Making smears for tick fever diagnosis

Tick fever or red water is caused by a group of parasites that are transmitted by the cattle tick. Tick fever parasites invade red blood cells and multiply, causing disease and in some cases killing stock. These parasites can be found and identified in a very small drop of **capillary** blood. When spread thinly as a smear on a microscope slide this blood can be stained, examined under a microscope and a diagnosis reported soon after receipt of specimens.

Other diseases can produce symptoms like tick fever. Examination of good quality blood smears is the best way to make a positive diagnosis of tick fever. You can then be advised on treatment and management strategies to control the disease and best protect the cattle at risk.

When to take smears

Take smears if tick fever is suspected or when animals show some of the following signs:

(1) Lethargy, depression, loss of appetite, weakness, reluctance to move from shade, fast respiration, muscle stiffness, manic behaviour.

(2) Any sign of fever – a healthy animal will have a morning temperature of around about 38.5°C; but expect higher temperatures in stock that have been standing in the sun or have been recently mustered. In general, if the temperature exceeds 40°C, it could be a fever.

(3) Red urine.

(4) Anaemia (pale membranes in the eye, mouth or vulva) or jaundice (yellow membranes).

Also take smears if you suspect tick fever in dead animals, or when carrying out post mortem examination, to confirm or exclude the presence of tick fever.

It is preferable to enlist the services of a veterinarian to investigate the cause of disease and/or death. However, if you can't access veterinary assistance it is well worthwhile making smears yourself to aid in a diagnosis. Care should be exercised when taking organ samples from dead animals. Wear gloves to avoid risk of infection and wash hands with disinfectant afterwards.

What you will need

- Clean glass microscope slides (frosted-ended ones are preferred as can label with pencil)
- A needle or pricker
- Tissue or paper towel
- Marking pen or pencil to identify animal or specimen
- Stiff cardboard to protect slides
- Protective packaging for mailing eg, a POSTpak® padded bag – do not send glass slides in plain envelopes as the mechanical sorting process will cause them to break.

How to make a thin blood smear

The tip of the tail is the best site for collecting capillary blood. **Venous or arterial blood is much less useful for diagnosis.** Clip the hair from the tail tip or fold the brush to expose the skin. Rub off any dirt or scurf.

FIGURE 1: With a milking action pull thumb and forefinger down the tail towards the tip. Holding this pressure, prick the tail tip using a blood lancet or needle and wait for a drop of blood to well up. Collect this drop of blood by touching the corner of a glass slide to it (this is called the *PUSHER* slide).

FIGURE 2: Place a clean slide on a flat rigid surface. Transfer a spot of the blood collected on the corner of the *PUSHER* slide to one end of this slide.

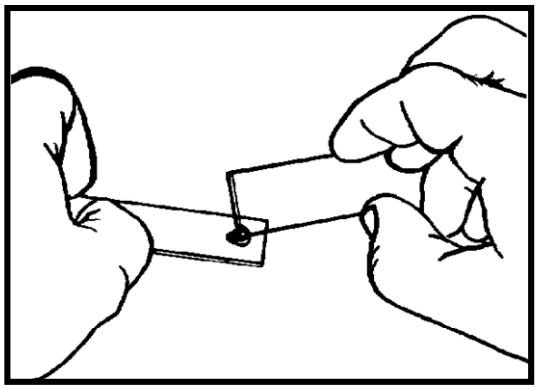


FIGURE 3: Wipe the end of the *PUSHER* slide until clean and dry.

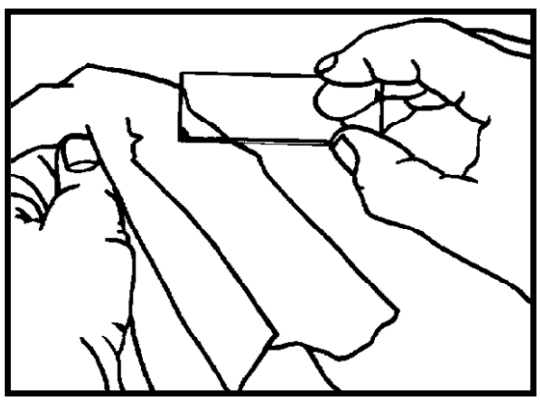


FIGURE 4: Holding the *PUSHER* slide at a 30° angle draw it back along the slide until the back bottom edge contacts the drop of blood.

