
- Keep contaminated overalls and other PPE gear, where they cannot contaminate other material and wash as soon as possible.

Vehicle

- When exiting a property, where possible clean from top to bottom. Thoroughly sweep contaminated material such as dust, dirt, mud and faeces off the vehicle.
- Scrub the tyres to remove any dust, dirt, mud and faeces from them. Fill the spray bottle with disinfectant solution and spray the tyres with disinfectant, especially if visiting more than one property on the same day.

Zoonotic disease risk assessment

When assessing livestock or wildlife, you must take precautions to reduce the risk of contracting a zoonotic disease. Always practice good hygiene and use appropriate Personal protective equipment (PPE) including gloves when handling biological samples.

Before the field visit

1. **Is it a routine visit or a disease investigation?**
   Disease investigations bear higher risks. Remember, the absence of apparent disease does not preclude the possibility of an animal harbouring a zoonotic disease agent.

2. **Collect relevant information.**
   Check clinical signs, previous history, origin of animals, risk factors and sick people to determine if a zoonotic disease may be present. With referred cases (from a private vet or one of our staff) contact the producer or the referrer to collect information.
   > **Note**: information on history/clinical signs may not be conveyed comprehensively or accurately.

3. **Prepare appropriate PPE.**
   If significant body or aerosolised fluids are present:
   - protect against splashes using goggles and a face shield
   - wear a P2 mask to protect against aerosolisation when using a bandsaw or other machinery
   - wear an impermeable apron over overalls or disposable impermeable overalls to protect against splashes
   - consider environmental contaminants like soil.

   Infection control precautions must be complied with at all times.
   - Cover skin cuts or abrasions
   - Wear closed in, waterproof footwear and protective outerwear around mud, soil and areas of pooled water
   - Wear gloves when handling soil or mud-soaked items and when sampling.
   - Use hand hygiene and remember sharps safety
   - Ensure your vaccinations are up to date:
     - Q-fever if working with cattle, sheep and goats
     - Influenza if working with poultry.
   - Always presume that specimens are infectious
   - Decontaminate containers.

If you suspect an exotic or reportable disease, contact the EAD hotline on 1800 675 888, your local Field Veterinary Officer, or our Diagnostics and Laboratory Services Duty Pathologist on 08 9368 3351.

Zoonotic disease risk in ruminants

Disease cannot be determined based on syndromic presentation alone. Use this guide when assessing if a zoonotic disease may be present and additional precautions may be required. **Do not use this guide in isolation.** Weigh up the context, risk factors, likelihood and consequences of each scenario when deciding which risk mitigations to apply.

Syndrome	Reproductive	**Cattle:** Bovine brucellosis; Leptospirosis; Listeriosis; Q fever, **Sheep**: *Brucella melitensis* • This is considered a syndromic presentation with a high risk of zoonotic disease. • Avoid or reduce contact with placenta, birth tissues, foetal membranes and aborted foetuses. • Pregnant staff must not attend.
	Gastrointestinal	**Cattle:** Campylobacteriosis; Cryptosporidiosis; Yersiniosis; Colibacillosis; Salmonellosis • Most zoonotic diseases that present with diarrhoea are transmitted via contact/ingestion of faecal contaminated material. Risk effectively mitigated with PPE.
	Neurological	**Cattle**: Bovine Spongiform Encephalopathy (BSE); Listeriosis **Sheep**: Scrapie; Listeriosis; Infection with hydatids • Extreme care. Caution with handling nervous tissue. Wear PPE for necropsy.
	Pruritis/skin lesions	**Cattle:** Ringworm; Pseudocowpox **Sheep:** Dermatophilosis; Orf (Scabby Mouth) • Most zoonotic diseases that present with pruritus or skin lesions and are transmitted via broken skin. • Risk can be effectively mitigated by using PPE. • Wash hands and equipment.
	Respiratory	**Cattle:** Bovine Tuberculosis **Sheep:** Tuberculosis (exotic) • Remove suspect animals from food chain. Wear PPE for examination of animals and necropsy.
	Sudden death	**Sheep and Cattle:** Anthrax • Requires further risk assessment – if bleeding from orifices present do not open carcass and seek further advice.

A visual guide to diagnostic sampling

Below is a guide to the base sample set required when completing a disease investigation in Ruminants.
Refer to document **'A visual guide to a ruminant animal postmortem'** for detailed postmortem instructions.

Antemortem Samples		
It is recommended the following is collected from live animals where live animals are available: • blood • faeces.		
If you are concerned about lead toxicosis, ARGT or cyanobacterial toxicosis, please refer to the specific sample guide for sampling instructions.	Collect 10ml of blood in each of the following tubes: • EDTA • plain clotted • lithium heparin.	Collect 50 g of faeces (If no live animals are available, faeces can be collected from the colon during the postmortem)

Sample type	Storage and transport
Blood (EDTA and Lithium Heparin)	• all to stand at room temperature • mix gently with anticoagulant • refrigerate (do not freeze)
Blood (Plain Clotted)	• allow to stand at room temperature • refrigerate once clotted.
Faeces	• refrigerate.
Fresh Tissues	• individual containers • refrigerate • do not freeze.
Fixed Samples (ratio 10:1 formalin to tissue)	• tissues can be pooled into a single formalin pot • keep at room temperature.

A guide to diagnostic sampling - postmortem samples

Collect fixed and fresh samples of <u>all lesions</u> noted during the postmortem

Below is the base sample set to be collected during a postmortem examination of a ruminant

Sample type	Tissue type	Sample to take
Fresh tissue	Brain	Refer to the **brain removal techniques** section.
	Lung	Collect one 3x3x3 cm section of fresh lung.
	Liver	Collect one 3x3x3 cm section (50 grams) of fresh liver ***Additional:*** Sterile E-swab can be taken.
	Ileal Contents	Collect 5 ml of ileal contents.
	Rumen Contents	Test rumen pH (normal 5.5 – 7.0). Collect 50 ml of rumen fluid and 50ml plant digesta.
	Kidney	Collect one 3x3x3 cm section of kidney (include medulla and cortex) or half a kidney chilled.
	Vitreous humour	Collect 1.5-2.0ml in a 2.0ml serum tube (freeze)
Fixed samples	Brain	Refer to the **brain removal techniques** section.
	Lung	Collect one 1 cm thick section of lung.
	Heart	Trim a 1 cm thick trasverse slice of mid-ventricular heart that includes both left and right ventricular free wall and interventricular septum.
	Liver	Collect one 1 cm thick slices of liver (left and right lobes).
	Duodenum, Jejunum and Ileum	Collect 2.5 cm tubes of duodenum, jejunum and ileum.
	Caecum and Colon	Collect one 2.5 cm piece of caecum and colon.
	Abomasum	Collect 2.5 x 2.5 cm pieces of pylorus and fundus.
	Rumen, Omasum and Reticulum	Collect 2.5 x2.5 cm pieces of rumen, omasum and reticulum.
	Kidney	Collect one 1 cm thick slice of kidney including the cortex, medulla and pelvis.
	Skeletal muscle	Collect 1 cm thick longitudinal slices of two muscle groups.

A guide to diagnostic sampling – blood smears

This guide on veterinary blood smears for diagnostic investigations is a key part of haematological testing. It will help you to perform blood smears for laboratory submission as well as helping you examine cell morphology and blood borne parasites.

Remember to include a detailed history when completing the DPIRD Diagnostic Laboratory Services submission form. If you suspect an exotic, reportable or zoonotic disease, contact our Diagnostic Laboratory Services sample receival or the duty pathologist prior to submitting samples on 0(8) 9368 3351 or DDLS@dpird.wa.gov.au.

Perform blood smears when you suspect:

- blood-borne protozoal or mycoplasmal conditions
- haemolytic conditions
- anaemia
- haematologic neoplasia.

