



Glossary of technical terms relating to diagnostic tests for tuberculosis and Johne's disease in deer

The following are alphabetical lists of diagnostic tests for tuberculosis (Tb) and for Johne's disease in deer, and some miscellaneous terms relating to diagnostic tests.

The Animal Health Board-approved tests used for Tb diagnosis in live deer are

- the skin tests (MCT and CCT)
- blood tests (BTB, ELISA, ETB)

Tests used for Johne's disease diagnosis in live deer are

- culture by BACTEC of faecal samples (including pooled faecal culture)
- blood tests (Indirect ELISA, PARALISA)
- PCR (blood samples).

After slaughter, body tissue samples can be tested for Tb and Johne's disease by BACTEC culture, PCR and/or histology.

Other publications produced by the Johne's Research Group include:

- **JRG Information Leaflet:**
"Johne's disease in farmed deer"
Dr C G Mackintosh, Invermay AgResearch
August 2002
- **JRG Bulletin One:**
"Johne's disease in New Zealand farmed deer. What does this mean for you and your farm in 2004?"
- **JRG Bulletin Two:**
"Detained carcasses: Johne's disease lymph node lesions in slaughtered deer and their implications"
- **JRG Bulletin Three:**
"Update of current research on Johne's disease in deer"
- **JRG Bulletin Four:**
"How to manage Johne's Disease"
- **JRG Bulletin Five:**
"Research Update"
 - * *Intra-uterine transmission of Johne's disease in farmed red deer*
 - * *Is Johne's disease common in wildlife on infected farms?*
 - * *Summary of the results of on-going Australian research on Johne's disease in sheep*
 - * *Update on JD research in Australia*

Tb tests

Skin tests

MCT – Mid cervical skin test.

Cost: Usually \$2 per test if large numbers of deer tested, and \$3 to \$6 for small numbers.

Turn-around time: 3 days

Practical application: The MCT is the basic or primary Tb skin test authorised by the Animal Health Board and conducted by an accredited tester. It is used to screen herds for Tb. Farmers are contacted when it is time to test their deer. A 10cm x 10cm patch is clipped on the neck, 0.1 ml bovine tuberculin is injected into the skin and the site is examined 72 hours later for any visible or palpable increase in skin thickness. Deer with bovine Tb usually give a positive result to the MCT, but avian Tb and Johne's disease can also cause deer to react to this test (ie false positive). When deer react positively to the MCT, the accredited tester and the District Disease Control Manager will, depending on circumstances, direct the farmer to:

- kill them on the farm
- send them to Deer Slaughter Premises
- or re-test for Tb using the CCT, ETB, BTB or bacterial culture of lesions.

CCT – Comparative cervical skin test.

Cost: \$10-15 per animal.

Turn-around time: Three days (plus 90-day stand-down period following positive MCT test).

Practical application: This skin test is an 'ancillary' test, ie it is approved by the Animal Health Board (AHB) for retesting deer that have reacted to the MCT. It can also be carried out as the first or primary test in some circumstances (especially in

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herds where there is a problem with false reactions to the MCT and there is little or no Tb present). The CCT is authorised and organised by the AHB in conjunction with the accredited tester. The CCT must not be carried out within 90 days of the MCT. Two sites are prepared by closely clipping two 10cm x 10cm patches on the neck, the skin thickness is measured and recorded and 0.1 ml of avian tuberculin (0.5 mg/ml) is injected into the skin at the upper site and 0.1 ml of bovine tuberculin (1.0 mg/ml) at the lower site. After 72 hours, the skin thickness at both sites is measured. A positive CCT test is one in which there is a reaction at the bovine site (ie an increase in skin thickness) of 2 mm or more and this reaction is equal to or greater than any reaction at the avian site. The animal must be culled as a reactor. Deer with bovine Tb usually react more at the bovine site. Deer with avian Tb and Johne's disease usually react more at the avian site, and the test is therefore considered to be negative.

Blood tests

BTB – A blood test for Tb. It is a combination lymphocyte-transformation and antibody (ELISA) test, and incorporates antigens to bovine Tb and avian Tb.

