

Making blood and organ smears for tick fever diagnosis

In Australia, tick fever is caused by a group of three parasites that are transmitted to cattle by the cattle tick. Tick fever parasites invade red blood cells and multiply, causing sickness and in some cases killing stock. These parasites can be found and identified in a very small drop of capillary blood from the tail tip in live animals. Examination of blood and/or organ smears can also be a valuable aid to post mortem diagnosis in animals that have died up to about 24 hours previously.

Red blood cells obtained from the capillaries or tissues are stained, examined under a microscope and a diagnosis reported within 24 hours of receipt of specimens. Since other diseases can produce symptoms like tick fever, a definite diagnosis allows, Biosecurity Queensland staff to advise on management strategies to deal with the current outbreak, avoid recurrence of the disease and protect the cattle at risk with an appropriate vaccination protocol.

When to take smears

Take smears if you suspect tick fever and 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 (101.5°F). If it exceeds 40°C (104°F), this is considered a fever. Expect higher temperatures for stock that have been standing in the sun or recently mustered.
3. Red urine
4. Anaemia (pale tissues) or jaundice (yellowing of tissues)

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 a disinfectant solution after taking samples.

What you will need

Smear making kits are available from TFC and most Biosecurity Queensland Offices within the cattle tick infested area of Queensland. Alternatively you will need:

- Clean glass microscope slides
- A needle or pricker
- Tissue paper
- Indelible marking pen to identify the origin of the smear (eg Cow 234, tail tip or brain etc). Some slides have frosted ends and can be written on in pencil
- Stiff cardboard to protect slides
- Protective packaging for mailing purposes e.g. a POSTpak® Padded Bag

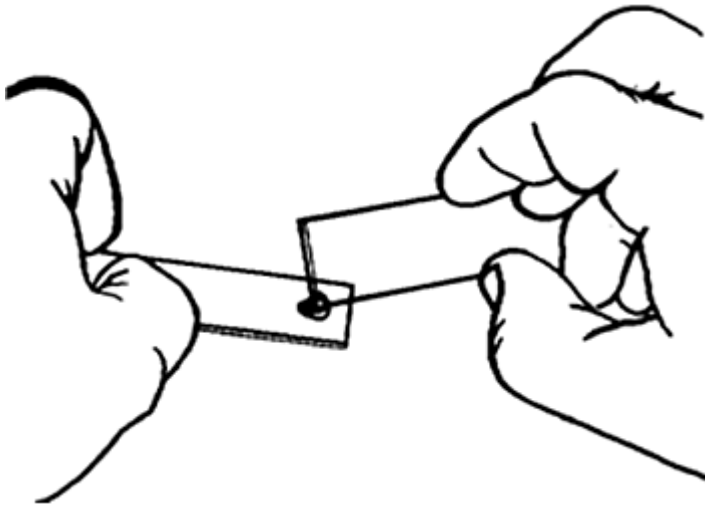
Do not send glass slides in plain envelopes as the mechanised mail sorting process will cause them to break.

How to make a thin blood smear

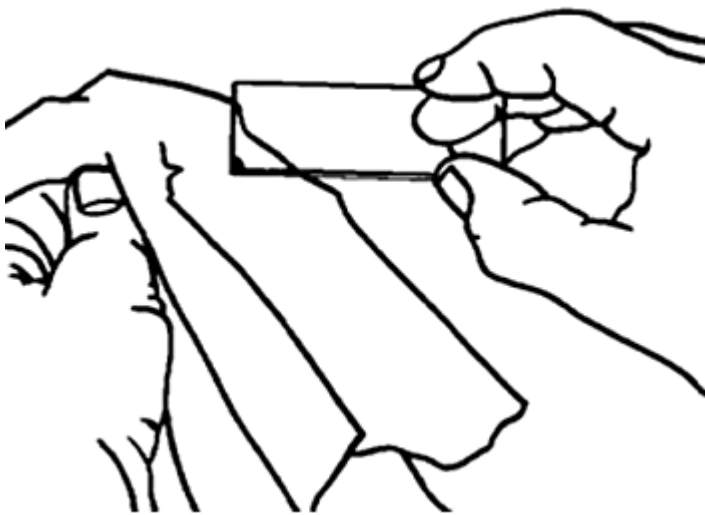
The tip of the tail is the best site for collecting capillary blood. **Venous or arterial blood is not as good for diagnosis.** Clip the hair from the tail tip or fold the brush to expose the skin. Rub off any dirt or scurf.