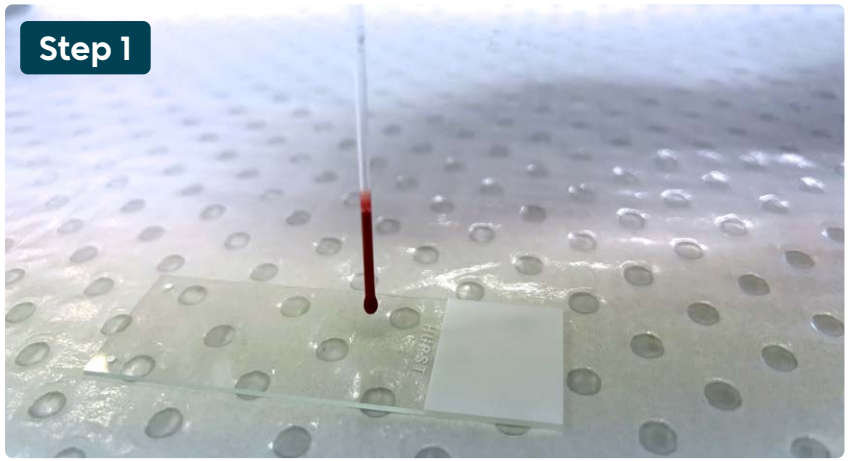
Equipment required for performing blood smears:

- microscope slides with frosted ends for writing on
- spreader slide – use a specific spreader slide with bevelled edges, or a second clean microscope slide
- capillary (microhaematocrit) tubes
- pencil to label the slides
- blood collected in an EDTA tube
- pot of sterile water to clean the spreader slide between uses.

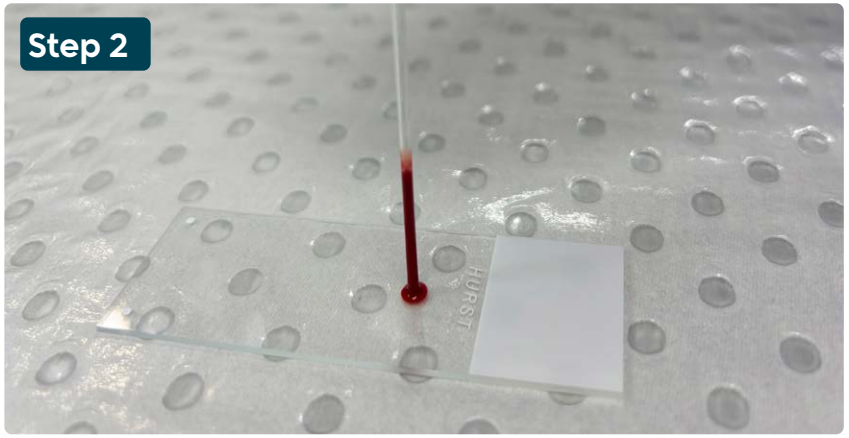
Top tips for making good smears:

Making great blood films takes practice; don't be discouraged by less than perfect smears. Submit all blood smears, perfect or otherwise, as areas of the film may be suitable for examination.

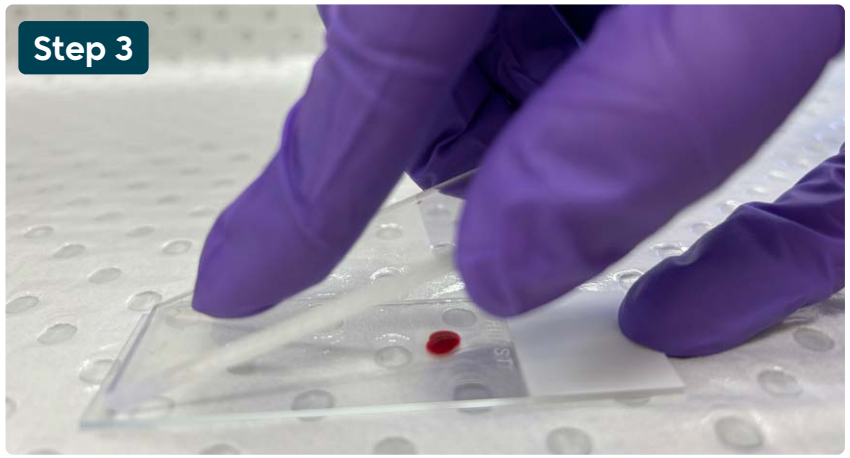
- Collect blood in an EDTA tube and make the smears when back at the clinic
- Use clean, high-quality microscope slides
- Aim for a blood droplet size of 4 mm diameter
- Optimise spreading speed for length and a good feathered edge
- Hold the spreader slide at 30-40 degrees to achieve optimal smear length
- Maintain even contact throughout the spreading motion.



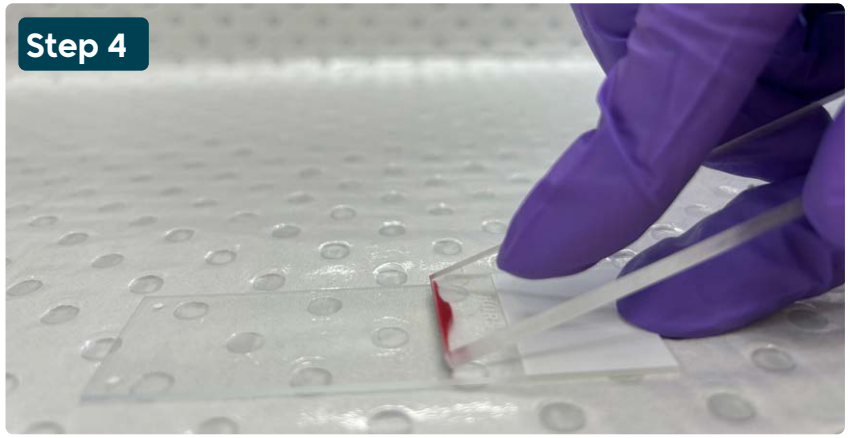
- Fill a capillary tube ¾ full with blood
- Hold the capillary tube vertically over the slide
- Allow 1 drop of blood to form on the end of the tube.



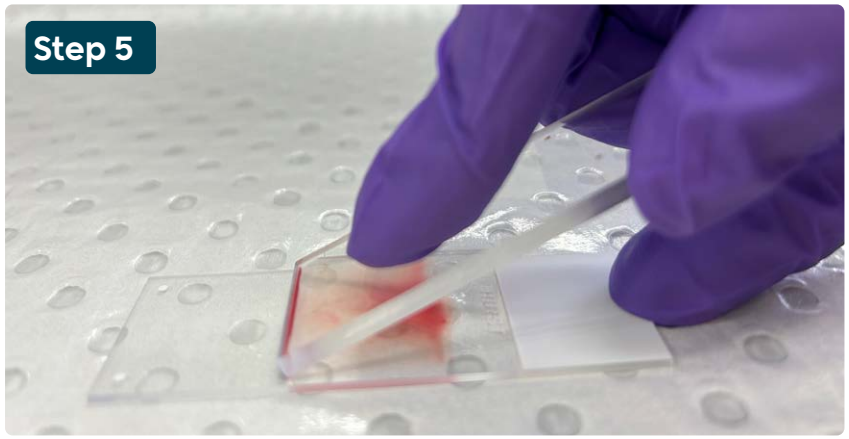
- The tube onto the slide about 0.5 cm from the frosted area
- Leave a drop of blood about 4 mm in diameter on the slide.



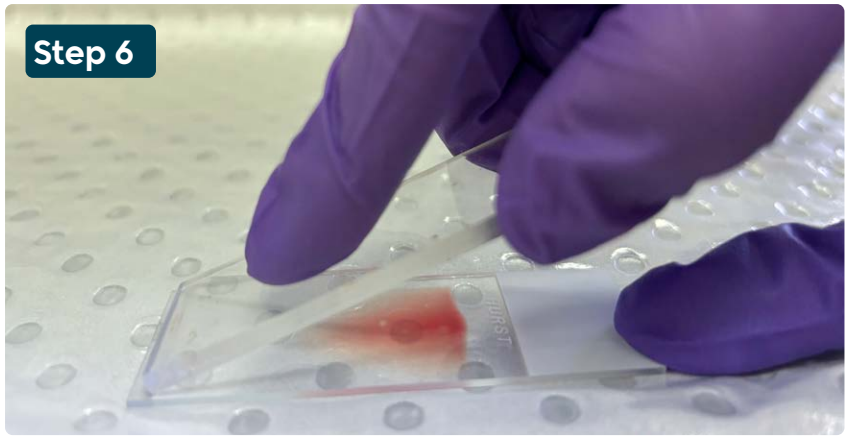
- Hold the spreader slide or second microscope slide at a 30-40 degree angle at the end of the slide (i.e. in front of the blood droplet)
- Ensure the short edge of the spreader slide is in even contact with the lower slide
- Pin the lower slide to prevent it from moving.



- Using a smooth motion, draw the spreader slide back through the entire drop of blood
- Allow the blood to spread evenly along the edge of the spreader slide.

