
**Republic of Namibia**
**Ministry of Agriculture Water and Forestry**

**Directorate of Veterinary Services**

# Protocol for Tuberculosis testing, using the Single Intradermal and Intradermal Comparative Tests

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# Protocol for Tuberculosis testing, using the Single Intradermal and Intradermal Comparative Tests

## 1. PURPOSE OF THE TEST

The standard method for detection of bovine tuberculosis is the tuberculin test, which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later. This may be performed using bovine tuberculin alone or as a comparative test using avian and bovine tuberculins. The tuberculin test is usually performed on the mid-neck, but the test can also be performed in the caudal fold of the tail. The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold. To compensate for this difference, higher doses of tuberculin may be used in the caudal fold.

Delayed hypersensitivity may not develop for a period of 3–6 weeks following infection. Thus, if a herd/animal is suspected to have been in contact very recently with infected animals, delaying testing should be considered in order to reduce the probability of false-negatives. As the sensitivity of the test is less than 100%, it is unlikely that eradication of tuberculosis from a herd will be achieved with only a single tuberculin test. It should be recognised that when used in chronically infected animals with severe pathology, the tuberculin test may be unresponsive. The tuberculin test has not been well validated in most non-bovid and non-cervid species.

The comparative intradermal tuberculin test is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other mycobacteria. This sensitisation can be attributed to the antigenic cross-reactivity among mycobacterial species and related genera. The test involves the intradermal injection of bovine tuberculin and avian tuberculin into different sites, usually on the same side of the neck, and measuring the response 3 days later.

The potency of tuberculins must be estimated by biological methods, based on comparison with standard tuberculins, and potency is expressed in International Units (IU). In several countries, bovine tuberculin is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU (±25%) in cattle. In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed, and in national eradication campaigns, doses of up to 5000 IU are recommended. The volume of each injection dose must not exceed 0.2 ml.

The purpose of the Single Intradermal Tuberculin Test (SITC) is to test cattle herds that have not been tested for bovine tuberculosis before.

The purpose of the Intradermal Comparative Tuberculin Test (ICTT) is to identify those cattle that are affected with bovine tuberculosis or capable of infecting other animals with bovine tuberculosis and to distinguish such animals from those that are not infected but which have become sensitised to bovine tuberculin as a result of exposure to cross-reactive antigens.

The comparative test, when carried out correctly, is highly reliable and has been assessed under certain conditions as 90-98% sensitive and 99.95% specific.

This reliability, however, is dependent upon the proper intradermal injection of the tuberculins (Bovine/Avian PPD) together with recording the accurate clinical observations together with characterisation, measurement and comparison of the reactions 72 hours later. The reliability is also influenced by the volume of tuberculin administered and by the site of delivery of the tuberculin (both

injections should be in the same plane in the middle third of the neck on a line parallel to the blade of the scapula – see picture below).

**Note**: The sub-cutaneous injection of tuberculin must be avoided as this will give rise to a false negative result in an infected animal and is also likely to lead to desensitisation of the site for a variable period.

## 2. TEST PROCEDURE

A correct injection technique is important. The injection sites must be clipped and cleaned. A fold of skin within each clipped area is measured with calipers and the site marked prior to injection. A short needle, bevel edge outwards and graduated syringe charged with tuberculin attached, is inserted obliquely into the deeper layers of the skin. The dose of tuberculin is then injected. A multi-dose syringe or multiple injection gun may be used provided that delivery of the volume and safety are assured. The dose of tuberculin injected must be no lower than 2000 International Units (IU) of bovine or avian tuberculin. A correct injection is confirmed by palpating a small pea-like swelling at each site of injection. The distance between the two injections (for the comparative test) should be approximately 12–15 cm. In young animals in which there is no room to separate the sites sufficiently on one side of the neck, one injection must be made on each side of the neck at identical sites in the centre of the middle third of the neck. The skin-fold thickness of each injection site is re-measured 72 hours after injection. The same person should measure the skin before the injection and when the test is read.

The syringes and needles used must be reserved for this purpose alone. Syringes must be clearly marked to distinguish between those used for avian tuberculin (red) and those used for bovine tuberculin (blue) when using the comparative test.

Veterinary practitioners must have properly identified, working syringes in their possession and immediately available when conducting the test. Spare needles, adaptors and identification thumb knobs must be carried at all times. Syringes must be emptied before commencing a test in a new herd. Syringes should be emptied at the end of each working day in order to prevent crystalisation of the tuberculin in the barrel, which could lead to syringe and/or tuberculin delivery problems.