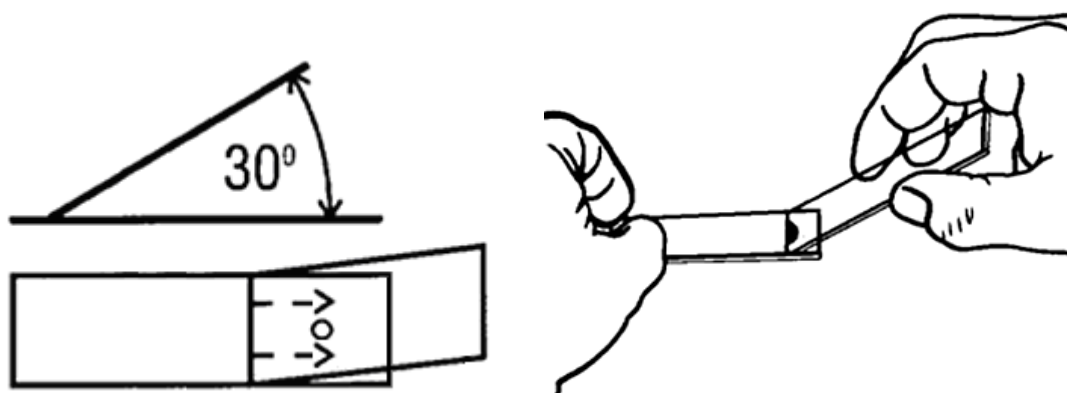
Step 1: With a milking action pull thumb and forefinger down the tail towards the tip. Holding this pressure, prick the tail tip with a needle and wait for a drop of blood to well up. Collect this drop of blood by touching the corner of a microscope slide to it. (We will call this the PUSHER slide).



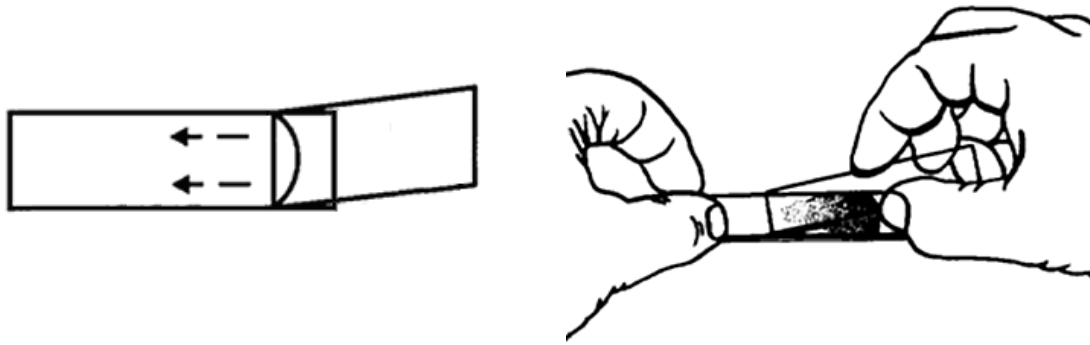
Step 2: Place a clean slide on a flat rigid surface. Transfer a spot of the blood collected on the corner of the PUSHER slide to one end of this slide.



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



Step 4: Holding the PUSHER slide at a 30° angle draw it back along the specimen slide until the bottom edge contacts the drop of blood. The drop will spread out along the edge of the pusher slide.