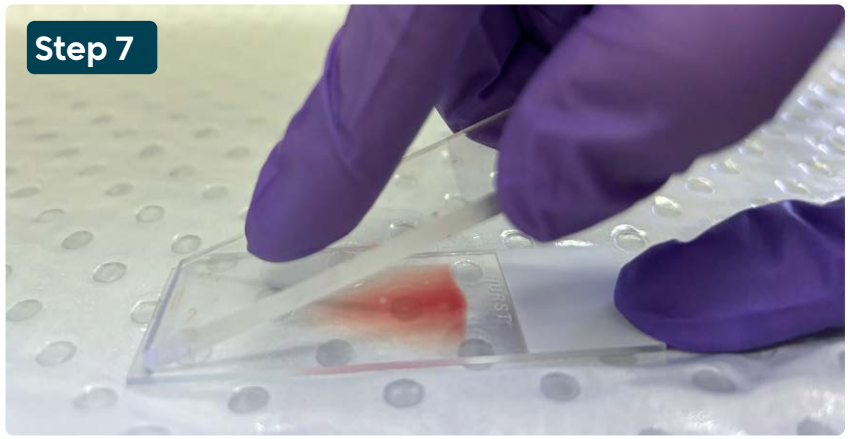


- Push the spreader slide forward along the length of the lower slide.

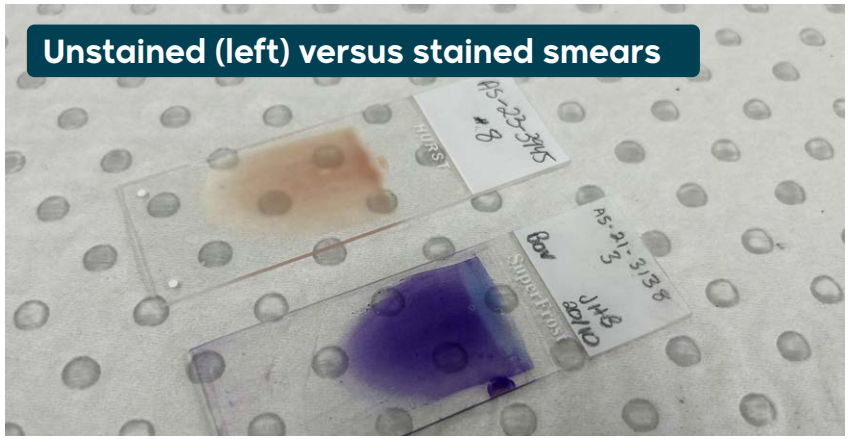


- Maintain a constant smooth motion, angle and even contact.

Note: blood is being dragged behind the spreader slide, not in front of the slide.



- An optimal smear is ¾ the length of the slide and has a feathered edge
- Leave the slide to air dry and make more smears if required
- Pack smears individually into slide holders.

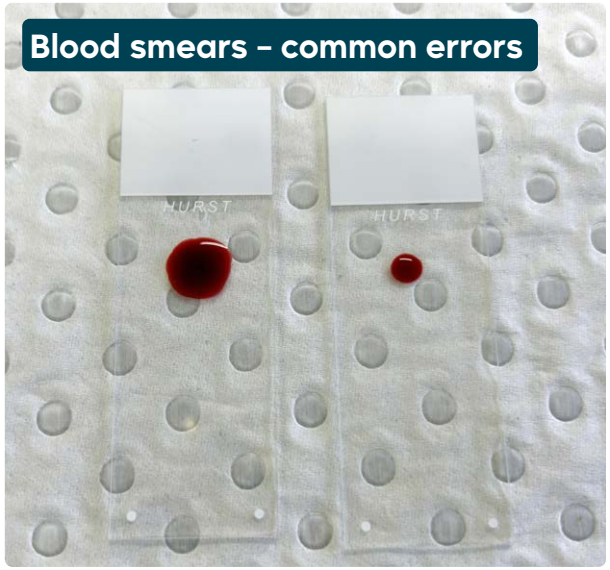


Do not fix or stain the smears – DPIRD Diagnostic Laboratory Services (DDLS) will do this under controlled conditions to optimise the staining process.

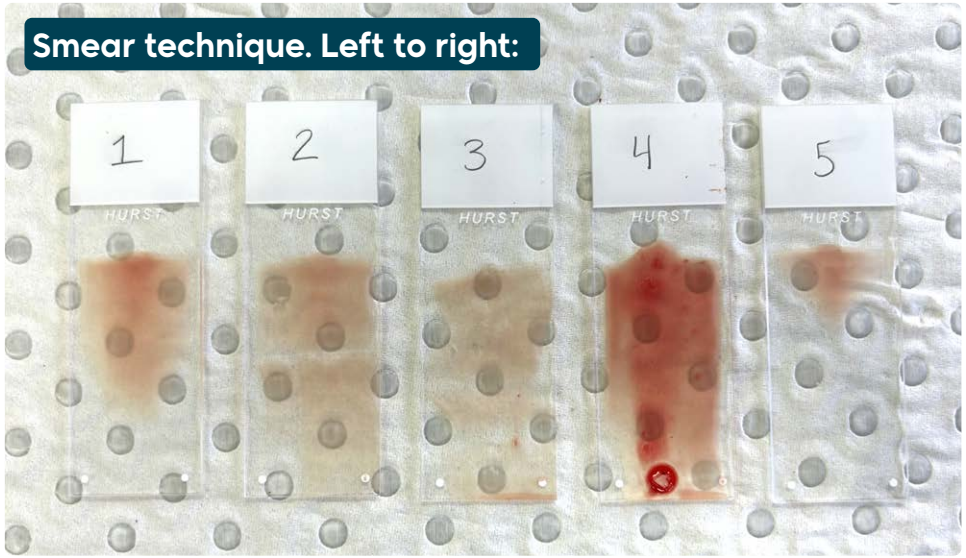
Troubleshooting blood smear errors

Problem	Solutions
Short smear	• Use a larger droplet of blood • Decrease the angle of the spreader slide • Decrease the speed of the spreader slide.
Long smear / no feathered edge	• Use a smaller drop of blood • Increase the angle of the spreader slide • Increase the speed of the spreader slide.
Thick smear	• Use smaller drop of blood • Decrease the angle of the spreader slide • Increase the speed of the spreader slide.
Thin smear	• Use a larger drop of blood • Increase the angle of the spreader slide • Decrease the speed of the spreader slide.
Smear has waves and ridges	• Maintain even contact and a smooth motion • Increase the speed of the spreader slide • Relax the wrist, reduce downward pressure on the spreader slide.

Reference: https://www.vet.cornell.edu/animal-health-diagnostic-center/laboratories/clinical-pathology/samples-and-submissions/hematology



The blood droplet on the left is too big and will result in a thick smear. The blood droplet on the right is the preferred size.

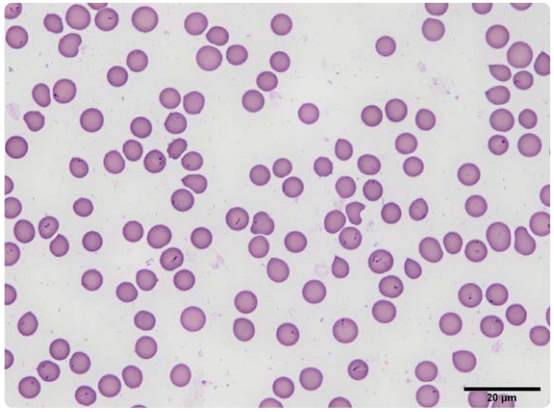


Slide 1 – perfect smear
Slide 2 – smear technique interrupted in middle
Slide 3 – smear was skewed
Slide 4 – blood droplet too thick
Slide 5 – smear too short.

Infectious diseases in Australian ruminants diagnosed by blood smear

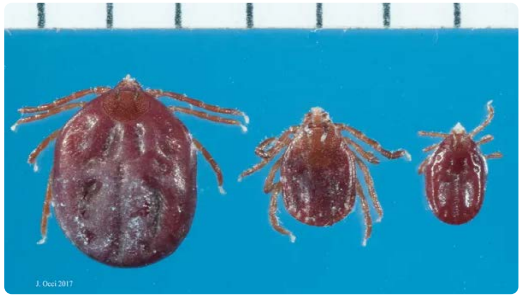
Disease	Species	Transmission	Disease status
Anaplasmosis	cattle	tick-borne	endemic – northern Australia
Babesiosis	cattle	tick-borne	endemic – northern Australia
Mycoplasma ovis (eperythrozoonosis)	sheep	iatrogenic, blood-sucking insects (midges, mosquitoes, flies)	endemic
Bovine anaemia due to *Theileria orientalis*	cattle	tick-borne	endemic to parts of eastern Australia and South west western Australia

Bovine erythrocytes infected with protozoal parasites from the *Theileria orientalis* group. The parasite is known as a piroplasm when it is within an erythrocyte. Piroplasms appear in erythrocytes from day 10 post-infection. Naïve, young, pregnant or immune-compromised animals may develop severe anaemia and mortalities can be as high as 30 per cent in a herd.



