The Enferplex TB test and the role of serology in TB diagnosis

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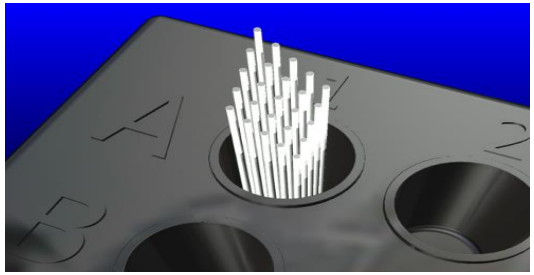
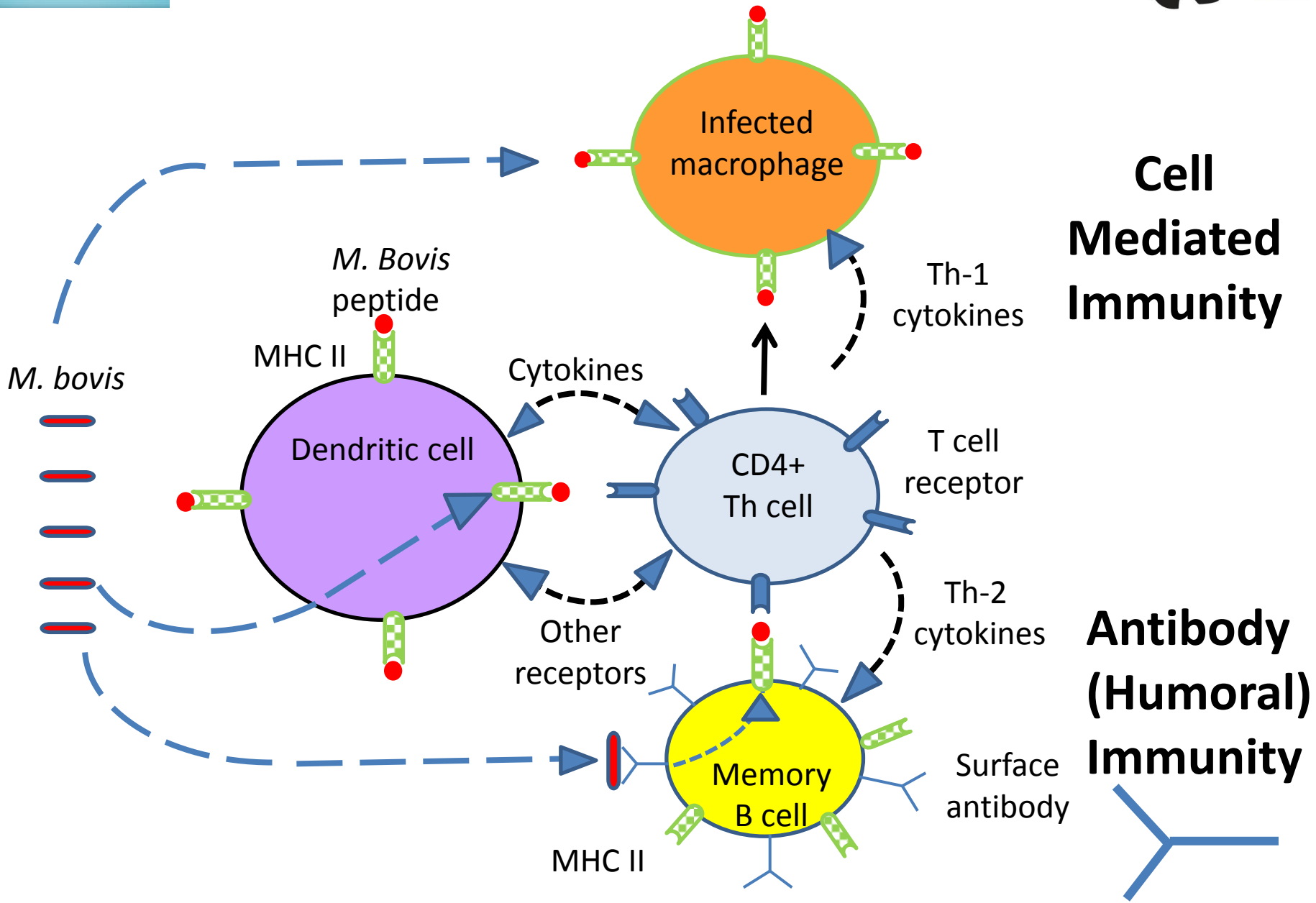
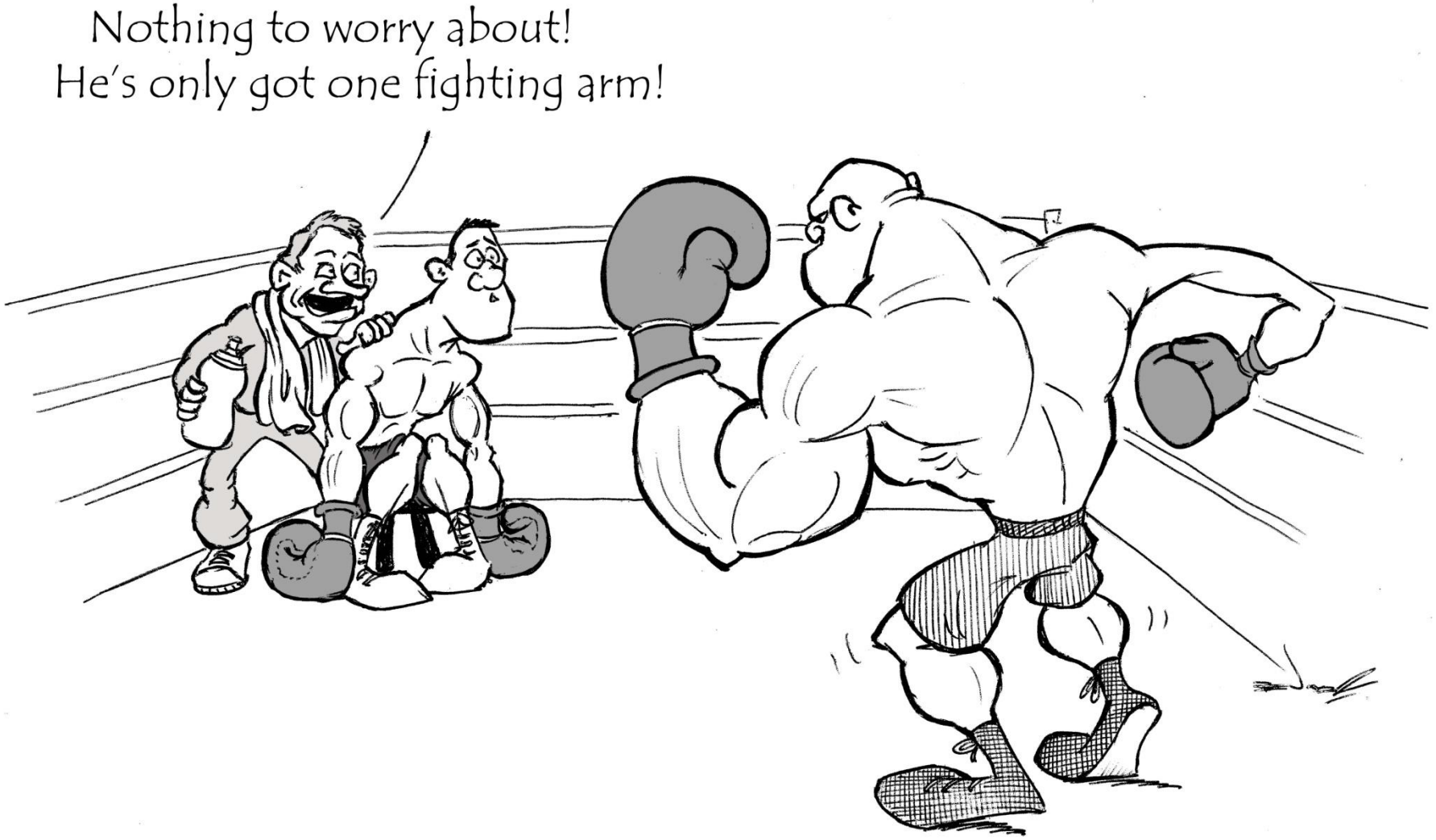


