



## Development and Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Bovine Tuberculosis

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## Detection of bovine tuberculosis

- **Skin Tests**

- Measurement of delayed-type hypersensitivity response to the intradermal injection of purified mycobacterial protein (PPD or tuberculins)
- Caudal Fold Test (CFT) or Single Cervical Test (SCT)
- Comparative Cervical Test (CCT)
- Standard or primary test: EU (64/432/EEC), OIE, USDA

- **Gamma-Interferon Test (BOVIGAM™)**

- Measurement of cell mediated immune response
- Ancillary test: EU (64/432/EEC), OIE, USDA
- Parallel/serial testing strategies
- Primary test in Mexico

## Detection of bovine tuberculosis

- **Serology Tests**
  - Measurement of serologic antibody responses to M. Bovis antigens
  - MPB70, MPB83, etc.
  - Not approved according to EU and USDA  
but listed at OIE

## Detection methods from Thermo Fisher Scientific for bTb:



## Bovigam™, bovine Gamma Interferon Test kit

- in vitro laboratory test for the diagnosis of bovine tuberculosis in cattle, sheep, goats, buffalo, bison and other bovidae
- monoclonal antibody-based sandwich enzyme immunoassay (EIA) for the detection of interferon- $\gamma$  (IFN- $\gamma$ )
- widely used as an ancillary test to the tuberculin skin test



## Tuberculin PPD for skin test

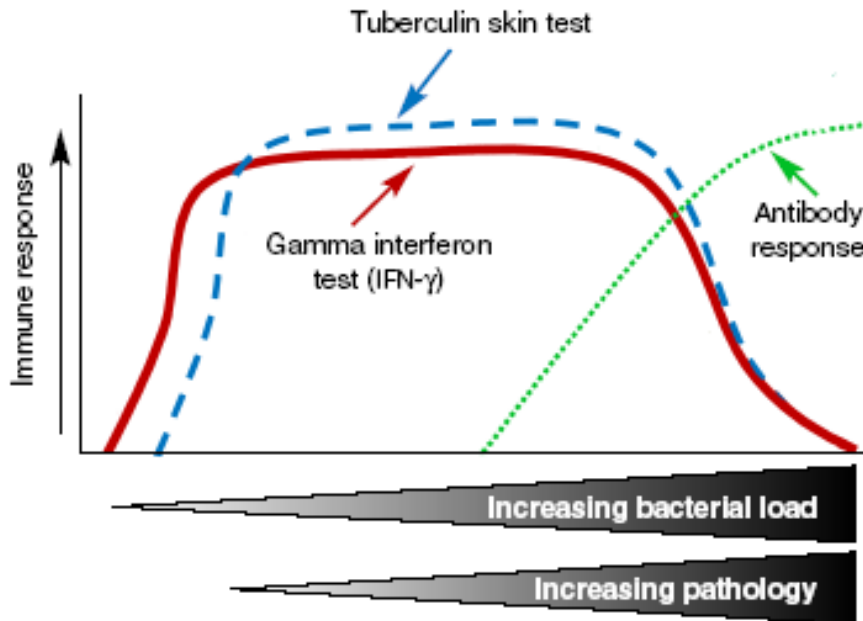
- High quality product – GMP facility (formerly Production Lelystad)
- Standardized product - Eu. Ph./OIE
- Matched Avian/Bovine Product (SICCT)
- Trusted by leading TB eradication programs (UK, Ireland, France)
- Potency tested in guinea pig assay and 2 - 3 assays on naturally TB infected bovines per year in Ireland



# Introduction

## Detection following experimental infection with *M. bovis* (Waters et al., 2011)

Test	dpi
BOVIGAM®	14 – 17
Serology	90 – 100



- CMI is the earliest immune response after infection with *M bovis*
- Antibody responses develop later than CMI responses but are a useful tool to complete the diagnostic portfolio for bTB testing
- Serological tests can be used in anamnestic animals and in very low prevalence areas as a confirmatory test

Vordermeier *et al.*, The Veterinary Record, July 10, 2004

## **Skin Test:**

- The only test approved for official herd testing as a primary test (OIE, EU, USDA)