Cost: Available from the Disease Research Laboratory, Otago University. (\$100/test*)

Turn-around time: 7 to 10 days.

Practical application: The BTB is approved by MAF as a primary test for Tb in deer, or as an ancillary test for retesting MCT Tb reactors. It is authorised by the Animal Health Board (AHB) and carried out 2 to 4 weeks after the MCT (skin test). A blood sample is collected in a green-top tube by the AHB-accredited Tb tester or farm veterinarian and sent to the Disease Research Laboratory, Otago University, for analysis. The sample must be tested within 48 hours of collection. The results are reported to the AHB's District Disease Control Manager and veterinarian. AHB policy requires that test-positive deer must be slaughtered. Sometimes the BTB result is reported as "equivocal" or "no data", which means the result is not clear, and the animal must either be blood sampled again within 15 working days for a further BTB, or sent for slaughter as a reactor. Often deer with JD give a false positive result to the BTB. Due to its high cost, the BTB has been largely superseded as a test for Tb by the ETB.

ELISA – An antibody-detecting blood test carried out in persistently (chronically) infected herds in deer that were negative to the MCT.

Cost: \$10 per sample.

Turn-around time: One week

Practical application: The ELISA is approved by MAF and authorised by the Animal Health Board as a parallel test on MCT-negative deer. It is most often used in persistently (chronically) infected problem herds to detect antibodies in those deer that are heavily infected but gave a false negative reaction to the MCT. It is carried out 2 to 4 weeks after the MCT. Blood samples are collected by an accredited tester or veterinarian in red-top tubes from deer that were negative to the recent MCT, and the test is carried out at the Disease Research Laboratory, Otago University. Deer that give a positive result to the ELISA must be slaughtered. As with the ETB and BTB, deer with JD can give false positive results with the ELISA, and so they must be killed.

ETB – An antibody blood test for Tb that detects a subclass of antibody (IgG1) resulting in improved sensitivity and specificity for Tb diagnosis in deer.

Cost: \$30*

Turn-around time: One week

Practical application: The relatively new ETB has been given interim approval by MAF and is authorised by the Animal Health Board (AHB) as an ancillary test to retest MCT reactors. It is carried out 2 to 4 weeks after the MCT. It has similar sensitivity and specificity to the BTB, but is quicker and cheaper. Blood samples (red-top tube) are taken by an accredited Tb tester or by a veterinarian for testing at the Disease Research Laboratory, Otago University. The ETB includes specific antigens for bovine and avian Tb and the official result is determined by the relative responses to these two antigens. Although it is not an official part of the test, the laboratory also checks for Johne's reactivity, as this can provide extra information about why an animal is a reactor. AHB policy is that if the bovine response is greater than the avian response, irrespective of the Johne's response, the animal must be culled as a reactor to preserve the integrity of the National Tb Scheme.

Johne's disease tests

Culture

BACTEC culture – The BACTEC method uses liquid culture in bottles that are placed in a BACTEC machine that monitors the growth of microorganisms. Tb and Johne's disease organisms tend to be very slow-growing, but the BACTEC system gives results much more quickly than earlier culture methods that used solid media and could take up to 16 weeks.

Cost: \$45 per sample

Turn-around time: 3 to 8 weeks

Practical application: BACTEC culture is a sensitive and specific means of testing for Tb and/or Johne's disease in samples from intestine, lymph nodes or lesions taken after slaughter on the farm. Fresh samples of Tb/Johne's-like lesions can also be collected at the Deer Slaughter Premises by the meat inspector or veterinarian and submitted to the diagnostic laboratory, along with a preserved sample for histology. If the histologist at the laboratory is unsure of the cause of the lesion, the fresh sample is sent to Wallaceville for BACTEC culture. BACTEC culture can also be used to test for Johne's disease in faecal samples from live deer, but this is less sensitive than the culture of tissue because not all infected deer shed organisms in their faeces.

PFC – Pooled faecal culture (PFC) involves taking faecal samples from up to 25 deer, mixing and taking a sub-sample for BACTEC culture.