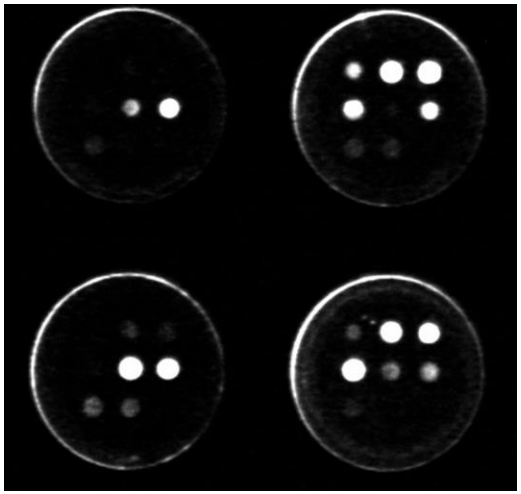
Immune responses to *M. bovis*

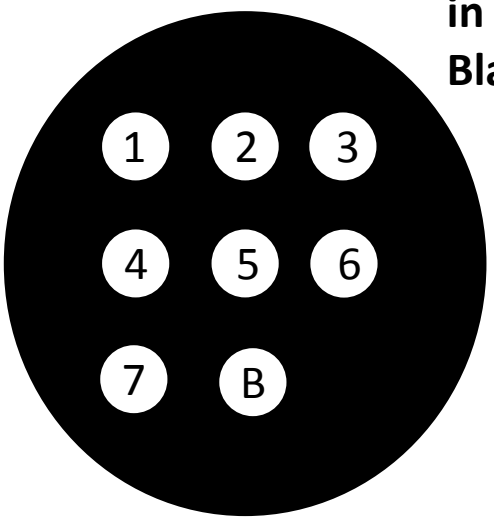



Nothing to worry about!
He's only got one fighting arm!

Enferplex Bovine TB antigen array

Antigen array layout





Antigens arrayed in spots 1 – 7
Blank spot B

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
Blank	300

2 Antigen Rule - positive result obtained against 2 or more of the 7 TB antigens is required before sample is labelled as "**POSITIVE**"

Enferplex results in cattle

- **Whelan *et al* (2008) IRELAND**

486+/522	Sensitivity	93.1%	VL, histopathology, and/or culture positive
1465-/1489	Specificity	98.4%	Free of TB for at least 5 years

- **Whelan *et al* (2010) UK**

76+/96	Sensitivity 79.2 %	SICTT +, VL, *M. bovis* +
91-/93	Specificity 97.9 %	SICCT-, no recent history of TB