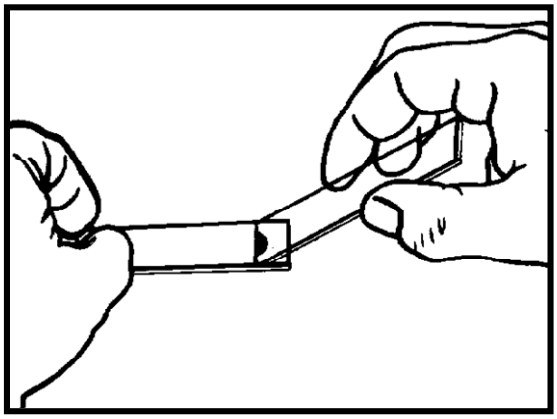


FIGURE 5: After the blood spreads along the back bottom edge of the PUSHER slide, move it smoothly forward in one quick movement. This should spread an even bullet shaped film of blood onto the specimen slide. Air dry the smear as rapidly as possible.

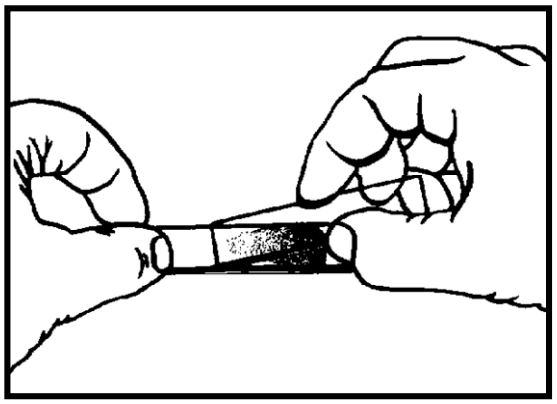
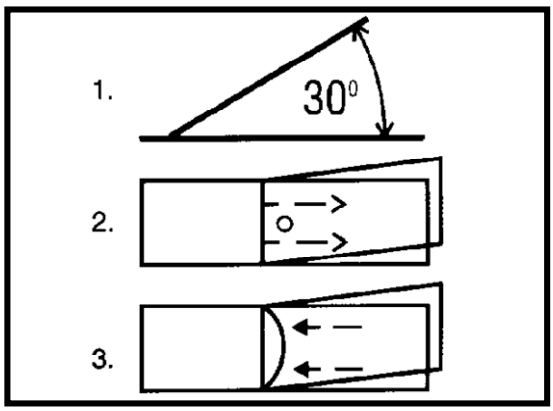


FIGURE 6: This shows the angle to hold, and the direction to move the *PUSHER* slide when making the blood smear on the specimen slide.



Thick smears

It is helpful for diagnosis to supply another spot of blood on an additional slide (e.g. **FIGURE 2**). This time, before it dries, use the corner of the *PUSHER* slide in a circular motion to spread the spot about 5-10mm in diameter, and thin enough to just see the hands of a watch through it. **Make sure it is thoroughly dry** before placing in a slide mailer or wrapping in tissue.

Smears from dead animals

A diagnosis can also be made from animals which have been dead up to 24 hours by taking blood and organ smears. Blood smears are more useful for the diagnosis of *Anaplasma* and *Babesia bigemina* and can either be made from unclotted blood in a vein, artery or heart, or can be made from blood squeezed out of cuts made in organs or muscles. Organ impression smears from dead animals, however, are a very useful aid to the diagnosis, particularly of *Babesia bovis*.

Selecting organs for smears

Organ smears are best made from freshly dead animals and should be made from brain, kidney, spleen, heart muscle and liver. Brain is particularly useful and should be collected from all dead animals (that is, freshly dead or even after 24 hours).

