How testing is performed

Measuring a test's quality

- **Sensitivity –** The probability that if the animal has the disease that the test will be positive.
- **Specificity –** The probability that if the animal does not have the disease that the test will be negative.
- **Gold standard** – a test which is 100% sensitive and specific.

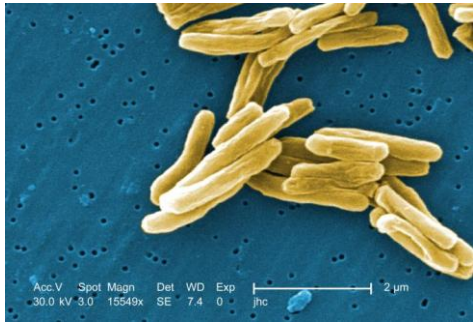
Testing for Bovine Tuberculosis

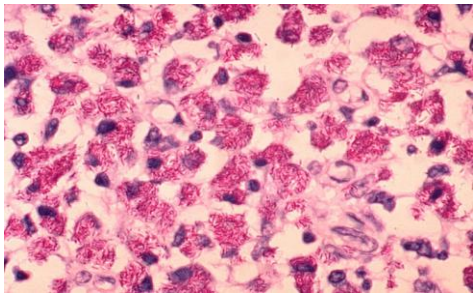
- It is very difficult to develop a ‘gold standard test for bTB which will sensitively and specifically detect all stages of infection from beginning to end.
- Tests for immune responses to TB, rather than directly for the organism itself, have the greatest flexibility and utility for detecting TB.
- However, immune responses to TB are very complex making good tests difficult to develop.

The main reasons for this are:

Testing for Bovine Tuberculosis

- The immune responses to M. bovis, are complicated and variable throughout the course of infection.
- There are at least 120 Mycobacterial species in the environment and they are very homogenous with very similar structural components therefore the proteins recognised by the immune system can be very similar too - this creates difficulties for developing good diagnostic tests.
- The biology / pathology of mycobacteria – M bovis is very slow growing, clinical signs can take months/years to develop and the bacteria are thought to be able to become latent (where infection remains in a dormant state).
- During this phase immune responses are at a low level and to test for the infection means that you have to stimulate the immune system to amplify the response.





The Enferplex TB Test

Serology – testing for BTb antibody

- Antibody responses were traditionally thought to be
- Slow to develop
- Only occurred towards the end stage of the disease process
- Were not present at high enough levels during the protracted ‘latent’ phase of the infection.

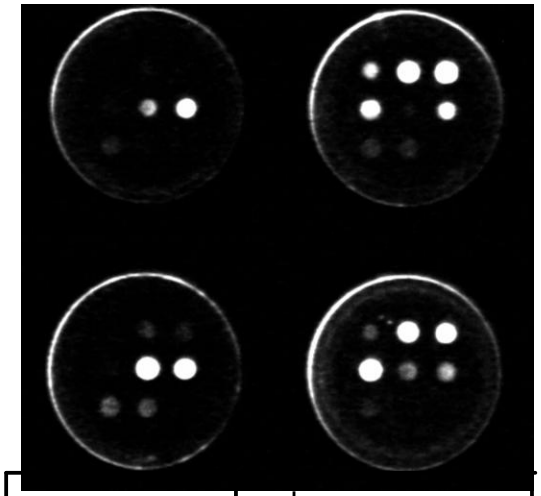
However, over the last 5-10 years new methods have shown that antibodies are produced early on after infection and can be diagnostically useful if tests are sensitive enough to detect them and the range of pure TB antigens which is used is broad enough.

The Enferplex TB Test

Serology – testing for BTb antibody

- Research has shown that antibody activity for different antigens develops at different times during the course of infection.
- This finding, along with the difficulties of differentiating between species discussed previously, means that use of multiple antigens to detect different antibodies is required.
- By doing so it is now possible to accurately diagnose TB.

The Enferplex TB Test



Ag ID	RLU
Ag 1	18750
Ag 2	45600
Ag 3	51400
Ag 4	33600
Ag 5	1500
Ag 6	31350
Ag 7	2300
Control	2500
Blank	300

The Enferplex TB test is a serological assay which identifies the presence of antibody to Mycobacterium bovis, the causal agent of for bovine Tuberculosis (bTB).

It does this by use of individual antigens – antigens are parts of the Mycobacterium which generate antibodies which then bind to the antigen.

There are seven different TB antigens in the test and these are placed separately as individual spots on the surface of the test well.

If antibody to bTB is present in the blood sample, then it will bind to the relevant antigen and the resultant reaction produces a luminescent reaction, the light from which can be measured and quantified as a number.

The Enferplex TB Test

Thresholds are set for each individual antigen spot and if the level of light is above this threshold, a positive reaction is deemed to have occurred.

