

PERFORMANCE OF THE ENFERPLEX TB ASSAY IN GB CATTLE AND ASSESSMENT OF POTENTIAL SUITABILITY AS A DIVA TEST

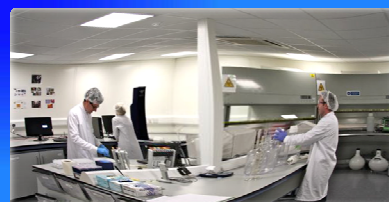
John Clarke,
Technical Manager



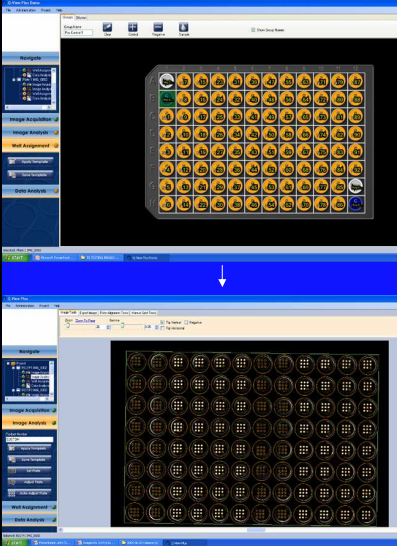
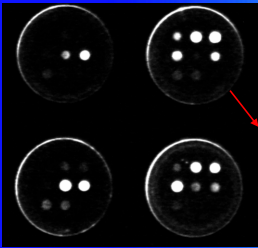
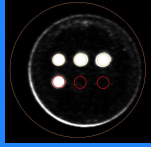
Who are Enfer Scientific?



- Established 1990
- Veterinary Diagnostics
- We have developed a number of ELISA based and Molecular assays for equine, bovine, ovine and porcine species
- Sample logistics and screening is one of our core competencies and we have been involved in several successful projects with the Irish Dept of Agriculture.
- We have a strong R&D focus (>30% of staff)



Results Processing

RLUs = 0 – 65,500

Ag ID	RLU
Ag 1	18750
Ag 2	45600
Ag 3	51400
Ag 4	33600
Ag 5	1500
Ag 6	31350
Ag 7	2300
Ag 8	2500
Ag 9	300

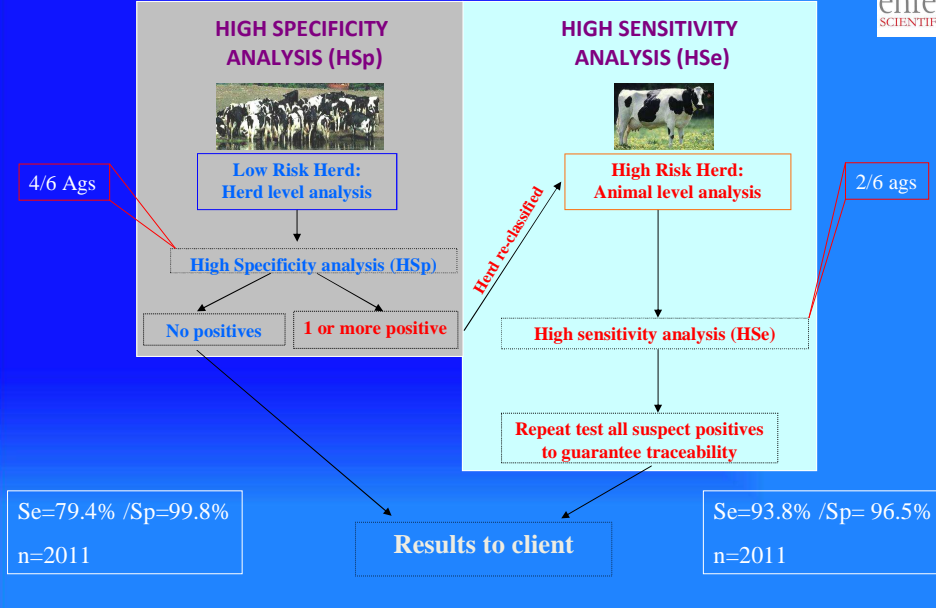
High signal

Background signal <1000

Excel Macro

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Interpretation of the Enferplex TB Assay