## **BOVIGAM Test:**

- Recognized by many authorities (USDA, FLI, EFSA, SENASA, APHA, DFAM etc.) as ancillary test to Skin Test
- OIE approval to use BOVIGAM™ as primary test and to confirm a herd free of TB after outbreak, since may 2015

## **Serology Test:**

- Not official tuberculosis diagnostic tool (but listed at OIE)

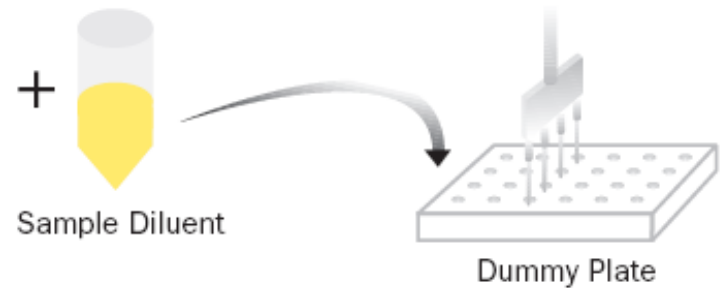


## ELISA

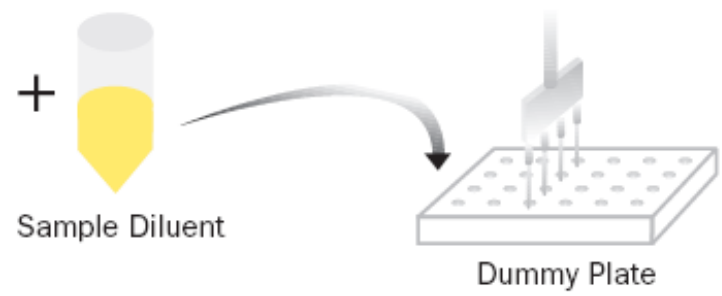
- Mycobacterium bovis antigen
- Blocking
- Serum Samples
- Conjugate
- Stop
- Measurement at  $\lambda = 450/620$  nm
- Analysis: Cut-Off 15% PP