Cost: \$75 for 10 – 25 samples (pooled)

Turn-around time: 3 to 8 weeks

Practical application: The PFC is useful for screening groups of deer for Johne's infection cost-effectively.

Blood tests

Indirect ELISA – This blood test has been developed by Gribbles' Laboratory, Palmerston North, for the diagnosis of Johne's disease in sheep, cattle and deer.

Cost: \$10

Turn-around time: 48 hr

Practical application: The Indirect ELISA is carried out on blood samples collected in a red-top tube. It is a useful test for Johne's disease in deer with clinical signs of the disease, although as with most antibody tests, deer infected with avian Tb can give a false positive result.

PARALISA – An antibody blood test for Johne's disease that uses two different Johne's antigens and an avian antigen.

Cost: \$20 for individual deer* and \$10 per sample for 100+*

Turn-around time: 1 to 5 working days

Practical application: The PARALISA (sometimes shown Paralisa) is a blood test (using blood collected in a red-top tube) that is available from the Disease Research Laboratory, Otago University. It can be used to test for Johne's disease antibodies in individual deer suspected of having the disease, to test deer being presented for sale, or to test breeding hinds so that affected hinds can be culled.

PCR

PCR – Polymerase chain reaction. This is a relatively quick test for a specific sequence of DNA (genetic material) in bacteria such as *Mycobacterium paratuberculosis* (the cause of Johne's disease) and *M. bovis* (a cause of Tb).

Cost: \$100 (AgResearch)

Turn-around time: One week

Practical application: The PCR can be used for Johne's disease or Tb diagnosis in body tissue samples (eg lymph node collected at meat inspection). It is not as sensitive as culture, but it is usually very specific if carried out carefully.

Histology

Histology – This involves the microscopic examination of very thin slices of body tissues like lymph node and intestine taken after death (ie post mortem).

Cost: \$65 for 3 to 4 tissues.

Turn-around time: 3 to 4 days

Practical application: Histological examination of samples taken at post-mortem at the Deer Slaughter Premises or on the farm is carried out by pathologists at Gribbles Laboratory. Histology can indicate the type of disease process that is present and its severity, and it can indicate or suggest the probable cause of the lesions. Tb or Johne's disease can only be confirmed by histology in combination with BACTEC culture or another of the more specific tests. If histology is carried out at the request of veterinarians at the Deer Slaughter Premises, the Animal Health Board pays; and if a veterinarian requests histology on samples taken at necropsy on the farm, the farmer pays.

Other terms relating to diagnostic tests

Sensitivity

The sensitivity of a diagnostic test is its ability to detect infected animals, ie its ability to give a positive result in animals that are infected. A very sensitive test will be able to detect animals at a very early stage of infection or when they are only suffering a very mild infection. Very sensitive tests give few “false negative” results (see below).

Specificity

The specificity of a diagnostic test is a measure of its ability to give a negative result in animals that are NOT infected. Very specific tests give very few “false positive” results.

True positive

A positive test result in an infected animal.

False positive

A positive test result in an uninfected animal.

False negative

A negative test result in an infected animal.

True negative

A negative test result in an uninfected animal.

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Note

- The laboratory fees shown under “Cost” and marked with an asterisk* are currently being reviewed.
- The laboratory costs shown are the basic laboratory fees and **do not** include the veterinarian’s or testing officer’s fees for collection of samples or interpretation.
- The laboratory costs shown were current in March 2005 but **are subject to change.**
- The turn-around times may vary depending on the number of samples submitted.
- The sensitivity and specificity of any test can be affected by a variety of factors including the way the tests are carried out, the age, gender, pregnancy status and genetic makeup of the animal, the degree of stress and inter-current disease experienced by the animals, the length of time the animal has been infected, the severity of the disease, the environmental conditions, the presence of environmental bacteria that are closely related to Tb and Johne’s organisms, variations in the diagnostic reagents and test materials etc. For these reasons, sensitivity and specificity are usually expressed as a range rather than a specific figure.
- The specificity and sensitivity ranges for all the deer Johne’s disease laboratory tests are still being established.
- All accredited Tb testers are authorised by the Animal Health Board.