HIGH SPECIFICITY ANALYSIS (HSp)

Low Risk Herd: Herd level analysis

4/6 Ags

High Specificity analysis (HSp)

No positives | 1 or more positive

Se=79.4% /Sp=99.8%
n=2011

HIGH SENSITIVITY ANALYSIS (HSe)

High Risk Herd: Animal level analysis

2/6 ags

High sensitivity analysis (HSe)

Repeat test all suspect positives to guarantee traceability

Se=93.8% /Sp= 96.5%
n=2011

Herd re-classified

Results to client

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Performance of the Enferplex TB Assay in GB Cattle and Assessment of Potential Suitability as a DIVA Test

Clare Whelan¹, Adam O. Whelan², Eduard Shuralev¹, Hang Fai Kwok³, Glyn Hewinson², John Clarke¹, H. Martin Vordermeier^{2*}

¹Enfer Scientific, Unit T, M7 Business Park, Newhall, Naas, Kildare, Ireland;

²Veterinary Laboratories Agency (VLA) – Weybridge, TB Research Group, Addlestone, Surrey KT15 3NB, United Kingdom;

³Fusion Antibodies Ltd., Springbank Industrial Estate, Belfast, BT17 0QL, Northern Ireland



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Study set-up – 3 parts

- Serum samples were collected from **96 SICCT-positive cattle** from known GB bTB incidence herds. All animals presented with visible lesions typical of bTB at slaughter and were culture-positive. } 1
- In addition, **93 sera** were collected from skin test negative animals from herds without recent TB history located in bTB non-endemic regions of GB. } 1
- A group of **39 male calves** (Holstein or Holstein crosses) were neonatally vaccinated with BCG Danish (Staten Serum Institut, Copenhagen, Denmark) and serum was collected 6 months post-vaccination. } 2
- A further group of 21 animals was composed of Holstein or Holstein-cross male calves that were experimentally infected via the intratracheal route with 2,000 CFU of the GB *M. bovis* cattle field isolate AF2122/97. Sampled monthly. } 3
- All samples in the study were coded by VLA until after results were reported



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Performance of the Enferplex TB assay in field animals.

Part 1

	Interpretation criteria:				
	Enferplex TB[level-5]	Enferplex TB[level-4]	Enferplex TB[level-3]	Enferplex TB[level-2]	Enferplex TB[level-1]
Sensitivity (n=96) ¹ % (95% CI)	77.1 (67.4, 85.0)	79.2 (69.7, 86.8)	81.3 (72.0, 88.5)	82.3 (73.1, 89.3)	86.5 (78.0, 92.6)
Specificity (n=93) ² % (95% CI)	100 (96.1, 100)	97.9 (92.4, 99.7)	94.6 (87.9, 98.2)	91.4 (83.8, 96.2)	79.6 (70.0, 87.23)

¹SICCT reactor animals, with visible lesions and positive *M. bovis* culture. ²Skin test negative cattle from TB-free herds.