At all times syringes must be clean and in perfect working order.

Before a test commences, it is essential to ensure that the loaded syringe does not contain any air and contains the correct tuberculin. The needle used should not protrude more than 3mm from the adaptor otherwise the tuberculin is likely to be injected subcutaneously. The needle should protrude at least 2 mm so that a successful intradermal injection is achieved.

A fresh plug of cotton wool, soaked in methylated spirits, must be placed in each syringe holster at commencement of the tuberculin test in each herd such that the needle of the syringe will make contact with and rest in the methylated spirits between each injection.

Calipers must be maintained in good working order. Both lugs, together with the thumb-piece, must be stable and both the millimeter measurements and the reference mark must be clearly legible. A suitable curved scissors, or other suitable clipping device, with a sharp cutting edge should be used and maintained in good working order.

## 3. EQUIPMENT CHECKLIST:

3.1 Boots, protective clothing and approved disinfectant (supply of a disinfectant officially approved and effective against M. bovis. To minimise the risk of the spread of infection, proper biosecurity and hygienic procedures, including disinfection, must be carried out before entering and on leaving each farm.);
3.2 Syringes x 3; (2 in use and 1 Spare syringe, with identification thumb knobs, needles, adaptors and spanner to change needle);
3.3 Cotton wool & Methylated spirits;
3.4 Calipers x 2-3 (for single or comparative testing) ;
3.5 Curved scissors/clipping device;
3.6 Correct documentation / forms;
3.7 Thermometer & stethoscope ( appropriate for the clinical examination of cattle);
3.8 Reactor Tags, (tag or other method of identifying reactors as supplied by DVS);
3.9 Tuberculin: Avian & Bovine tuberculin ppd provided by Central Veterinary Laboratory (CVL) or State Veterinary Office (SVO). Ensure that both tuberculins are within the expiry dates and record the batch number and expiry dates for each test (this may be relevant should there be a problem subsequently or legal challenge to the test). The tuberculin should be kept refrigerated between 2 to 8°C and protected from light until the date required. Not more than a single day's supply of tuberculin should be kept un-refrigerated at any time. Tuberculin must only be used on the day on which the vial is opened. Used vials should be returned to the practice centre for safe disposal and should not be discarded on farms;
3.10 Cool Box with ice to keep tuberculin cold.

## 4. TESTING

It is important to note that a minimum of 42-60 days is required between consecutive intradermal tuberculin tests.

### 4.1 DAY 1 (INJECTION)

**4.1.1 Verify Ear Tag numbers:**Before commencing a test the testing veterinary official must be satisfied as to the identity of each animal being tested and also personally take the caliper readings. When not personally recording the details of animal identity, test measurements etc., it is the responsibility of the testing veterinary official , who will be expected to be in a position to certify the test, to assure him/herself of the accuracy of such recording. It is essential to record or verify each ear tag number in full.

All animals on the herd-profile must be accounted for at a herd test

Under no circumstances is the testing of unidentified animals permitted.

**4.1.2 Clinical and other observations** or other treatments likely to have a bearing on the results of the test must be recorded linked by tag number reference to the individual animal(s).

**4.1.3 Syringes must be disinfected** after use (dismantled and sterilized at end of day), between herds and also after every 100 animals (by filling with alcohol, leaving for 5 minutes and then rinsing out at least twice with distilled water)

**4.1.4 Filling of syringes**:

Withdraw the plunger of the syringe to approximately the 0.5ml mark. The needle is then inserted into the bottle and the air expelled into the bottle. This increases the pressure inside the bottle and makes the filling of the syringe easier and faster. The syringe and bottle must be held upright and the plunger is slowly withdrawn, drawing the tuberculin into the syringe. When all the tuberculin is in the syringe, then one dose is injected back into the bottle. This should appear as sharp solid jet of fluid. If there is any air in the syringe then the air can be seen as it is injected into the bottle. If air is present then extra doses must be injected into the bottle until no air is present in the syringe at all. (Do not expose tuberculin to sunlight whilst filing syringe)

**NB:** If any air is left in the syringe then a full dose will not be injected into the animal and the test could show false negative results. When injecting and a dribble is seen on withdrawal it is a sure sign of air in the syringe.