Step 5: Move the PUSHER slide smoothly forward in one quick movement. This should spread an even bullet shaped film of blood onto the specimen slide.

Thick smears

It is helpful for diagnosis to supply another spot of blood on an additional slide (e.g. step 2). This time, however, before the blood dries, use the corner of the PUSHER slide in a circular motion to spread the spot of blood in a circle about 5-10mm diameter, or thin enough to just see the hands of your watch through it. Make sure the smear is thoroughly dry before packing to mail (see below).

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. Do **not** apply a coverslip or allow slides to stick together, as smears will be unsuitable for examination.
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.

Collection Procedure 1 – *useful if collecting whole brain for other reasons (e.g. histology, TSE surveillance etc).*

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.

Collection Procedure 2 – *particularly useful if you suspect Tick Fever but do not need to collect brain tissue for any other purpose (this is a simpler technique as you do not need to create a large opening in the skull to remove the brain).*

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 and pulling back on plunger to ‘suck’ tissue into syringe/needle hub.

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 blood or organ smears

It is important that smears of reasonable quality are taken as it is very difficult to make a diagnosis on poor-quality smears.

- Collect only clean blood from the tail tip. Avoid contamination with dirt, water, scurf, faeces or urine.
- Keep hands off the surface of the specimen slide.
- Keep specimen slide clean and dry – both before and after preparing the smear
- Spread the drop of blood before it starts to clot.
- Cells in the smear can lyse (rupture) when smears take too long to dry or are affected by moisture or condensation. Therefore dry smears as rapidly as possible and do not refrigerate.
- Grey matter has numerous capillaries and is the target when making brain smears. When using the core sampling technique adjust the needle depth accordingly to capture this tissue.
- Smears which have been exposed to formalin or formalin fumes cannot be used for parasite identification. Therefore, pack smears in an airtight container if formalised tissues are also being sent.
- Protect smears from flies as they will destroy the specimen.
- Wherever possible, blood smears from dead animals should be submitted as well as organ smears. Organ smears are frequently unsuitable for diagnosis of *Anaplasma marginale* and *Babesia bigemina* infections.
- Identify the smear – slides with “frosted” ends can be easily labelled with pencil.
- Common faults include: smears are too thick; smears have been stuck together when wet; or organs are too decomposed.

Transporting slides

Allow the slide to **dry completely**, then place in a slide mailer or wrap individually in tissue.

Please do not stick slides together.

If more than one slide is being submitted, animal identification can be written on the slide with an indelible marking pen, on frosted slides with lead pencil, or on the tissue wrapping.

Pack slides in a protective case or between 2 sheets of stiff cardboard and place in a padded mail bag so they do not break in transit.

Where to send blood or organ smears

Smears can be sent to the nearest diagnostic laboratory or direct to the Tick Fever Centre for examination.

Diagnostic centres

Brisbane

Specimen Receipts, Biosecurity Sciences Laboratory
665 Fairfield Road, Yeerongpilly QLD 4105
Postal address: Locked Mail Bag 4, Moorooka QLD 4105
Telephone: 07 3362 9471
Facsimile: 07 3362 9440

Toowoomba

Specimen Receipts, Animal Disease Surveillance Laboratory
203 Tor Street, Toowoomba QLD 4350
Postal address: PO Box 102, Toowoomba QLD 4350
Telephone: 07 4688 1351
Facsimile: 07 4688 1195

Townsville

Specimen Receipts, Tropical and Aquatic Animal Health Laboratory
180-202 River boulevard, Oonoonba QLD 4811
Postal address: PO Box 1085, Townsville 4810
Telephone: 07 4722 2624
Facsimile: 07 4778 4307

Tick Fever Centre

280 Grindle Road, Wacol Qld 4076
Telephone: 07 3898 9655
International: +61 7 3898 9655
Facsimile: 07 3898 9685
International: +61 7 3898 9685
Email: tfc@deedi.qld.gov.au