

AlpacaMAP

Appendix 13

Pooled Faecal Culture

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The selection of animals for this test needs to be made as follows:

- The sample is selected from all alpacas that are 12 months of age and older.
- Pools comprise single faecal pellets from each of up to five (5) alpacas.
- The maximum number of animals in very large herds that are screened by PFC is 300 (ie 60 pools).
- In a herd with fewer than 210 alpacas over 12 months of age, all of these animals must be tested (ie 42 pools).
- A guide to the total number of animals to be sampled in herds with between 210 and 300 animals is outlined in Appendix 9.
- Animals must be selected in proportion to the numbers of eligible animals in the different mobs in the herd.
- All introduced males 12 months of age and older are to be included (as a separate pool).
- Any breeding animals introduced to the herd from a herd of a lower status (e.g. in an MN2 herd from an MN 1 herd) must be tested, unless it is more than 2 years since they were introduced or the herd of origin has since achieved the same herd status.
- The alpacas to be sampled should first include any in poor condition and then the remainder selected systematically.
- The identities of all animals contributing to each pool must be recorded.

Faecal sample collection for Pooled Faecal Culture submissions

- One faecal pellet is required from each alpaca selected for sampling. If no pellet can be collected, the animal should be identified and collection attempted when the

remaining alpacas in that pool have been sampled. Alternatively, a substitute sample from an additionally selected alpaca may need to be collected.

- Collect one pellet from each alpaca into a sterile plastic jar with a maximum of 5 per jar.
- Ensure all pools have faecal material from a minimum of 3 alpacas per pool. (For instance, if there are 57 alpacas to sample from one mob, collect 10 pools of 5 from 50 animals and one pool of 4 and one of 3 from the remaining 7).
- Change gloves for collection of faeces between each pool to prevent cross-contamination of pools.
- To avoid contamination of a pool from an alpaca from which a sample cannot be collected (and which therefore will not be identified with the pool), change gloves after attempting to sample such animals.
- If an alpaca has soft/watery faeces, collect an amount similar to a normal pellet.
- Ensure proper labelling of each pool to enable identification of alpaca which have contributed to that pool. Record ear tag number/colour or brand details for each group of alpacas contributing to a pool.
- Keep samples cool in an esky with ice bricks. Send to the laboratory as soon as possible so that samples reach the laboratory within three days of collection. Keep samples at 4°C in a domestic refrigerator if there is likely to be a delay in transport.

A Sample Identification Form for PFC Testing is provided with this manual and is also available from the Animal Health Australia website at

[http://www.animalhealthaustralia.com.au/programs/jd/maps\\$/alpacamap.cfm](http://www.animalhealthaustralia.com.au/programs/jd/maps$/alpacamap.cfm)

It is not compulsory to use this form and veterinarians may wish to use an alternative recording format.

Investigation of positive radiometric (Bactec) pools

Sample or Maintenance Testing using PFC includes screening using radiometric (Bactec) culture and additional testing of any Bactec positive pools.

If growth is detected in Bactec culture these pools require additional testing:

1. Polymerase Chain Reaction (PCR) on the Bactec culture
2. Subculture from the Bactec sample onto solid media, and if necessary, additional PCR testing.

Pools which are confirmed positive on solid media and which are PCR positive on either the Bactec culture or the solid media subculture are definitive for *M. paratuberculosis* and result in a positive sample test (i.e. infected).

If pools are positive on PCR of the Bactec culture but negative on subculture on solid media, alpacas contributing to those pools must be identified and held in a secure area, preferably isolated, as soon as possible and must be subjected to a Faecal Culture Follow-up test, as set

out below, within one month of the notification of a positive result, or within a timeframe agreed to by the CVO.

The status of all animals contributing to a Bactec positive pool must be clarified before a herd's status can progress. No tested alpacas are to be disposed of or slaughtered unless it is done as part of a follow-up investigation by the Approved Veterinarian, or the Sample Test has been completed.

7. Faecal Culture Follow-up Test

The Faecal Culture Follow-up Test must be carried out as follows:

PCR +ve, Solid media –ve, All alpacas present

Where one or more pools are positive on PCR of Bactec culture but negative on subculture on solid media and ALL alpacas which contributed to the relevant PFC pools are present, follow-up testing will be undertaken by individual faecal culture as follows

- Collect faecal samples from the alpacas that comprised the particular pool(s) and submit these for individual faecal culture. Faecal samples must be individually identified
- If all of the repeat individual faecal cultures are negative, the result will be considered as a negative Sample Test.
- If any of the repeat individual faecal culture results in the confirmation of M.ptb on solid media subculture AND by PCR, the Sample Test will be considered positive.
- If any of the repeat individual faecal cultures are positive on PCR of Bactec culture but negative on subculture on solid media, the CVO will determine the further investigation that is most appropriate in the circumstances taking into consideration the very low incidence of JD in alpaca since the mid-1990s and the history of the particular herd.

PCR+ve, Solid media –ve, All alpaca NOT present

Where one or more pools are positive on PCR of Bactec culture but negative on subculture on solid media and all alpaca which contributed to the relevant PFC pools are NOT present, the Faecal Culture Follow-Up Test must be carried out as described above plus additional pools must be tested as detailed below:

- If one or two alpacas in a pool are missing, one additional pool derived from the same mob(s) as the missing alpaca(s) must be tested as part of the Faecal Culture Follow-up Test.
- If between three and five animals in a pool are missing, two additional pools derived from the same mob(s) as the missing alpaca(s) must be tested as part of the Faecal Culture Follow-up Test.
- If the composition of the mob(s) which contributed to the PCR +ve, Solid Media –ve pool(s) containing missing alpaca(s) has changed, or if more than five animals in a pool are missing, a full Pooled Faecal Culture Sample Test must also be conducted as part of the Faecal Culture Follow-up Test.

- The Approved Veterinarian must ensure that where possible any deaths of sampled animals prior to results being available or a Faecal Culture Follow-up Test being initiated are investigated. It is also important that adequate records are kept to allow further sampling of alpaca from the appropriate mob if any animals are missing.