$$PP = 100 * \frac{OD\ sample - OD\ NC}{OD\ PC - OD\ NC}$$

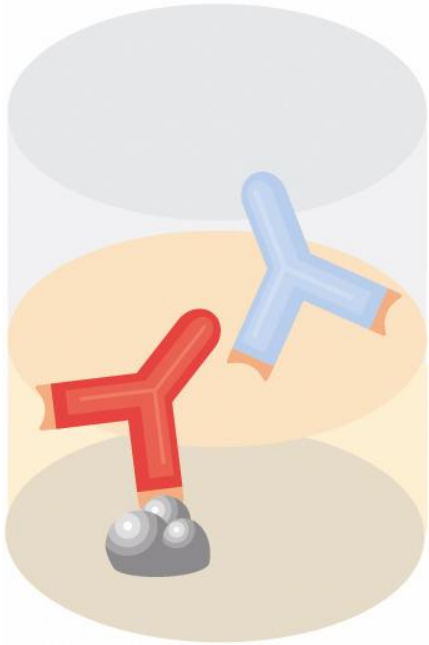
## Sample Preparation



Pos. Con  
Neg. Con

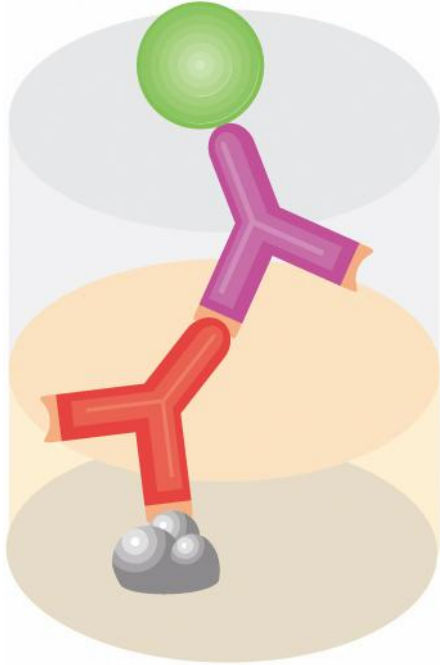


## Sample incubation



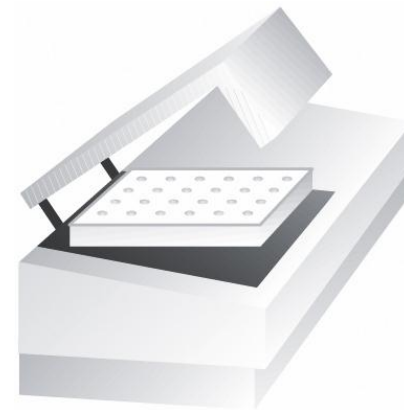
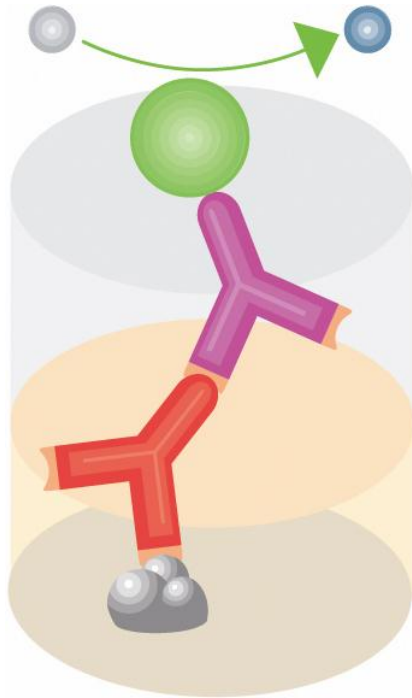
- Transfer of controls and samples from the dummy plate to the Test Plate.
- Final dilution 1:100
- Sample incubation for 60 minutes
- Washing of plate

## Conjugate incubation



- Adding of POD labelled detection antibody
- Conjugate incubation for 60 minutes
- Washing of plate

## Detection



- Adding of substrate (TMB) for 15 minutes
- Stopping of color development by adding of Stop Solution
- Reading of the optical density at  $\lambda = 450 \text{ nm} / 620 \text{ nm}$

## Determination of Sensitivity and Specificity

Sensitivity / Specificity – results

- Data from 835 samples
- Cut-off defined with ROC

Cut-off (PP)	Sensitivity %	95% CI	Specificity %	95 % CI
> 15 %	65.99	60.3 - 71.4	98.34	96.9 - 99.2

Origin	total samples	Status	
		negative	positive
APHA 105	105	105	0
APHA 99	99	99	0
IRL	50	50	0
USDA 300	300	200	100
APHA positive	11	0	11
UCD 150502	38	0	38
Chamau	56	56	0
APHA 138	138	0	138
USDA spec.	38	31	7
Σ	835	541	294

## Determination of the Analytical Specificity

Status	Number of samples	Result Neg / Pos	Origin
<i>M. Paratuberculosis</i> positive	48	48 / 0	USDA (7), NL (10), IRL (31)
<i>M. kansasii</i> positive	8	8 / 0	USDA
<i>M. avium</i> positive	8	8 / 0	USDA

No Cross Reactivity was detected

## Determination of Variances

	Plate	Mean OD	Stdev	% CV	Variance
Positive sample	1	0.850	0.026	3.12	Intraplate variance
	2	0.944	0.028	2.95	
	3	0.948	0.023	2.44	
	1 - 3	0.914	0.052	5.70	Interplate variance
Negative sample	1	0.021	0.001	4.72	Intraplate variance
	2	0.021	0.001	5.02	
	3	0.022	0.001	6.20	
	1 - 3	0.022	0.001	6.19	Interplate variance



# Conclusion

The PrioCHECK™ Tuberculosis Complex Ab is a suitable and reliable tool for confirmatory testing of bovine tuberculosis.

The determined sensitivity of 66 % and specificity of 98 % makes it an ideal tool to complement the diagnostic portfolio for the detection of bovine tuberculosis.

# Acknowledgements

- Prof. Dr. Martin Vordermeier, Animal and Plant Health Agency (APHA)
- Prof. Dr. Eamonn Gormley - University College Dublin
- Jeffrey T. Nelson, DVM, MPH, DACVPM, USDA · APHIS
- Ray Waters, U.S. Department of Agriculture
- Prof. Dr. Hans-Rudolf Wettstein, ETH Zürich



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