



Animal &
Plant Health
Agency

APHA Briefing Note 14/22

Polymerase chain reaction (PCR) test for detection of *Mycobacterium bovis* in post-mortem tissue samples

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Purpose

1. To inform Official Veterinarians (OV) in Great Britain (GB) and Approved Tuberculin Testers (ATT) in England of APHA's new *Mycobacterium bovis* (*M. bovis*) polymerase chain reaction (PCR) test.

Background

2. APHA has recently validated and adopted a new PCR test that can detect *M. bovis* directly from tissue samples collected at post-mortem inspection. The major advantage of this method is that it typically takes only three weeks for the laboratory to generate a result compared with protocols using microbiological culture, which typically take 6-22 weeks.
3. The *M. bovis* PCR test is initially only being used in GB for tissue samples from carcasses of:
 - TB slaughterhouse cases in cattle and non-bovines i.e. animals routinely sent for private commercial slaughter that have suspicious lesions of TB at routine meat inspection;
 - Non-bovine animals such as camelids, goats, pigs, sheep and farmed deer that are removed as TB test reactors, direct contacts or clinical TB suspects, and cases where suspicious TB lesions are identified on diagnostic post-mortem examination in a veterinary laboratory; and

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- Domestic pets (cats and dogs) and exotic species of animals (e.g. in zoological collections) submitted to APHA for laboratory investigation.
4. After a period of operation and review of its performance, APHA will consider wider use of the PCR test, e.g. for routine detection of *M. bovis* in post-mortem samples taken from skin and blood test positive cattle.
 5. In APHA's validation study, the *M. bovis* PCR test produced equivalent results to the traditional microbiological culture method, for both bovine and non-bovine tissue samples.
 6. In a very small proportion of cases, it may not be possible to obtain a valid PCR test result. In those instances, APHA will revert to bacteriological culture to attempt to grow *M. bovis*. APHA will inform keepers if this applies to samples from their herd/flock.
 7. An important limitation of this test is that it is not possible to apply whole genome sequencing (WGS) directly on the *M. bovis* target DNA sequence that is amplified through PCR testing (i.e. the PCR product). WGS is only possible with DNA obtained from a pure microbial culture. This means that APHA will not be able to rely exclusively on the PCR test for case management and epidemiological analyses of TB breakdowns.
 8. Culture will only be carried out where WGS is required to allow full analyses of the breakdown and identify the probable source of infection. APHA will not report culture results unless they are used for the confirmation or negation of *M. bovis* infection i.e. in the rare instances where the PCR test has not provided a valid result. The additional WGS analysis of positive PCR samples will not interfere with or delay APHA's decisions to manage the TB breakdown.

Impact on management of TB incidents

9. When a **bovine slaughterhouse case** is identified, the herd of origin's official TB free status is suspended, which means that bovines cannot move on or off the holding except under a licence issued by APHA. Previously, tissue samples were taken from the carcass and submitted for microbiological culture, which can take up to 22 weeks for a result. In the meantime, the herd remains under movement restrictions and a skin test (check test) of the herd may be carried out pending the final culture result.
10. Instead of relying on culture results for confirmation or negation of *M. bovis* infection, PCR testing is now carried out on the lesions sampled from the carcass of the slaughterhouse case. PCR test results are typically available within three weeks and, if negative, herd movement restrictions could be revoked sooner instead of

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waiting for culture results. This limits the negative impact of movement restrictions on cattle herds and (in England and Scotland only) eliminates in most cases the need for a check test. PCR test positive results will allow for more timely deployment of additional disease control measures, such as source or spread tracing and application of interferon-gamma testing where applicable.

11. Similarly to bovines, the report of TB-like lesions from carcasses of **non-bovine animals** detected at post-mortem examination in a veterinary laboratory, or during routine post-mortem meat inspection in the slaughterhouse, lead in most cases to the application of herd/flock movement restrictions.
12. PCR test results are typically available within three weeks, which means that if the result is negative APHA may be able to revoke herd movement restrictions sooner. In the rare cases where a valid PCR test result cannot be obtained, movement restrictions will remain in force until negative culture results are received.
13. The PCR test will also be used by APHA to detect *M. bovis* in non-bovine animals that have been removed as TB test reactors or direct contacts whenever APHA considers that the procedure is necessary in addition to a post-mortem examination. In these cases, even if no typical TB lesions are identified at post-mortem examination and the PCR test result is negative, additional testing will still be required before movement restrictions can be lifted.
14. OVs and ATTs should refer to specific instructions on the APHA Vet Gateway for further detail about management of TB breakdowns.

Action

15. OVs and ATTs should familiarise themselves with the updated instructions on the APHA Vet Gateway website <http://apha.defra.gov.uk/vet-gateway/> and signpost any queries from clients about the PCR test for *M. bovis* to the TB hub website or APHA.

Further Information

16. Detailed information about APHA's PCR test for *M. bovis* can be found on the TB hub website: <https://www.tbhub.co.uk/>