How to make visceral organ smears

1. Make a fresh cut in the organ (kidney, heart muscle, liver, spleen)
2. Squeeze out a little fresh blood and prepare a smear as for thin blood smear (as above); or if there is not enough blood ooze to prepare a thin smear, make impression smears by lightly applying the freshly cut surface of a piece of organ to the surface of the slide in several places.
3. Dry the slide by either waving it in the air or, if conditions are cold or wet, gently heat over a flame (for example, a lighted match) or use a car heater.
4. Identify the slide, and once dry, follow instructions below for packing and transport.

How to make brain smears

A brain smear is the most important sample to assist with the diagnosis of *Babesia bovis* in dead animals. *Babesia bovis* is the cause of most tick fever outbreaks, so time spent collecting the brain sample is time well spent.

Brain Collection Procedure 1 – *useful if collecting whole brain for other reasons.*

1. Remove the top of the skull using an axe or similar tool, or split the skull in two lengthwise.
2. Using scissors or a scalpel blade, slice off a tiny piece of grey matter from the cerebrum. This is the grey coloured layer (under the tough membrane covering the brain) over most of the two large cerebral hemispheres.

Brain Collection Procedure 2 – *particularly useful if you suspect Tick Fever but do not need to collect brain tissue for any other purpose, as only requires a small hole to be made in the skull.*

1. Drive a large nail or drill a hole through the skull into the brain, just off to one side of the midline in the flat ‘forehead’ portion of the skull.
2. Use a large needle and syringe to extract a sample of grey matter by inserting the needle through the hole into the brain. Do not insert too deep or it will penetrate the blood vessel free white matter under the grey matter.
3. Pull back on plunger to suck tissue into the syringe/needle hub. This tissue can then be expelled onto a clean glass slide.

Brain Smear Preparation Procedure

1. Place a piece of grey matter (about the size of a match-head) about 2cm from the end of the specimen slide.

2. Take a second slide and lay it on top of the grey matter, but with the long axis at right angles to the first.

3. Press down and move the top slide sideways to the far end of the lower slide (this crushes the brain tissue and spreads it thinly).



4. Dry slide by either waving it in the air or by warming if conditions are cold or wet. Do not stick slides together or apply any coverslip as smears will be unsuitable for examination.

Identify slide, and once dry, follow instructions below for packing and transport.

Hints for better diagnostic results

(1) Collect only clean blood from the tail tip. Avoid contamination with dirt, water, scurf, faeces or urine.

(2) Spread the drop of blood before it starts to clot. Thin is better than thick.

(3) Rapidly dry slide (eg by waving in the air).

(4) Do not stick wet slides together and do not apply coverslips.

(5) Keep hands off the surface of the specimen slide.

(6) Keep specimen slides clean and dry and protect from flies.

(7) Do not refrigerate prepared smears.

(8) Smears which have been exposed to formalin or formalin fumes cannot be used for parasite identification. Please keep formalin away from smears.

Transporting slides

Pack slides separately in a slide mailer once completely dry.

Slides can also be individually wrapped in tissue once dry and then packed between 2 sheets of cardboard and place in a padded mail bag so they do not break in transit.

If more than one slide is being submitted, animal identification can be written on the frosted surface of the slide with a pencil.

Specimen advice sheet

Complete all sections of a specimen advice sheet (including clinical signs) as the information provided here will assist in making a diagnosis. Details of age, breed, numbers affected, vaccination history and whether the animals are home bred or introduced are particularly important; as well as a description of the clinical signs.

Where to send smears

Specimen Receipt, Biosecurity Sciences Laboratory

Loading Dock 12

39 Kessels Rd,

Coopers Plains Qld 4108

Telephone: 07 3276 6062

Facsimile: 07 3216 6620

Further information

For further information on tick fever and its diagnosis, contact:

Tick Fever Centre

280 Grindle Road,

Wacol Qld 4076

Telephone: 07 3270 9600

Facsimile: 07 3270 9685

Email: tfc@daf.qld.gov.au

Website: www.business.qld.gov.au (search for 'tick fever')