Example only.
Not a diagnostic resource

Haemaphysalis longicornis **(common bush tick)**



Ticks are vectors for many protozoal and mycoplasmal parasites. For *Theileria* spp. transmission in the tick is known to be trans-stadial or life stage-to-life stage. A larva or nymph stage tick transmits the parasite to the next animal it feeds on. Trans-ovarial transmission (transmission from infected females to their larvae) does not occur. Control of ticks and good sanitation when using needles and surgical equipment minimises the introduction of these infectious parasites into naïve populations.

Disease investigation sampling guide: lead exposure and toxicosis

Lead is highly toxic, particularly in cattle and lead toxicosis is a clinical condition still noted throughout Western Australia. In addition to animal health concerns, lead residues in livestock and livestock products pose risk to human health and to Western Australia's ongoing access to international markets.

Clinical suspicion

Clinical suspicion is based on clinical signs and/or evidence of access to lead sources. Young animals are more susceptible than adults and commonly present with acute clinical signs. Adult animals typically present with a subacute syndrome. Chronic lead poisoning is less common. Some animals may be lead affected without displaying clinical signs.

Clinical signs

Nervous signs

- Blindness
- Ataxia
- Salivation
- Head pressing
- Convulsions
- Tremors
- Death

Gastrointestinal signs

- Anorexia
- Constipation
- Diarrhoea

Other signs

- Anaemia
- Sudden Death

Common on-farm lead sources

- Lead batteries
- Painted surfaces
- Paint tins
- Sump oil
- Grease or oil filters
- Linoleum
- Caulking or putty

If you are concerned about lead toxicosis, please follow the sample sets on the next page.

Sample type	Sample requirements
Antemortem samples	
Blood	EDTA: 10 ml whole blood per animal (preferred) or Lithium Heparin: 10 ml whole blood per animal, not separated. **Other tube types like plain clotted and SST or plasma/serum are not suitable.**
Milk	10 ml per animal
Postmortem samples	
Liver	50 g Fresh
Kidney	50 g kidney – Fresh
Bone	5 cm distal rib – Fresh Clean bone before submission
Lead particulate matter	Check rumen, reticulum or omasum
Environmental samples (if present)	
Paint – Scrapings, flakes, or power	Approximately 5 cm^2
Liquids* – Waters	Minimum 10 ml. **Note: Oil and grease are not accepted for testing**
Feeds and mineral mixes	Minimum 100 g
Soil*	Minimum 10 g

*Tested at external laboratory

Post-mortem guide

This is a visual guide to a thorough ruminant post-mortem examination. This guide was developed by the Department of Primary Industries and Regional Development (DPIRD) to assist veterinarians in safely performing a ruminant post-mortem and collecting diagnostic samples. Using correct post-mortem sampling techniques will increase the likelihood of a definitive diagnosis in disease investigations. Please refer to document **'A visual guide to diagnostic sampling'** for specific sample requirements.

Post-mortem approach

Observe, describe and photograph all lesions found during the postmortem. Where possible, sample transitional zones from healthy to diseased tissue for histopathology.

Equipment required:

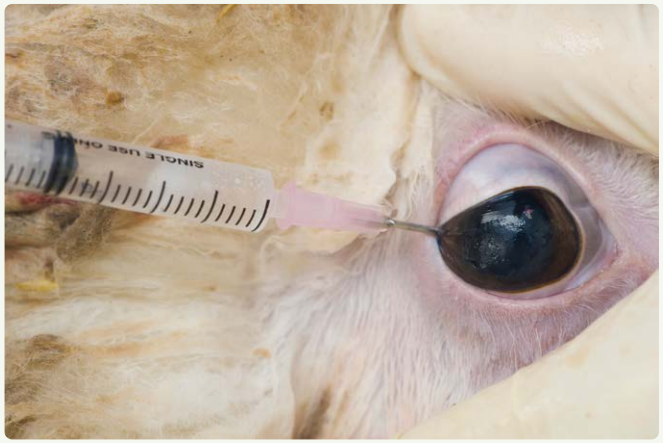
- Standard postmortem kit including boning and skinning knives, scissors, scalpel blades, forceps, laboratory submission forms, permanent markers
- Dry swabs, swabs in media, specimen jars for individual fresh samples, large containers for pooled formalin fixed samples
- Large pruning secateurs to cut through ribs and jaw
- Bone saw, small axe and hammer for brain removal
- Personal protection equipment including overalls, gloves, mask, knee protectors.

Laboratory samples

Base sample set

Blood (fresh and fixed samples is sufficient to diagnose common endemic diseases present in Western Australia.

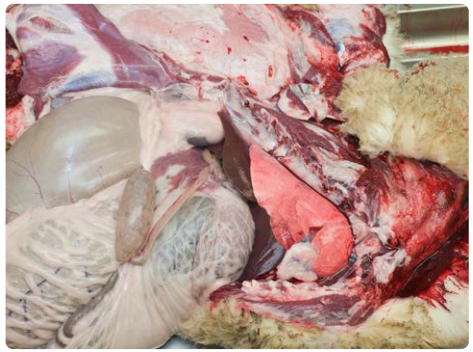
Collection from recently dead animals (< 24 hours)



Collect vitreous humour from the posterior chamber of the eye. Collect 1.5-2ml in a 2ml serum tube and freeze.

Post-mortem procedure (page 1 of 4)

Step 1:



Expose the thorax and abdomen

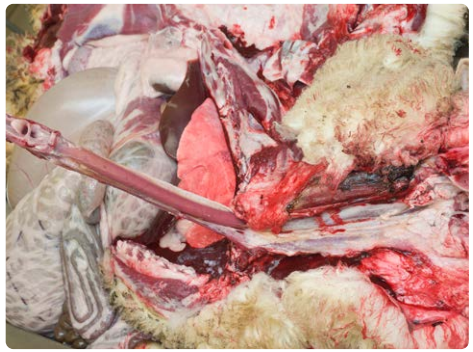
Lay the animal in left lateral recumbency. Reflect the right forelimb and hindlimb. Carefully incise abdominal muscles to expose the abdominal organs without rupturing the intestine or forestomachs. Using rib cutters, cut the rib cage along the ventral and dorsal aspects to expose the thoracic contents

Step 2:

Expose the oral cavity

Extend the incision up the neck to the chin. Using rib cutters, cut through the mandibular symphysis to expose the oral cavity. Check for lesions

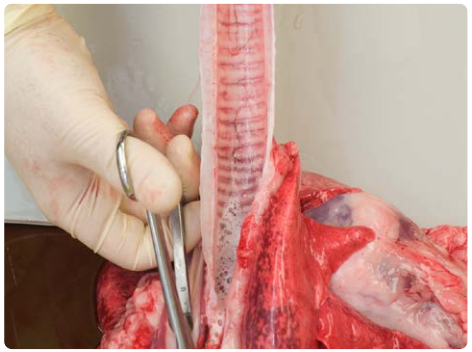
Step 3:



Dissect the neck

Hold the tongue and dissect through the hyoid apparatus to release the pharynx and larynx. Continue dissecting the oesophagus and trachea down to the thoracic inlet

Step 4:

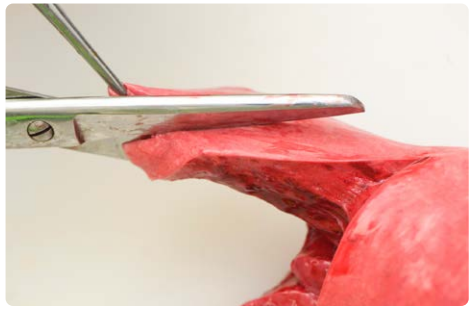


Open the trachea

Using scissors, cut down the trachea extending along the major brochi into the lungs. Check for the presence of lung worms