A test where only a single antigen spot reacts to the presence of antibody (Fig. 1) does not mean that the animal is definitely infected as we see this type of response in animals from TB-free herds and countries.

A positive reaction which indicates that the animal is infected is considered to have occurred if two or more of the individual antigen spots react to the presence of antibody (Fig. 2).

At this interpretation the test has maximum sensitivity and, in camelids, is 97% specific. As animals respond to more antigens the test becomes more specific until when four antigens are positive the test is 100% specific

Figure 1: Schematic example of test where only one antigen is positive (red) – this result would be interpreted as negative to bovine tuberculosis

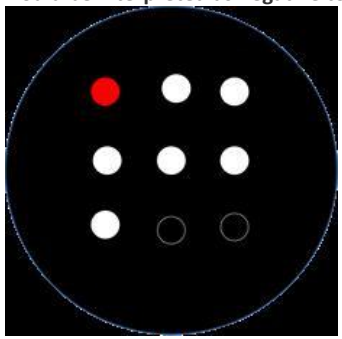


Figure 2: Schematic example of test where two antigens are positive – this result would be interpreted as positive to bovine tuberculosis at the two antigen level

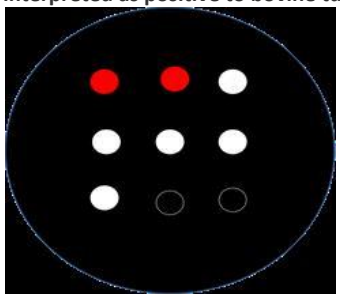
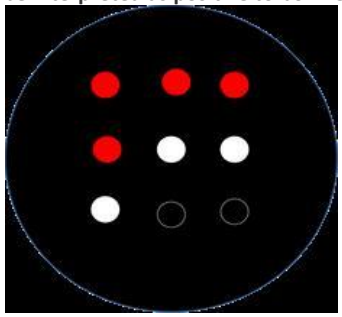


Figure 3: Schematic example of test where four antigens are positive – this result would be interpreted as positive to bovine tuberculosis at the four antigen level



Performance of the test

- The AHVLA/BAS study suggested that there was no statistical difference between the available serological tests with a sensitivity for all the tests

Test	n/total	% Sensitivity	95% CI	n/total	% specificity	95% CI
Stat-Pak	35/52	67.3	54.5 - 80.8	8/306	97.4	95.6 - 99.2
DPP	30/52	57.	44.3 - 71.1	10/306	96.7	94.1- 98.4
Idexx	36/52	69.2	56.7 - 81.7	8/306	97.4	95.6 - 99.2
Enferplex*	32/48	66.7	53.4 - 80.0	8/306	96.9	94.8 - 99

- So why the Enferplex test?
- As with all herd screening for disease (as opposed to individual animal testing), the need to maximise specificity is essential, if avoidance of the risk of false positive diagnoses is to be achieved.

Why the Enferplex test?

- The advantage of the Enferplex theoretically over the other serological tests stems from greater specificity due to a quantitative result as opposed to the qualitative results and due to each antigen being analysed separately as opposed to being mixed.
- Using the four-antigen cut-off, the Enferplex test is considered on current evidence to achieve, when used in isolation, close to 100% specificity
- Furthermore, the sensitivity of the test may have been downgraded inadvertently due to the trial design.
- The societies recognise that the development and understanding of serological testing for bTB in camelids is still in its infancy.
- As the Enferplex is based on quantitative, rather than qualitative outputs, and relies on seven antigens, the potential to refine the test, to both maximise sensitivity and specificity, is far higher than with the other tests.

The use of a statistical package to aid diagnosis

- The need for such a statistical approach stems inherent uncertainty in testing for disease in individual animals. Although at the four-antigen level the high specificity of the Enferplex test (which is close to 100%) implies that any positive result can safely be interpreted as confirmation of disease, situations where a proportion of the herd tests positive only at the two-antigen level (with no positives at the four antigen level) need to be addressed.
- At the two antigen level, the specificity of the test is currently estimated as 96.9%.
- This implies that there is significant risk of the occurrence of false positives, which would be highly problematic under a strict herd screening approach.
- Ignoring positive tests, on the other hand, would risk overlooking true TB herd infections.

The use of a statistical package to aid diagnosis

- The statistical package developed by Surefarm Ltd and reviewed and approved by Defra addresses these issues.
- For a given herd population size, the package determines the upper bound for the number of animals that could test positive at the two antigen level while the herd would still be considered highly likely to be disease free (because a diseased herd would almost certainly have generated more positives).
- In cases where small numbers of animals are tested, a high degree of confidence that all the animals in the herd are disease-free may not be attainable.
- In these cases, any positive test will need to be treated as potentially a true positive however Defra do plan to repeat test such animals prior to any final decision.