**Site of Injection**

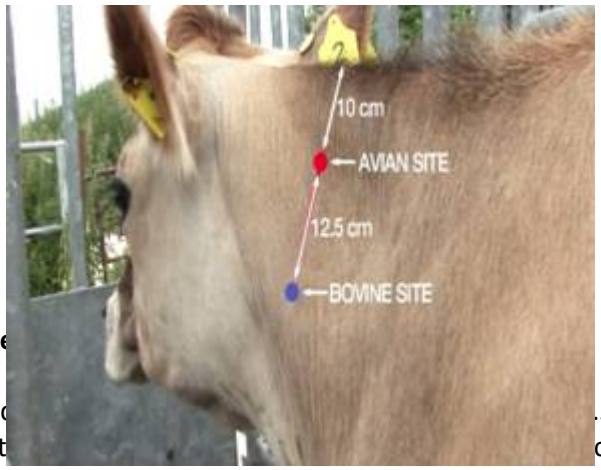
It is known that the skin of an animal does not show the same degree of sensitivity to tuberculin everywhere on the body. There is an increase in sensitivity from the back towards the front and from the bottom to the top of the body.

The neck is thus used as the preferred site of injection and the shoulder or back must NOT be used. For accurate and consistent testing the injection site is very important. The approved injection sites are situated at the border of the anterior and middle thirds of either side of the neck.

The upper site (for avian tuberculin) is about ten centimeters (10cm) below the crest.
The lower site (for bovine tuberculin) should be between twelve(12) and fifteen (15) centimeters from the upper site, in the same plane along a line drawn parallel with the ridge on the scapula (the representational diagram below is a guideline only).In calves under six weeks of age, or in animals where there is insufficient space to inject both tuberculins into the same side of the neck, the tuberculin should be injected, one on each side of the neck (avian on the left, bovine on the right), at corresponding sites in the centre of the middle third of the neck.

For animals, which have non-associated lumps or swellings adjacent to or obstructing the injection site(s) on the presenting side of the neck the tuberculin should be injected into the opposite side and recorded linked by tag number reference to the individual animal.

**The test should be done on the left side of the neck if possible**.




**4.1.5 Site Pre**

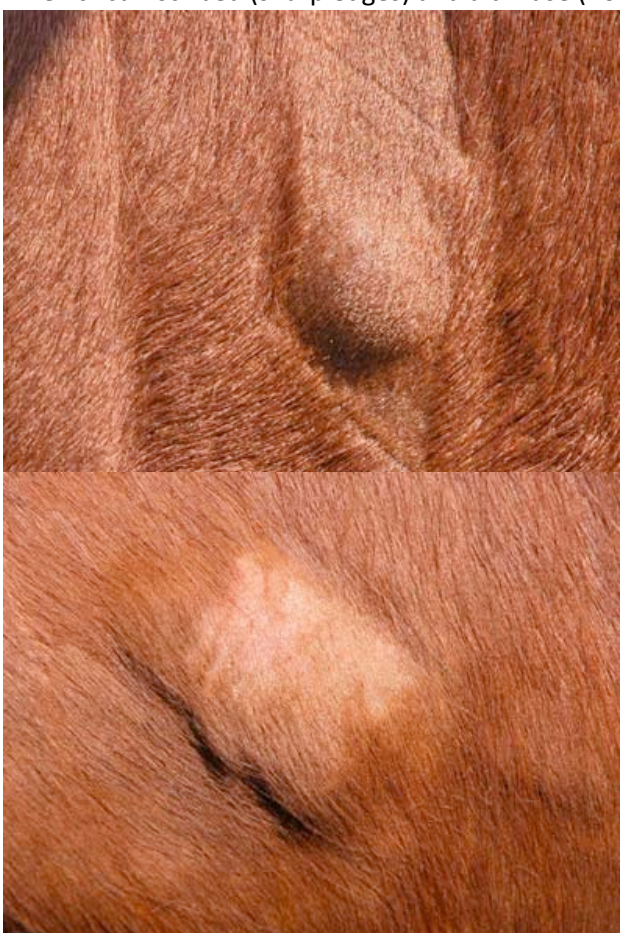
The selected .5cm in diameter) and cleansed i.e. any dirt ce of any abnormalities at the injection site(s) should be recorded (not under Clinical remarks) at the time and linked by tag

number reference to the individual animal. The presence of skin tuberculosis should always be recorded as a clinical remark.