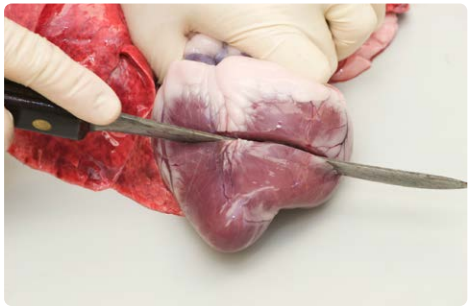
Post-mortem procedure (page 2 of 3)

Step 5:



Sample the lungs

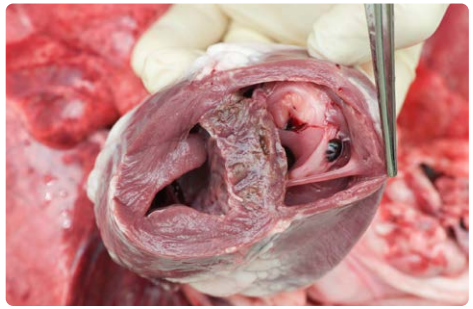
Step 6a:



Dissect the heart

Make two transverse sections (1 cm apart) two-thirds of the distance from the apex of the heart

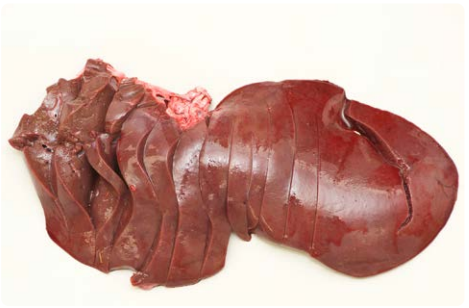
Step 6b:



Dissect the heart

Examine the A-V valves through the exposed ventricles. Sample the heart

Step 7:



Dissect and sample the liver

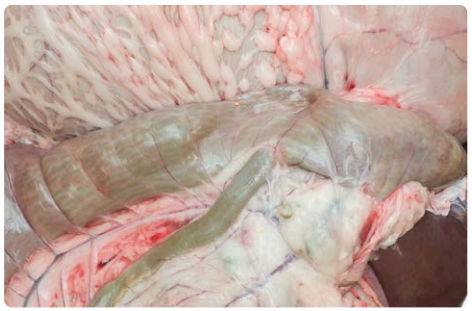
Make multiple slices through the liver to detect any lesions not grossly visible. Sample the liver

Step 8a:

Dissect the intestines

Sample the duodenum and jejunum

Step 8b:



Dissect the intestines

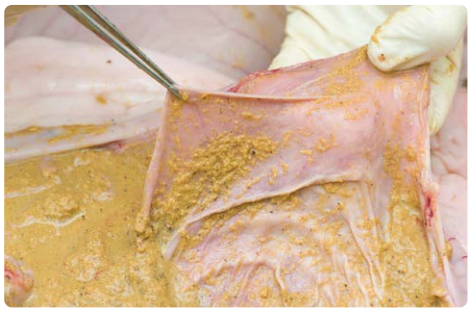
Expose the ileo-caecal junction by lifting the small intestines over the dorsal aspect of the carcase. Sample the ileum and ileal contents

Step 8c:

Dissect the intestines

Sample the caecum and colon

Step 9a:



Examine the abomasum

Examine the abomasum for the presence of Haemonchus parasites, hyperplasia or nodular changes. Sample the abomasal tissue

Post-mortem procedure (page 3 of 3)

Step 9b:

Examine and sample the rumen

Test rumen pH (Normal 5.5-7.0). Examine the contents for intact leaves of poisonous plants. If ARGT is suspected, sample rumen fluid

Step 9c:

Examine and sample the forestomach

Empty the forestomachs and examine the ventral rumen mucosa for evidence of rumenitis. The ventral pillars are often affected. Sample the rumen

Step 10:

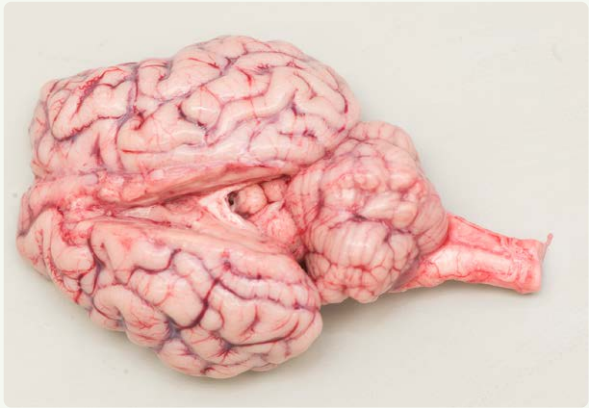
Examine and sample the kidneys

Step 11:

Examine and sample the muscles

Slice hindlimb musculature looking for areas of pallor suggestive of nutritional myopathy. Sample the skeletal musculature

Step 12:



In some circumstances, the brain and spinal cord are required for diagnostic purposes. Refer to the brain removal techniques section of this guide.

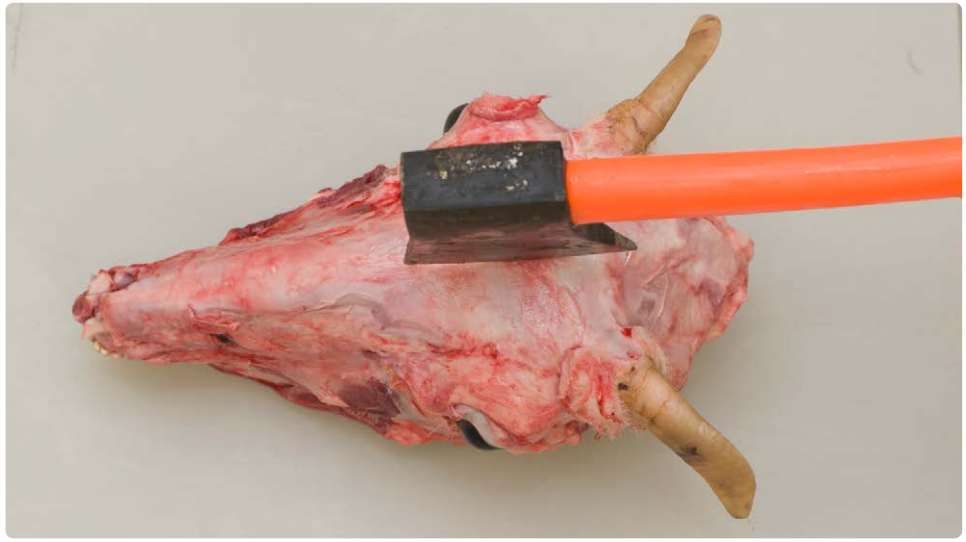
Brain removal techniques

This is a visual guide of two brain removal techniques that are fast and easy to perform in the field or laboratory. This guide helps you in safely removing brain samples that are suitable for routine diagnostic and transmissible spongiform encephalopathy (TSE) exclusion testing. Using correct brain sampling techniques will increase the likelihood of a definitive diagnosis in disease investigations. Always use PPE.

If you suspect an exotic or reportable disease, contact the EAD hotline on 1800 675 888, your local Field Veterinary Officer, or our Diagnostics and Laboratory Services Duty Pathologist on (08) 9368 3351.

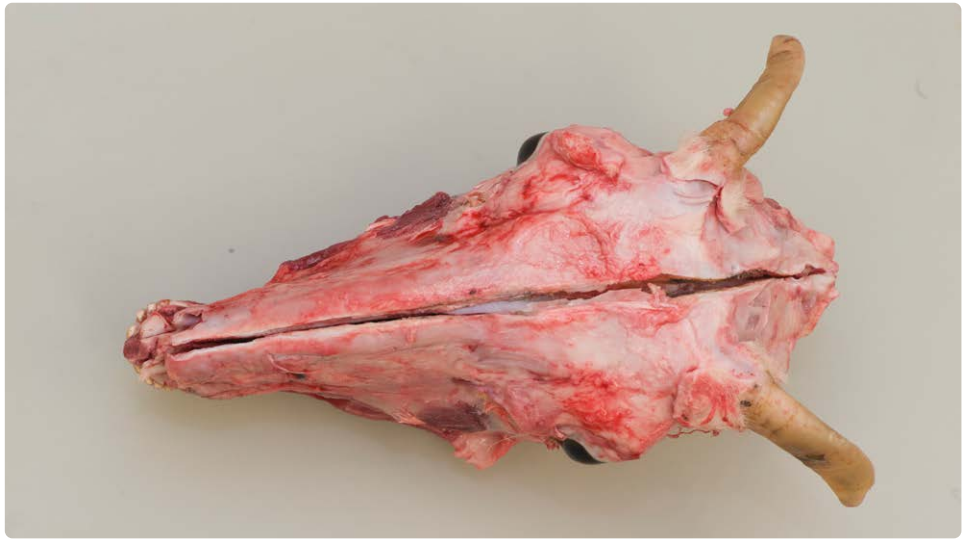
Method 1: Longitudinal craniotomy

Step 1:



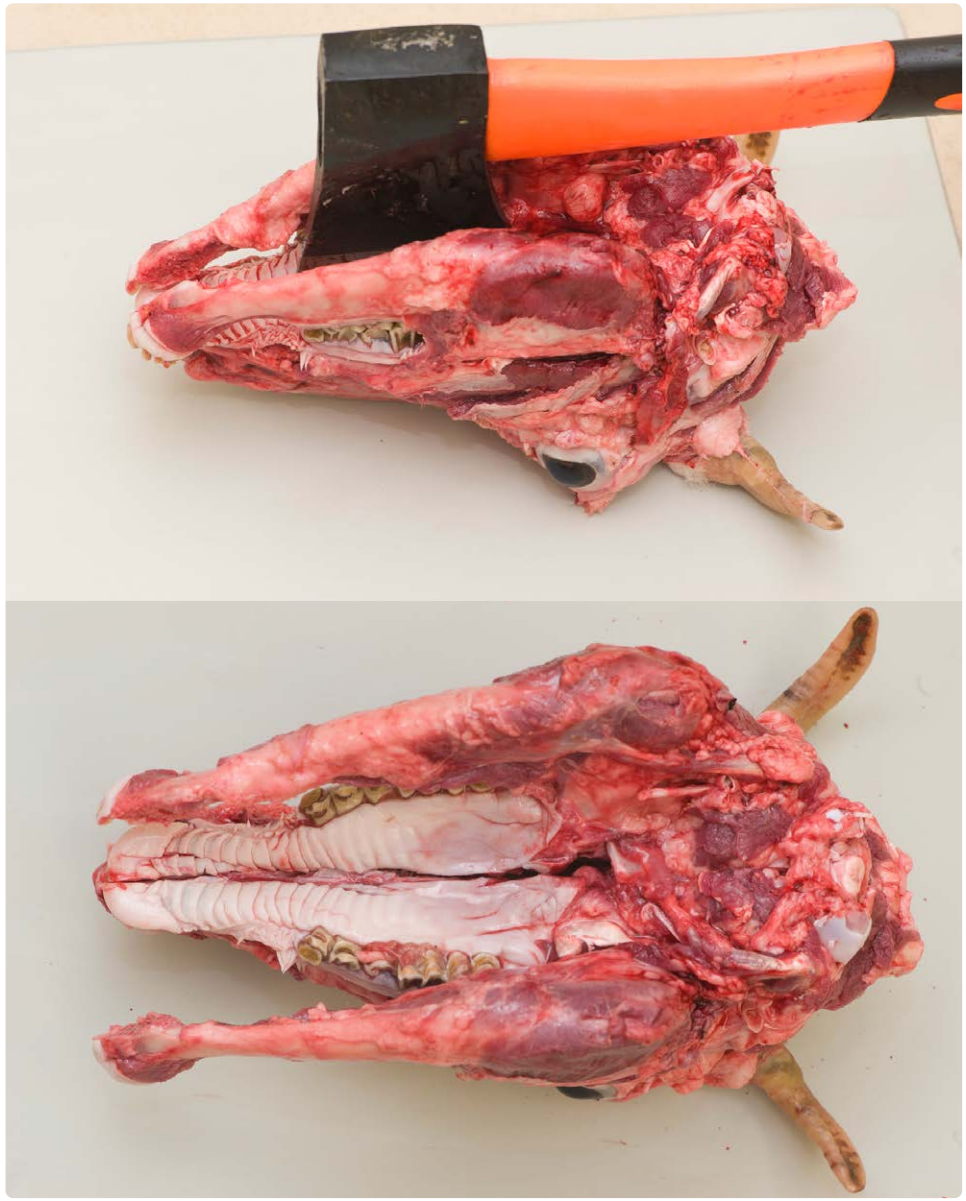
Remove the head at the atlanto-axial joint. Remove the tongue and pharynx to expose the hard and soft palate. Split the mandibular symphysis and place the head on a non-slip surface such as a rubber mat or onto the ground, using the separated mandible to improve stability.

Step 2:



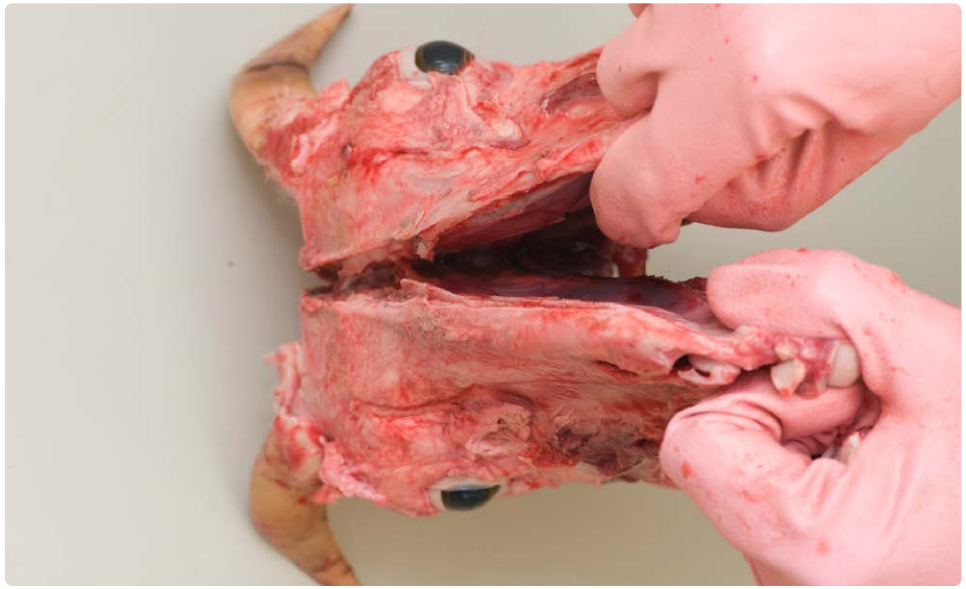
Using a hatchet and a mallet, make a dorsal midline cut through the bone from the nose to the foramen magnum. Place hatchet blade at cutting site and use mallet to split the bone. Do not swing the hatchet. The head does not need to be skinned, but making an incision through the skin along the proposed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

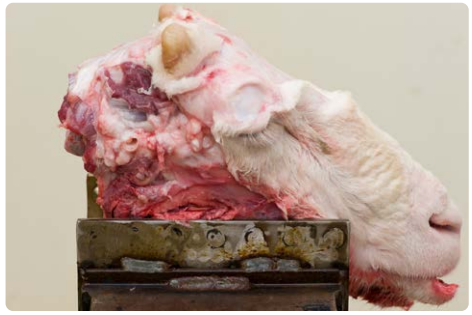


Stand the head upright on the foramen magnum and use a knife to sever any remaining attachments. Lever the nasal bones apart, using the hatchet to increase leverage and split the skull. If nasal bones feel as if they will snap, check all the bone is cut, especially between the occipital condyles.

Method 2: Transverse craniotomy (not advised for TSE sampling)

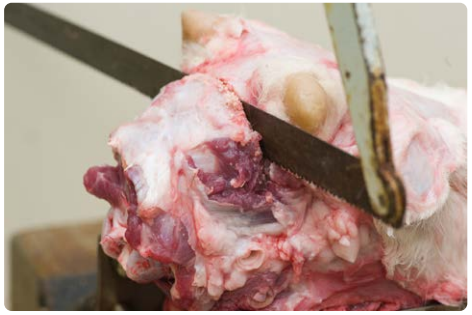
When using the transverse craniotomy technique in **cattle**, it is advisable to leave the head attached and immobilise for sawing by tensioning nose grips to a vehicle or other solid object. Alternatively, flex the neck and tie the nose grips to the hock. In small ruminants, removing the head is recommended.

Step 1:



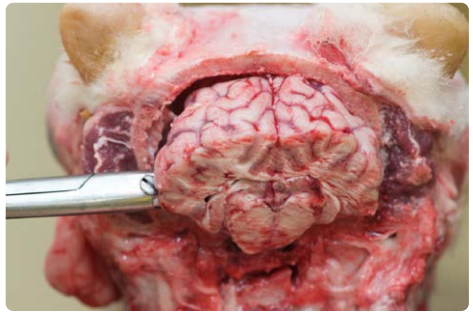
Stabilise the head for sawing by placing it on a non-slip surface or mat. The head does not need to be skinned, but a knife cut through the skin at the intended line of sawing is recommended.

