Animal & Plant Health Agency

APHA Briefing Note 27/23

Comparative (Head-to-Head) Study of the use of the Enferplex and IDEXX antibody tests alongside the Gamma-interferon test in OTFW breakdowns in England and Wales

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Purpose

1. To inform Official Veterinarians working in England and Wales of an APHA study of the diagnostic performance of the two World Organization for Animal Health (WOAH) approved TB antibody tests Enferplex & IDEXX alongside the Gamma-interferon ('Gamma') blood test in TB breakdown herds in England and Wales during 2023 and 2024.

Background

2. The Gamma blood test has been used in GB cattle to supplement the tuberculin skin test since October 2006, mainly as a mandatory test in TB breakdown herds with lesion or culture-positive animals. It was approved and registered by the WOAH in 2015 and it remains the supplementary blood test of choice for TB in cattle in the UK, Ireland and other EU countries with a bTB eradication programme.

3. The Gamma blood test has a higher sensitivity (90%) than the skin test (81%), meaning it can detect additional infected cattle before they become skin test-positive. The Gamma blood test has a specificity of 96.5%, less than that for the skin test (99.98%), which is why the Gamma blood test is used to supplement the skin test in OTFW breakdown herds, where the priority is to maximise sensitivity of infection detection to help resolve the TB breakdown.

4. The Gamma blood test can also detect some cattle that are unresponsive to the skin test. Using the Gamma blood test in conjunction with the skin test in parallel interpretation, therefore, can identify additional infected cattle compared to the skin test alone and so contribute to the more rapid elimination of TB from the herd.

5. More recently, antibody tests, traditionally found to have low sensitivities compared to the Gamma blood test, have become available for TB blood testing of cattle.

6. The IDEXX antibody test (IDEXX Laboratories, Maine, USA) was listed by the WOAH in 2012 as a supplementary test for bTB. It was then validated for use in GB by APHA and has been used since 2018 - less frequently than the Gamma blood test, largely as a third-line test. It is a simple, relatively inexpensive ELISA test with a modest reported sensitivity of 65% and a specificity of 98%.

7. The IDEXX test is currently used by APHA in England on a discretionary basis, usually targeted to defined groups of cattle in herds with persistent TB breakdowns and OTF status withdrawn (OTFW) that have not been resolved after combined skin and Gamma blood testing. The expected benefit of using the IDEXX antibody test in this way is the identification of further infected individuals that are skin and Gamma blood test-negative. The IDEXX test has also been used in breakdown herds where infection is suspected or known in youngstock under six months old, which are not eligible/suitable for Gamma blood testing.

8. In Wales the IDEXX test is often used together with the Gamma blood test in certain mandatory scenarios, e.g. rapid re-testing of severe Inconclusive Reactors (IR) as part of an exit strategy for TB breakdown herds

9. The Enferplex antibody test (Enfer Scientific Laboratories, Ireland) gained WOAH approval in 2019 as a supplementary test for TB in cattle. It is a non-statutory test not yet approved by Defra or the Welsh or Scottish Governments for statutory TB testing of cattle, although it can be used on a private basis in England subject to prior permission from APHA. There is also an ongoing, separate project investigating the use of Enferplex under specific circumstances in Wales.

10. The Enferplex test measures serum antibody responses to 11 different *M. bovis* antigens (‘spots’) in a multiplex ELISA format and it has two test interpretations; High Sensitivity and High Specificity. According to the data sheet published by the WOAH (see also O’Brien et al., 2023, https://www.nature.com/articles/s41598-023-28410-9), the Enferplex test (High Sensitivity interpretation) has a reported test sensitivity of 76% - 94% in confirmed infected cattle (depending on time elapsed between skin testing and blood sampling – the highest sensitivity achieved within 30 days of the skin test), and a specificity of 98% - 99%.

11. An animal is considered TB positive on the Enferplex test on either interpretation if the serum sample reacts with two or more spots, as per test manufacturer’s instructions, .

In this study, any animal with a positive outcome for either High Sensitivity or High Specificity test interpretation will be treated as an Enferplex test-positive.

Description of the APHA Head-to-Head Study

12. Overall the study aims to compare the performance of each of these tests in a limited number of infected herds and assess how combining them may affect the outcome. The data obtained from this study will help inform how these supplementary antibody tests could be deployed in TB breakdown herds in the future.

13. This study provides herd owners with an opportunity to maximise the likelihood of identifying all TB infected animals in their herd, reducing the likelihood of leaving animals with undisclosed infection in the herd when the movement restrictions have been lifted.

14. Owners of cattle herds in England and Wales undergoing mandatory Gamma blood testing due to a new (no TB breakdown in the last 5 years), recurrent or a persistent (> 18 months' duration) TB breakdown with Officially TB Free Status withdrawn (OTFW) may be asked whether they would be interested in taking part in this study.

15. APHA will provide them with an information letter containing information regarding the project and the additional antibody testing requested.

16. OTFW eligible herds in Wales will need to have had at least one animal presenting visible lesions on post-mortem inspection and/or where *M. bovis* have been detected by the TB Polymerase Chain Reaction (PCR) test or bacteriological culture.

17. If a keeper agrees to participate in the study, APHA will collect an additional blood sample per animal at the time of Gamma blood test sampling, during a single test round. The additional blood sample will be tested in an APHA laboratory for TB antibodies using the Enferplex and IDEXX tests.

18. In order to participate in the study, herd owners will need to agree in writing to the removal of all Gamma test negative cattle that are positive to any of the two antibody tests (Enferplex or IDEXX). This will be in addition to the statutory removal of all the cattle positive to the Gamma blood test in the same herd, irrespective of their antibody test results (i.e. results of the three blood tests will be interpreted in parallel). IDEXX test positive animals undergo statutory removal in Wales.

19. All test-positive cattle will attract the usual compensation payments (including any reductions in compensation when they apply) and will be removed from their herds within the usual timescales.

20. The injection of bovine tuberculin triggers a transient boost of specific antibodies in *M. bovis*-infected cattle, which enhances their detection. Therefore, blood sampling for this

project (for Gamma, Enferplex & IDEXX tests) will need to take place at the same time/same day within 10-30 days after the injections of tuberculin in order to take advantage of the antibody boost.

21. Samples for antibody testing will ONLY be taken from cattle sampled for a whole or part herd parallel Gamma test, e.g. youngstock < 6 months old not eligible for the Gamma test will not be sampled for antibody testing.

22. This project requires a single round of antibody testing to be performed in conjunction with the Gamma blood test. If additional rounds of Gamma blood testing are necessary on a given herd, APHA will not take an extra blood sample for a second Enferplex and IDEXX test under this project. Any individual cattle that require to be Gamma resampled e.g. samples were rejected or did not have a valid result will not have further samples taken for antibody testing.

23. The study will conclude once 2300 cattle from new breakdown herds and 2300 cattle from recurrent or persistent breakdown herds have been tested. No more than 250 cattle from each participating herd will be sampled. The number of samples needed from persistent/recurrent TB breakdowns in Wales, for this project, have almost been collected.

Action

24. If following discussions with one of your clients whose herd is undergoing an eligible TB breakdown, you both feel that the herd could benefit from participating in this study, please ask your client to contact APHA in order to discuss their potential participation.

Further Information

25. Please contact APHA:
    - on 03000 200 301 or TB.Advice@apha.gov.uk, in England
    - on 0300 303 8268 or APHA.CymruWales@APHA.gov.uk, in Wales