### 4.1.6 Measurement of Skin Thickness

Before injection, a fold of skin at each of the injection sites and within the clipped area must be taken between the forefinger and thumb and accurately measured to the nearest millimeter using a calipers; and the measurements recorded linked by tag number reference to the individual animal.

A well circumscribed (sharp edges) and a diffuse (no distinct edges) lesions



### 4.1.7 Injection Technique

The needle should be introduced, bevel outwards, into the skin in such a manner as to ensure the intradermal delivery of the tuberculin. This usually requires the insertion of the needle at a narrow angle to the skin. The insertion of the needle at a right angle to the skin will generally

result in a subcutaneous injection being made. Such injections give rise to false negative results and must be avoided. Considerable pressure on the plunger of the syringe is usually necessary to make an intradermal injection. Absence of resistance to the flow of the tuberculin is an indication that it has not been injected intradermally, or that the syringe is leaking or improperly loaded.

If there is any doubt about either of the injections being delivered intra-dermally, a further injection should be made, preferably at a corresponding site on the other side of the neck.

Each injection must be confirmed; by palpating at the site a small pea-like swelling that is created by a properly administered intradermal injection.

The dose of tuberculin injected must be no lower than 2000 International Units (IU).

### 4.2 DAY 2 (72 HOURS +/- 4 HOURS POST-INJECTION)

#### 4.2.1 Reading of the Tuberculin Test

The test must be completed by the VO who commenced the test, on the same holding, and using all the data recorded contemporaneously on Day 1. Any deviation from this must be for very exceptional reasons and have the advance permission of the State Veterinarian in charge.

Each animal must again have its eartag number verified in full and its measurements, reactions, clinical signs and any other observations immediately recorded and correlated to the measurements and remarks recorded on Day 1 linked by tag number reference to the individual animal.

Each site where tuberculin was injected must be examined, palpated and measured. Measurements must be taken carefully by placing the calipers across the broadest width of any response present, without applying undue pressure, and recording the findings.
**N.B.** all measurements must be rounded up to the next whole millimeter. Any additional remarks must be recorded immediately and correlated to the measurements and remarks recorded on Day 1 linked by tag number reference to the individual animal.

Clinical signs directly associated with a reaction to the tuberculin must be recorded, contemporaneous with the time of reading, linked by tag number reference to the individual animal. These signs include the presence of oedema, exudative necrosis, heat, pain or swelling at the individual injection site and/or heat, pain or swelling of the related prescapular lymph node.

The presence of diffuse or extensive oedema, necrosis, heat, pain at the bovine injection site and/or swelling of the lymphatic ducts in the region or the related pre-scapular lymph node are regarded as clinical signs and always indicative of likely tuberculosis infection. Animals showing such reactions to bovine tuberculin or with diffuse or extensive oedema, necrosis, heat or pain at the injection site must always be deemed as reactors, irrespective of the measurements recorded.

The purpose in observing clinical signs, characterising reactions to the tuberculins and considering herd and animal histories and the status of contiguous herds is to facilitate the

identification of animals, which may be infected but which have not been identified as reactors to the tuberculin i.e. False Negatives.

The most reliable information is obtained by measuring the increase in skin thickness as this is measured in millimeters with the calipers. This measurement is objective and not subject to human judgement such as signs of pain, heat, redness oedema etc.
The increase in skin thickness is therefore one of the main criteria used in interpretation. It is however of vital importance that ALL SIGNS ARE TAKEN INTO ACCOUNT when interpreting a test result.

**4.2.2 The interpretation of reactions:**

**Single intradermal test**

In the single intradermal test (which requires a single injection of bovine tuberculin), the reaction is commonly considered to be negative if only limited swelling is observed, with an increase of no more than 2 mm and without clinical signs, such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes. The reaction is considered to be inconclusive if none of these clinical signs is observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm. The reaction is considered to be positive if clinical signs, as mentioned above, are observed or if there is an increase of 4 mm or more in skin-fold thickness. Moreover, in *M.-bovis*-infected herds, any palpable or visible swelling should be considered to be positive. Sometimes a more stringent interpretation is used, particularly in a high risk population or in-contact animals. Animals that are inconclusive by the single intradermal test should be subjected to another test after an interval of 60 days to allow desensitisation to wane (in some areas 60 days for cattle and 120 days for deer are used). Animals that are not negative to this second test should be deemed to be positive to the test. Animals that are positive to the single intradermal test may be subjected to a comparative intradermal test or blood test. Any retest should be performed in accordance with the local or national control programmes standard.