Step 2:



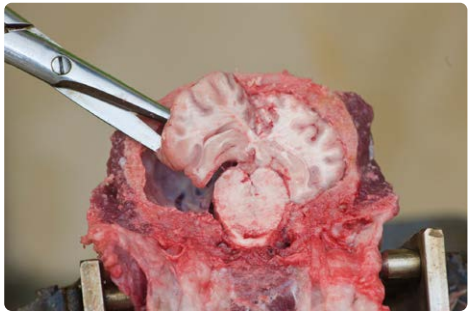
Begin sawing vertically in a line 1 cm rostral to external ear canal and extend the cut through the bone until the cranium hinges apart. Remove the head at the atlanto-occipital joint if still attached.

Step 3:



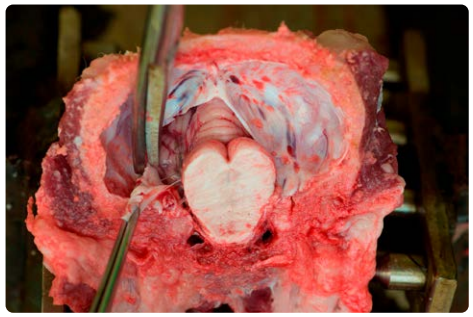
Begin with the rostral half of the skull. Using curved scissors, cut the ventral nerve roots and the olfactory bulbs. Remove the rostral brain.

Step 4:



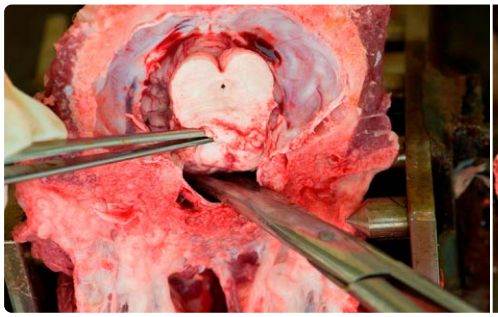
Shell out the occipital lobes in the caudal half of the skull to expose the opaque tentorium cerebelli.

Step 5:

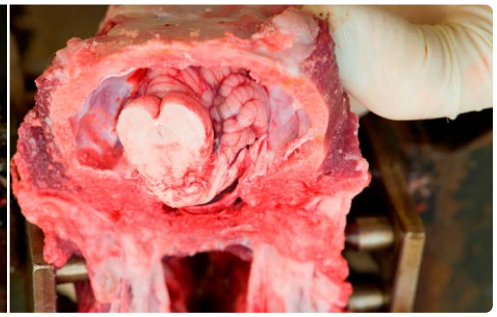


Cut the tentorium to expose as much of the cerebellum as possible.

Step 6:

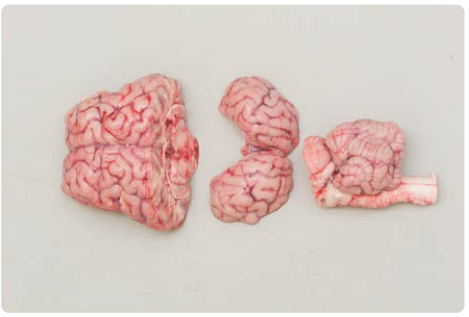


Using scissors, cut the cranial nerves on the ventral and lateral surfaces inside the cranium and then around the cut spinal cord at the foramen magnum.



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 2-3 cm spinal cord and submit these sections fresh. Fix remainder of brain in 10% buffered formalin.

Brain sampling for TSE exclusion

Brain samples required for the National TSE Surveillance Program include:

Sheep:

1. fresh dorsal cerebellum
2. fresh spinal cord, 2–3 cm in length
3. fix the rest of the brain and brainstem whole.
4. Optional: small section of 1 frontal lobe fresh sample

Cattle:

1. fresh spinal cord, 2–3 cm in length
2. fix the rest of the brain and brainstem whole.
3. Optional: small section of 1 frontal lobe fresh sample

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 1 L pot filled to the top with formalin
  - cattle brain – use a 2 L pot filled to the top with formalin.
- Allow the brain to fix in the formalin pot at room temperature.

Check case meets TSE criteria (listed on TSE lab submission form). Eligible case must have two or more of the presenting clinical criteria, and fall within eligible age criteria:

- Cattle > 30 months, < 9 years.
- Sheep > 18 months, preferably < 5 years.

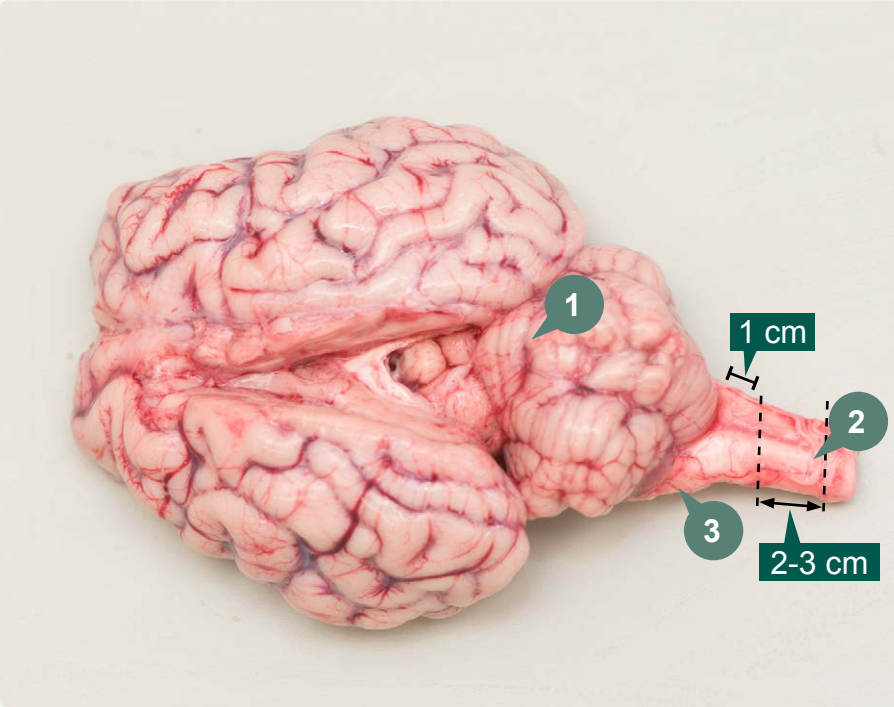


Figure 1 Collection of fresh brain TSE samples

1. Fresh dorsal cerebellum (no more than dorsal one-third of cerebellum) – sheep only
2. Fresh spinal cord, 2–3 cm in length – cattle and sheep
3. Take care not to damage the obex. Leave intact and fix with the whole brain and brainstem.

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.

