3.1 Tuberculosis testing

Tuberculin Tests—Background Information

1. For many decades, intradermal tuberculin testing has been the most commonly used diagnostic procedure for detecting bovine tuberculosis in live animals. The basis of tuberculin testing is the induction of a delayed hypersensitivity reaction to the intradermal injection of tuberculin, a protein purified derivative of a laboratory culture of *Mycobacterium bovis (M.bovis)*. A positive reaction is manifested by erythema and induration at the injection site appearing at 8–12 hours post injection, and peaking between two (2) and seven (7) days.

2. Factors which may influence the sensitivity and specificity of the test include:

    a) type of tuberculin, (bovine, avian, or human);
    b) site of injection (cervical or caudal);
    c) quantity of tuberculin used;
    d) late pregnancy/postpartum;
    e) state of nutrition; and,
    f) whether or not the animal is infected.

3. Some cattle infected with *M. bovis* fail to respond to the injection of tuberculin (false negatives). The number of cattle in this category is a measure of the sensitivity of the particular test, sensitivity being the probability of the test correctly identifying as positive those cattle which are truly positive. False negative responses may be observed in cattle which are debilitated, in the very early or late stages of the disease, postpartum, or otherwise stressed, or cattle desensitized by tuberculin injected within the previous 8–60 days.

4. Similarly some cattle, which are not infected with *M. bovis*, respond to the injection of tuberculin (false positive). The number of cattle in this category is a measure of the specificity being the probability of the test correctly identifying as negative those individuals which are not infected. Most false positive responses in cattle are due to the animal having been exposed to antigens similar to or shared with those in the tuberculin used in the test.

Caudal Fold Tuberculin Test

5. The caudal fold tuberculin test is used for routine screening and diagnostic or export tests of cattle, bison, sheep, or goats at low risk of having tuberculosis.

6. The test is an intradermal injection of 0.1 mL of bovine purified protein derivative (PPD) tuberculin in the caudal fold of cattle, bison, sheep, or goats with observation and palpation at 72 ± 6 hours. Animals cannot be tested if they have been injected with tuberculin within the previous 60 days.

7. Equipment and supplies required:
   a) bovine PPD tuberculin provided by the CFIA district office. Store in a cool (4°C) dark place and do not use after the expiry date;
   b) 1 mL disposable tuberculin syringe with a needle calibre 26 of 3/8";
   c) absorbent cotton;
   d) CFIA/ACIA 1524 on which to record the test and the animal identification.

Note: *Only fill the syringes with tuberculin at the time of injection, never in advance*.

8. Do not conduct the test without having the animal adequately restrained. Restrain all animals sufficiently to permit the accurate identification of the animal and proper injection of tuberculin. Both confirmation of identification of the animal and palpation of the injection site are required to interpret the test results. The veterinarian performing the injection must interprete the tuberculin test result.

9. The injection site is the caudal fold, distal to the base of the tail, well away from the hairline, in the centre of the fold. Note any abnormalities found near the injection site on the test record so that they will not be mistaken for tuberculin responses. Either left or right fold may be used, but be consistent and always record which fold was injected.

10. Clean the caudal fold with dry cotton or cotton dipped in saline water (never use alcohol). Inject 0.1 mL of tuberculin intradermally, lifting the tip of the needle slightly after insertion to ensure that it is clearly outlined just under the skin. Following the injection, the injected tuberculin should be visible as a palpable bleb. Use a fresh sterile needle for each animal tested.

11. Observe and palpate the injection site at 72 ± 6 hours post injection. Raise the tail to stretch the caudal fold slightly and palpate the length of the caudal fold with the thumb and index finger of the other hand. Record test results on the CFIA/ACIA 1524 form as follows:
    a) no change in the tissue at the point of injection - "no reaction," decision "negative."
    b) **any** change in the tissue at the point of injection, **notify the district veterinarian immediately**.

Mid-cervical Intradermal Test in Cervids

12. The mid-cervical intradermal test is the standard test for tuberculosis in cervids (deer, elk.).

13. Inject 0.1 mL of bovine PPD tuberculin in the mid-cervical area of cervids with observation and palpation at 72 ± 6 hours. In the case of animals for export to the U.S., do not repeat this test on the same animal at intervals of less than 90 days.

14. Equipment: as in paragraph 7 plus electric clipper with a #40 head and an indelible felt marker.

15. Adequately restrain all cervids to be tested to ensure adequate preparation of the injection site, proper application of the tuberculin injection, correct recording of animal identification and correct reading of the test.

16. Use clippers to clip an area in the mid-cervical region approximately 8 cm square. Mark the site of injection. Inject 0.1 mL of tuberculin intradermally, lifting the tip of needle slightly after insertion to ensure it is clearly outlined under the skin. Following the injection, the injected tuberculin should be visible and palpable as a definite bleb.

17. Observe and palpate the injection site at 72 ± 6 hours post injection. The response at the skin injection site is frequently diffuse as opposed to the circumscribed response on caudal fold test in cattle. Good lighting and restraint is essential. Record responses to the test on form CFIA/ACIA 1524 as follows:

    a) no change in the tissue at the point of injection - "no reaction," decision "negative."
    b) **any** visible or palpable change in the tissue at the point of injection, **notify the district veterinarian immediately**.

Post-Axillary Test

18. For export testing of New World camelidae only, at the request of the importing country.

19. The intradermal test is performed at the post-axillary site in camelidae. Responders to the test are determined by relatively small changes in skin thickness, and therefore it is imperative that a regimented approach be taken in applying this test. Measurements will be influenced by skin tension (related to restraint), by the amount of pressure placed on the skin by the calipers, and by the amount of skin picked up for measurement. Make every effort to standardize the conditions for both injections and readings.

20. Do not repeat this test on the same animal at intervals of less than 90 days.

21. Equipment: as in paragraph 7. above, plus a caliper capable of accurately measuring to less than 0.1 mm.

22. Adequately restrain all camelidae to be tested to ensure adequate preparation of the injection site, proper tuberculin injection, correct recording of animal identification, and proper reading of test. Use clippers to clip an area in the axillary region approximately 8 cm square. Mark the site of injection, and palpate and record any unusual findings.

23. Using the dial caliper measure the marked spot three times. Record the average of the measurements.

24. Inject 0.1 mL of tuberculin intradermally, lifting the tip of the needle slightly after injection to ensure it is clearly outlined under the skin. Following the injection, the injected tuberculin should be visible and palpable as a definite bleb.

25. Observe and palpate the injection site at 72 ± 6 hours. The response at the skin injection site is frequently diffuse as opposed to the circumscribed response on the caudal fold test in cattle. Using the dial caliper, measure the marked spot three (3) times. Record the average of the measurements. Record responses to the test on the test report form as follows:

    a) if no change in the tissue at the injection site, or less than 1.5 mm increase in average thickness, record "no reaction" decision "negative;"

    b) if there is a visible or palpable reaction including 1.5 mm or greater increase in average skin thickness, record the decision as "suspicious" and **notify the district veterinarian immediately.**