n=96 confirmed positive animals

n=93 confirmed negative animals

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Part 1

HSp cut-off

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Part 1

HSe cut-off

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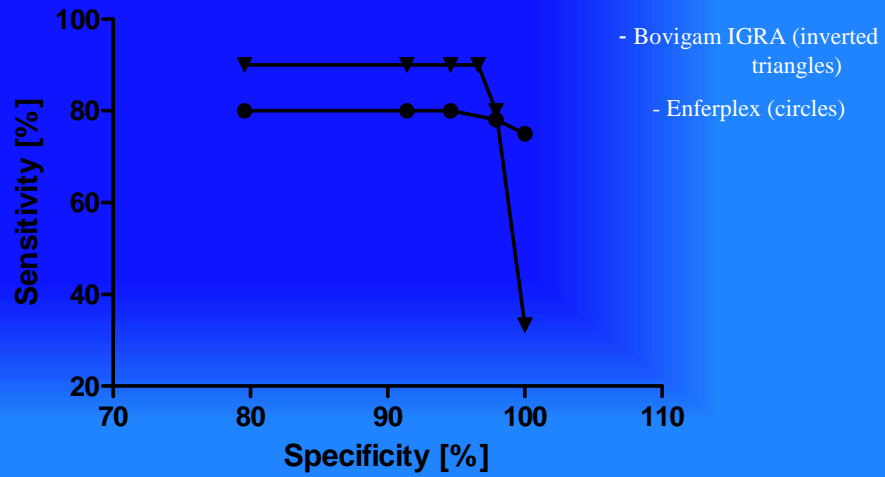
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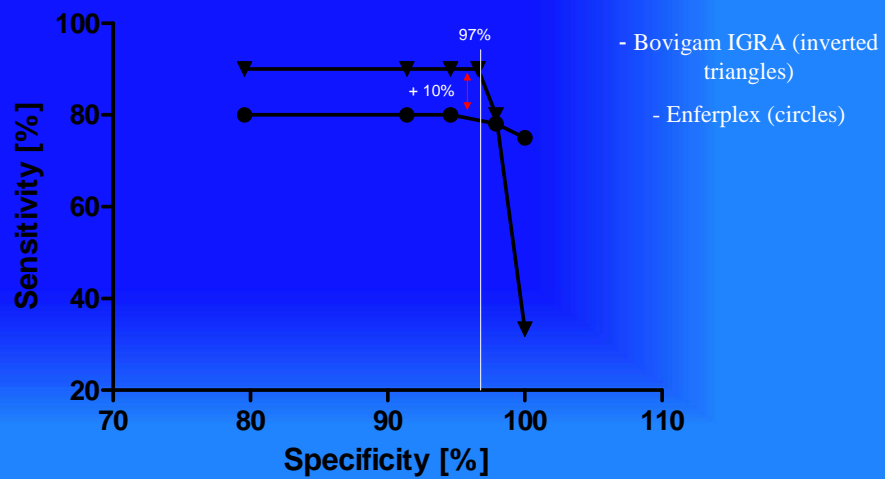
Comparison of Bovigam IGRA and Enferplex TB immunoassay sensitivities. Part 1



n = 30

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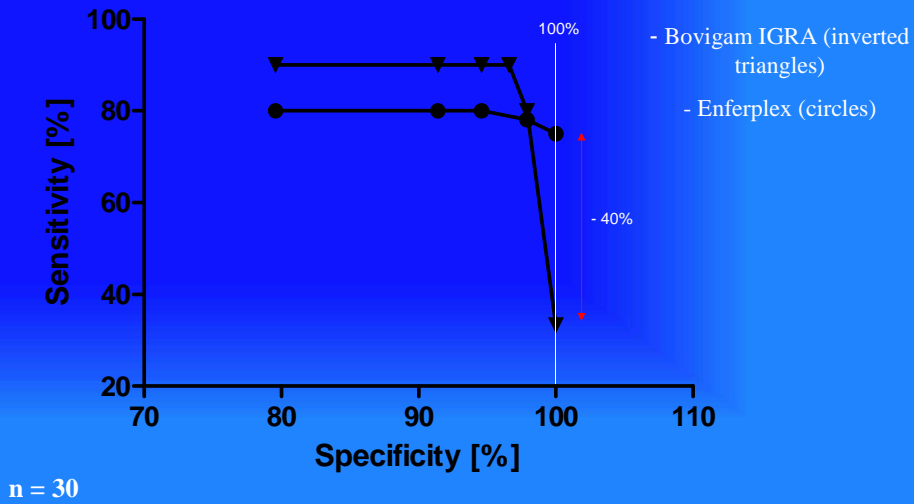
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Comparison of Bovigam IGRA and Enferplex TB immunoassay sensitivities. Part 1