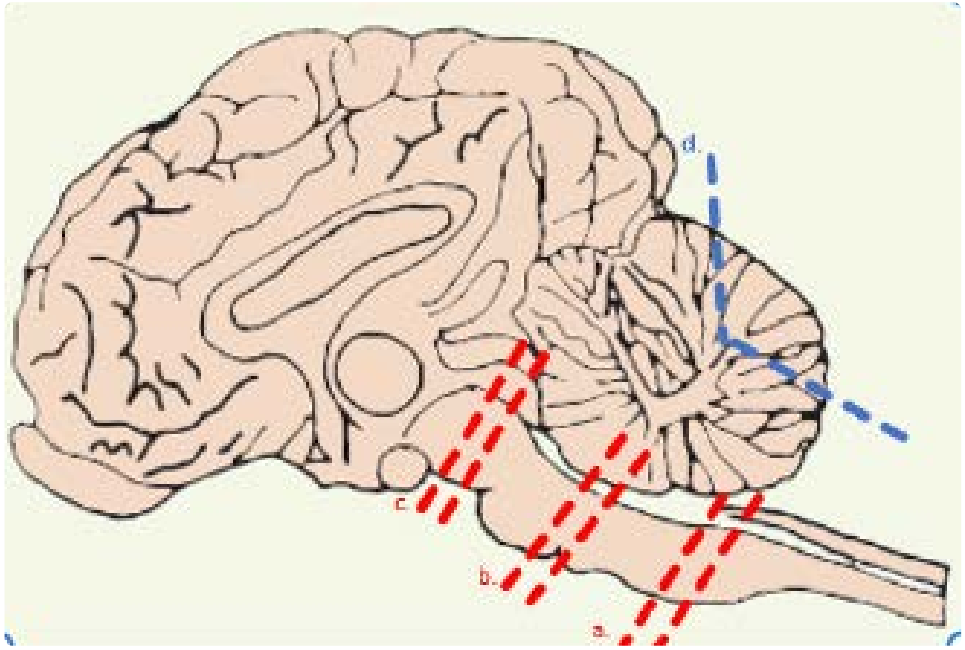
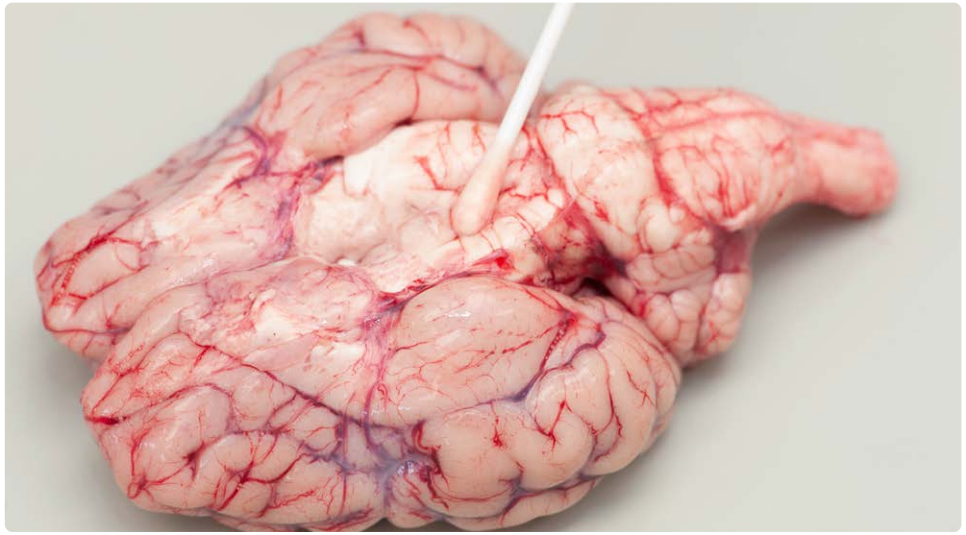


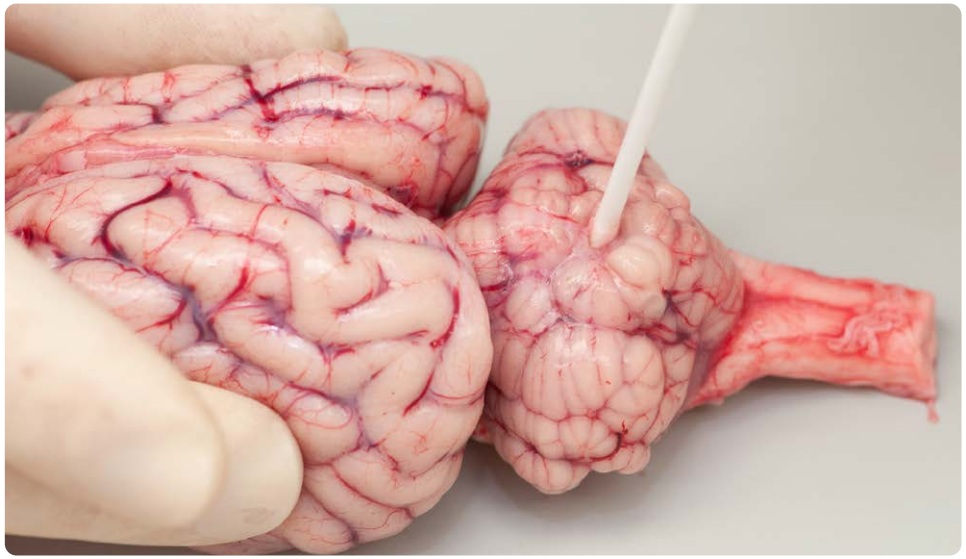
Figure 2 Key regions for TSE exclusion.

a. Obex region
b. Caudal cerebellar peduncles
c. Midbrain through rostral colliculi
d. Dorsal cerebellum, **sheep only**.

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 inserted vertically to minimize damage to the TSE sites.

Sampling for Annual Rye Grass Toxicity (ARGT)

Clinical signs of ARGT

- Neurological signs exacerbated by stress/movement
- Horses may stop eating, have muscle tremors or convulse
- Uncoordinated movements
- Animals may fall over
- Convulsions are strong muscular movements in fallen stock
- Pregnant stock may abort

Testing for ARGT

Sample type	Sample requirements
Rumen fluid	50mL of rumen fluid in jar fresh
Faeces	50g of faeces in pot fresh
Hay	1kg of representative hay sample
Grain	1kg of representative grain sample
Pasture	Min 100g of randomly collect rye grass sample from paddock

Submission of samples

The faeces and rumen content should be kept chilled and submitted in separate labelled, sealed containers.

The hay/grain or pasture should be submitted in a clearly labelled, well sealed plastic bags with sample identification, submitters name and contact details.

All samples should be submitted to the administration DPIRD 3 Baron Hay Crt Kensington for DPIRD diagnostic laboratory services.

Sampling for Toxic Algae

Clinical signs of toxic algae poisoning

- Difficulty breathing
- Muscular weakness
- Paralysis
- Sudden death
- Rapid carcass deterioration

Water sampling procedure

Note: Some toxic algae can be zoonotic so take care and wear gloves

Toxic algal blooms tend to be on the surface of the water and can look like a paint or oil film

- Collect water from the surface in an area where the bloom is visible (if present) in watertight plastic or glass bottle
- 100mL is sufficient up to 500mL is suitable
- Allow some air at the top of the bottle
- Thoroughly wash the outside of the bottle

Submission of samples

- Label bottle with water source and submitter name and contact number
- Keep sample chilled at less than 4˚C
- Do not freeze sample
- All samples should be submitted to the administration DPIRD 3 Baron Hay Crt Kensington for DPIRD diagnostic laboratory services

Veterinary sample packaging guide

This guide has been developed to assist you in safely packaging biological samples for transport and laboratory submission.

1

- Please ensure all labelling is legible.
- Clean jars of all external contamination.
- Faecal samples are to be packaged in a screw top container.

2

- Check the lids are tightly sealed. Please tape around the lids to reduce leaks.

3

- Remove all needles, scalpels and gloves.
- Submit fluid samples in sterile jars or plain tubes.

4

- Submit blood tubes in foam racks wrapped in plastic film or held together with rubber bands. Wrap single blood tubes in absorbent padding.

5

- Place slides in a slide transport box and seal these in a ziplock bag. This prevents formalin fumes causing cellular artefacts.

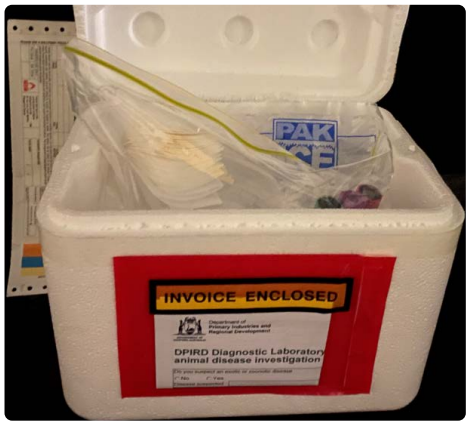
6

- Use absorbent material (paper, cotton wool) to line the esky in case of leaks. Note: rigid containers such as eskies are required

7

- Place all samples in the esky with cool packs or ice bricks. Do not place samples directly onto ice bricks as this can damage samples.

8



- Seal the esky with tape.
- Place the laboratory submission form in an envelope or zip locked bag and tape to the side of the esky

9

- Place the courier label on the top of the esky and notify the courier of sample collection requirements

Contact the DPIRD Diagnostics and Laboratory Services (DDLS) duty pathologist on 08 9368 3351 or your local field veterinary officer to discuss the case, sampling and charge exemptions.

If you suspect an exotic, or reportable disease, contact EAD hotline on 1800 675 888, your local Field Veterinary Officer or DPIRD Diagnostics and Laboratory Services (DDLS) Duty Pathologist on 08 9368 3351. Always use personal protective equipment including gloves when handling biological samples and practice good hygiene.

PO014-25 AUGUST 2025