**Intradermal comparative test**

In the interpretation of the intradermal comparative test, a reaction is usually considered to be positive if the increase in skin thickness at the bovine site of injection is more than 4 mm greater than the reaction shown at the site of the avian injection. The reaction is considered to be inconclusive if the increase in skin thickness at the bovine site of injection is greater than the avian reaction with a difference of less than 4 mm. The reaction is considered to be negative if the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection. This interpretation scheme is used in European Union (EU) countries and is recommended in Council Directive 64/432/EEC (EU, 1980). Sometimes a more stringent interpretation is used.

**4.2.3 Identification of Reactors**

All animals classified as reactor must be identified as such, and these reactors must be reported to DVS without delay.

- Animals Missing from Day 2:

An animal, recorded as injected with tuberculin on Day 1 and for which no Day 2 skin measurements or tuberculin response details is recorded, will be regarded as not 'read' and the test will therefore be treated as incomplete and thus ordinarily herd status may not be certified. In such cases, the herd status will, at a minimum, be suspended until it can be clarified by means of test or otherwise. If an animal recorded on Day 1 is not presented for reading on Day 2, the keeper should in the first instance be queried as to the absence of the animal, the explanation recorded in the clinical remarks column and the DVS informed immediately (if the animal died on farm the DVS may wish to arrange for examination of the injection site and/or a post-mortem).

- **Removal of Test Materials from Holdings:**
  It is essential that the residue of all test materials employed in the test procedure including syringe parts, used tuberculin vials, needles, cartons and other items are gathered and removed at the time of leaving the holding.
  The safe and proper disposal of such materials in compliance with relevant legislation is the responsibility of the testing veterinary practitioner.

**4.2.4 Positive reactors**

Inconclusive or positive reactors must be isolated from the rest of the herd and must be re-tested in not less than 60 days using the comparative intradermal test or the Gamma interferon test.

Animals testing positive on the re-test must be reported to DVS without any delay.

**Annex 1**

**BOVINE TUBERCULOSIS SAMPLING FORM AND HERD HEALTH QUESTIONNAIRE**

**DIRECTORATE OF VETERINARY SERVICES**

| | | | |
|---|---|---|---|
| SV District | | Date | |
| Ref No. | | Magisterial District (1st four letters) | |
| Farm Name | | Farm No. | |
| Owner | | Telephone No. | |
| Postal Address | | Veterinary Official | |

**LIVESTOCK POPULATION ON THE FARM**

| **SPECIES** | **NUMBER** | **BREED** |
|---|---|---|
| Cattle | | |
| Sheep | | |
| Goats | | |
| Game | | |
| Others | | |

**DETAILS OF THE HERD TESTED**

Breed: …….. Stock Brand: ……

| | |
|---|---|
| Number of cattle in total | |
| Number of cattle older than 6 weeks | |
| Number of animals tested | |

**HISTORY**

Have any of the conditions listed below been diagnosed in cattle on the farm or at the abattoir:

| Condition | YES | NO | Don't know | Number of cases |
|---|---|---|---|---|
| Chronic coughing | | | | |
| Gradual loss of condition/ stunted growth | | | | |
| Enlarged lymph nodes | | | | |
| Granulomatous lesions in the lungs and lymph nodes of the thorax | | | | |
| Granulomatous lesions in the intestines | | | | |
| Other | | | | |

Comments: ……
……
……..

**BOVINE TUBERCULOSIS TESTING FORM**

**DAY ONE DATE: ...../...../..... DAY TWO DATE: ...../...../.....**

| Ear tag number | Sex and Category (breeding female/male | Breed | DAY ONE SKIN THICKNESS AVIAN | DAY ONE SKIN THICKNESS BOVINE | DAY THREE SKIN THICKNESS AVIAN | DAY THREE SKIN THICKNESS BOVINE | Clinical signs: oedema, exudation, necrosis, pain, lymph node swelling, etc. | Result |
|---|---|---|---|---|---|---|---|---|
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Notes: 1. To be completed by the Veterinary Official each time when testing cattle.
2. This form should be completed for each herd separately

..... .....
Name of Veterinary Official Signature

..... .....
Name of Supervising veterinarian Signature