Vaccinated animals - DIVA potential. Part 2

Test performed	% test positive (95% CI)	Numbers test positive	P-values ⁴
EnferplexTB ¹	0 (0/9)	0/39	NA
SICCT, standard ²	66.6 (49.8, 80.9)	26/39	< 0.0001
SICCT, severe ²	87.2 (72.5, 95.7)	34/39	< 0.0001
Bovigam ^{IM} , PPDB - A ³	38.5 (23.4, 55.4)	15/39	< 0.0001
Bovigam ^{IM} , ESAT6/CFP10 ³	7.7 (1.6, 20.8)	3/39	0.2403

Neonatal BCG vaccination, serum samples were collected 6 months post-vaccination 2-5 weeks after SICCT application.

¹Animals tested negative applying any of the 5 interpretation criteria.

²SICCT using standard or severe interpretation of the test.

³Bovigam IGRA test using PPDB-B and PPDB-A (cut-offs: PPDB minus PPDB-A > 0.1 OD450 values) or cocktail of ESAT-6 and CFP-10 derived synthetic peptides (cut-off: peptide cocktail minus nil antigen control > 0.1 OD450 values).

⁴Statistical analysis: Fisher's exact test compared to EnferplexTB assay. To account for multiple comparison errors, a P-value smaller than 0.0125 was used to define statistical significance. NA, not applicable.

n = 39

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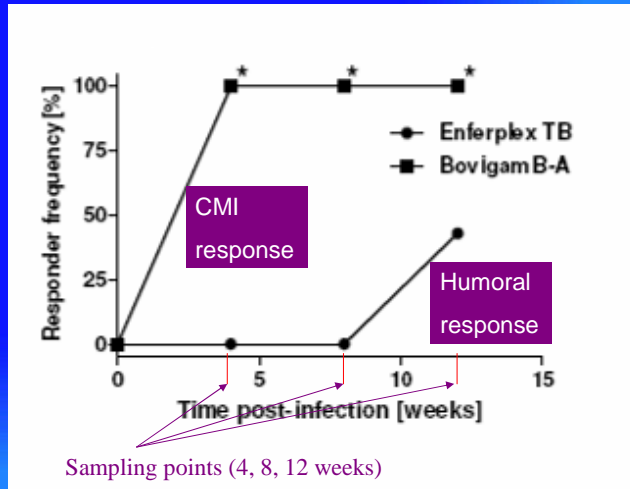
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n = 39

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Kinetic of Bovigam IGRA and Enferplex TB multiplex responses in experimentally infected cattle. Part 3



Experimentally infected via the intratracheal route with 2,000 CFU of the GB *M. bovis*

n = 21

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Points to consider

- The use of the SICCT and IGRA assays together significantly increase the proportion of diseased animals that are detected and one would expect a similar outcome using the Enferplex TB assay in combination with skin tests and/or IGRA assays.
- However, further comparative test validations are required to fully appreciate whether the multiplex serology assay can be used alone as a DIVA test, or in combination with these tests probing cell-mediated immunity to maximize sensitivity and specificity.

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Main conclusions

- The present study has re-affirmed the Enferplex TB assays high sensitivity (77%) even at maximal specificity levels (100%).
- Our study also demonstrated that the Enferplex TB assay is a potential assay that would have considerable logistical advantages over blood tests using cellular immunity as read-out, and could therefore contribute to the cost-effective application of a cattle vaccine policy.
- Furthermore, it potentially could be used in combination with SICCT and BOVIGAM IGRA ante-mortem tests as part of a diagnostic programme aimed at detecting animals at different disease stages.

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Acknowledgements

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Enfer Scientific

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Thank you

