Performance Characteristics of a Multiplex Serology Test for Bovine Tuberculosis in Cattle

Gordon Harkiss[1] , Amanda O'Brien [2], Alastair Hayton[3], Keith Cutler[3], Andy Adler[3], Darren Shaw[4], Clare Whelan [2], John Clarke[2], Neil Watt[1]

[1]MV Diagnostics Ltd, Roslin Innovation Centre, Edinburgh, U.K., [2]Enfer Scientific Ltd, M7 Business Park, Naas, Ireland, [3]Synergy Farm Health Ltd, Crewekerne, UK.,[4]Roslin Institute, University of Edinburgh, Easter Bush, UK.

Background

- Failure to eradicate Bovine Tuberculosis (bTB) may be partly due to the relative lack of sensitivity of the commonly-used Single Intradermal Comparative Cervical Tuberculin (SICCT) test and Interferon Gamma (IFNγ) tests, and their focus solely on Th1 cell mediated responses.
- We have developed a multiplex serological test for bTB. The test is based on a standard indirect ELISA format with two differences: the wells have 11 M. bovis antigens spotted onto the bottom surface rather than coated with a single antigen; the endpoint is chemiluminescence rather than colour development. These two factors result in increased sensitivity compared to colourimetric ELISA tests.
- The test sensitivity was assessed using serum samples from cattle that were positive by the SICCT test, IFNγ test, presence of visible lesions at post mortem, culture of *M. bovis*, or a combination of these. The blood samples were taken 4-30 days after SICCT to take advantage of the known 'boosting' effect of the injection of PPD. A high sensitivity and high specificity settings of the test were established by adjusting cut-offs for eradication or screening purposes respectively. The specificity was determined using sera from herds that were deemed to be free of bTB on the basis tuberculin test history, long term freedom from bTB.

Results

Diagnostic specificity

bTB-free animals	Number of animals	Statistical Variable*	High Sensitivity setting	High Specificity setting
Non-boosted	4258	DSp CI	98.4% 98.0 – 99.0	99.7% 99.5 – 99.8
Boosted	339	DSp CI	98.8% 97.0 - 99.5	99.1% 97.4 - 99.8

Kappa analysis

Bovine TB infection status	Kappa value*	95% CI	Interpretation
M. bovis culture and bTB free	0.82	0.79 – 0.86	**Almost perfect agreement**
Visible lesions and bTB free	0.94	0.93 – 0.95	Almost perfect agreement
SICCT and bTB free	0.92	0.91 – 0.93	**Almost perfect agreement**
IFNγ and bTB free	0.90	0.89 – 0.91	Almost perfect agreement

Diagnostic likelihood ratio analysis

Bovine TB infection status	Positive LR	Negative LR	DOR
M. bovis culture and bTB free	58.8	0.062	**952.7**
SICCT and bTB free	59.1	0.060	1020.3
IFNγ and bTB free	56.4	0.100	**551.5**

The results showed:

- **The diagnostic specificity was 98.4% and 99.7% using the high sensitivity and high specificity settings of the test respectively.**
- **The diagnostic sensitivity was between 93.9 in M. bovis culture positive animals and the relative sensitivity was 90.0 – 96.1% depending on the comparator used at the high sensitivity setting.**
- **Kappa values showed almost perfect agreement between the test results and diagnostic comparators used.**
- **Positive and negative diagnostic likelihood ratios of 56.4 – 58.8 and 0.060 – 0.100 were obtained respectively using 3 different comparators indicating that the test provides good diagnostic evidence of the infection being either present or absent respectively (Caraguel & Colling, 2021).**
- **The test detected infected animals missed by the SICCT and IFNg tests.**
- **There was a good correlation between the number of antigens recognised by antibody and the presence of visible lesions in SICCT positive, IFNg positive or in-contact SICCT negative animals.**
- **The use of different antigen rules might allow a risk based approach to managing bTB infection in breakdown herds.**

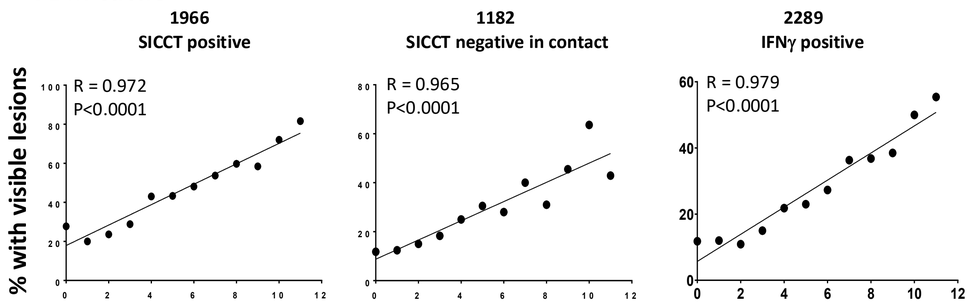
Diagnostic sensitivity

bTB infection status POSITIVE by:	Number of animals	Statistical Variable*	High Sensitivity setting	High Specificity setting
M. bovis culture Boosted	214	DSn CI	93.9% 89.9 - 96.4	93.9% 89.9 - 96.4
Visible Lesions Boosted	1179	RSn CI	96.1% 94.8 - 97.1	93.3% 93.3 – 95.9
SICCT test Boosted	1949	RSn CI	94.3% 93.2 - 95.2	91.9% 90.6 - 93.0
IFNγ test Boosted	1341	RSn CI	90.0% 88.3 - 91.5	85.6% 83.6 - 87.4

Relative Sensitivity in SICCT or IFNg negative in contact animals from bTB infected herds

Bovine TB infection status	Number of animals	Statistical Variable*	High sensitivity setting	High specificity setting
SICCT negative VL positive	143	RSn CI	88.8% 82.6-93.0	83.2% 76.2-88.5
IFNγ negative VL positive	55	RSn CI	83.6% 71.7-91.2	74.6% 61.7-84.2

Relationship between the number of antigens recognized by antibodies and VL in cattle



Risk based approach to bTB management using different antigen rules in SICCT-positive cattle

Antigen Rule	Total number VL + NVL (n = 1789)	Percentage with VL	Percentage of total VL	*Odds ratio for having VL	Dsp
0 or 1	126	23.8	3.0	-	-
2 or more	1840	53.7	97.1	3.7	98.43
3 or more	1768	55.0	95.5	3.9	99.41
4 or more	1713	55.9	94.0	4.1	99.79
5 or more	1635	56.6	91.0	4.2	99.88
6 or more	1411	58.8	81.5	4.6	99.95
7 or more	1061	62.7	65.3	5.4	99.98
8 or more	806	66.0	52.3	6.2	100
9 or more	522	69.9	35.9	7.4	100
10 or more	339	74.9	26.4	9.6	100
11	118	81.4	9.4	14.0	100

- **Significance/Impact**: Enferplex Bovine TB Antibody test shows high sensitivity and specificity and detects infected animals which are missed by SICCT and IFNg tests. This could prove useful in managing bTB outbreaks.
- The correlation between the number of antigens recognised by antibody and the presence of visible lesions suggests that a risk based approach could be applied to identify animals mostly likely to be shedding bacteria. This could be used to selectively remove high risk animals in a way that is sustainable when there are large numbers of seropositive animals.
- **Reference**

Caraguel & Colling, 2021. *Rev. Sci. Tech. Off. Int. Epiz.*, 40 (1), 299-309).