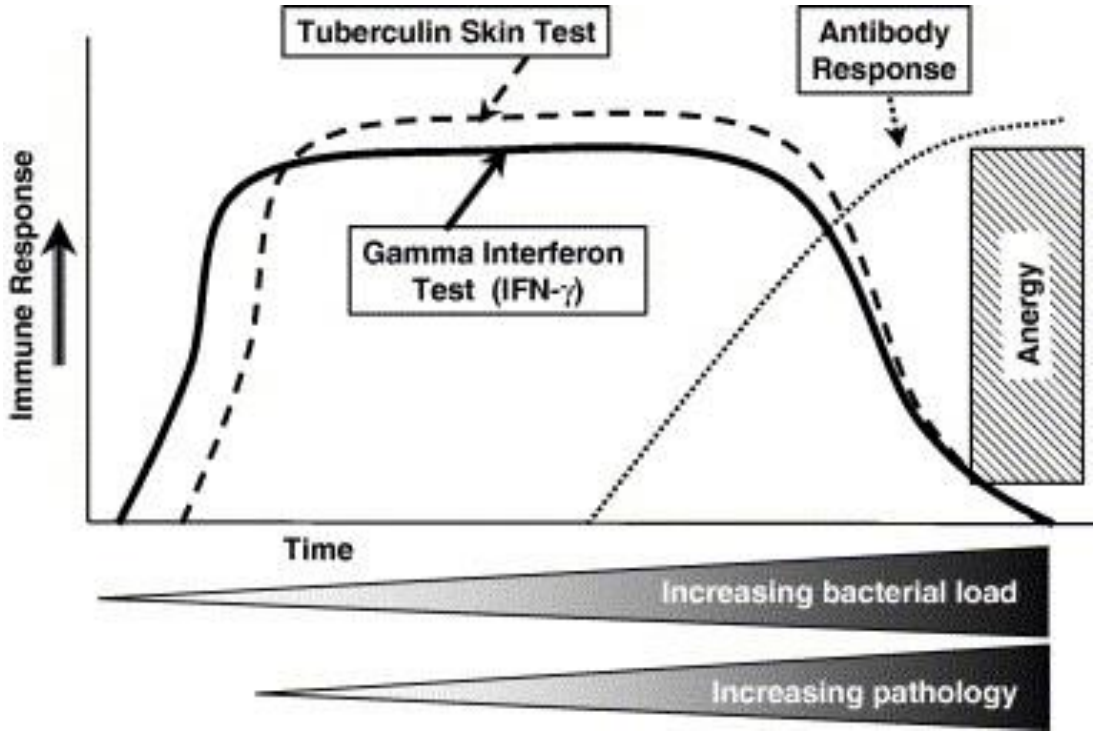
- **Casal et al (2014) SPAIN**

28+/33	Sensitivity 85%	*M. bovis*+ and/or VL
16+/18	Sensitivity 95%	VL
59-/60	Specificity 98.3%	SICTT-, no recent history of TB

Propositions

1. Enferplex antibody test picks up infected animals missed by cell mediated tests (skin test; IFNγ test)
2. Combined use of CMI and Enferplex antibody tests give higher sensitivity than either alone
3. Combined use of CMI and Enferplex antibody tests will be lead to faster more efficient eradication of infection
4. Enferplex could be used to test the infection state of badgers

Is there any evidence that antibody tests detect infected animals missed by CMI tests?



The Interferon-gamma field trial: Background, principles and progress
Vordermeier, Goodchild , Clifton-Hadley, de la Rua Dominech
Vet Record 155: 37-38 (2004)

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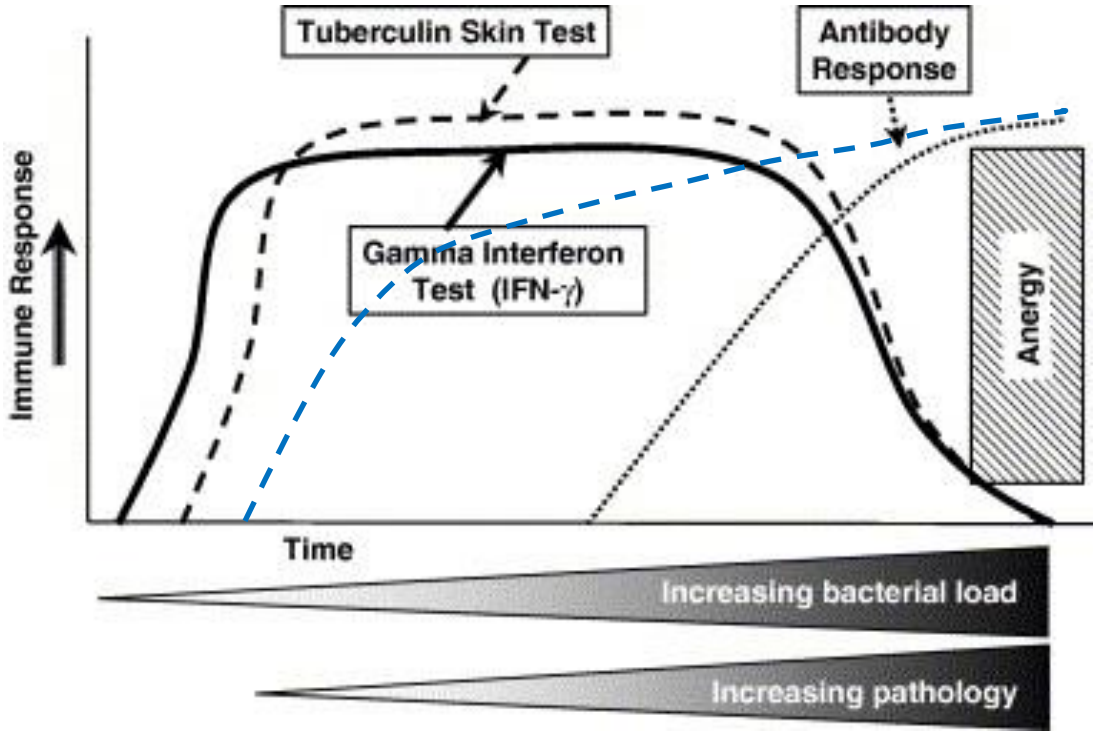

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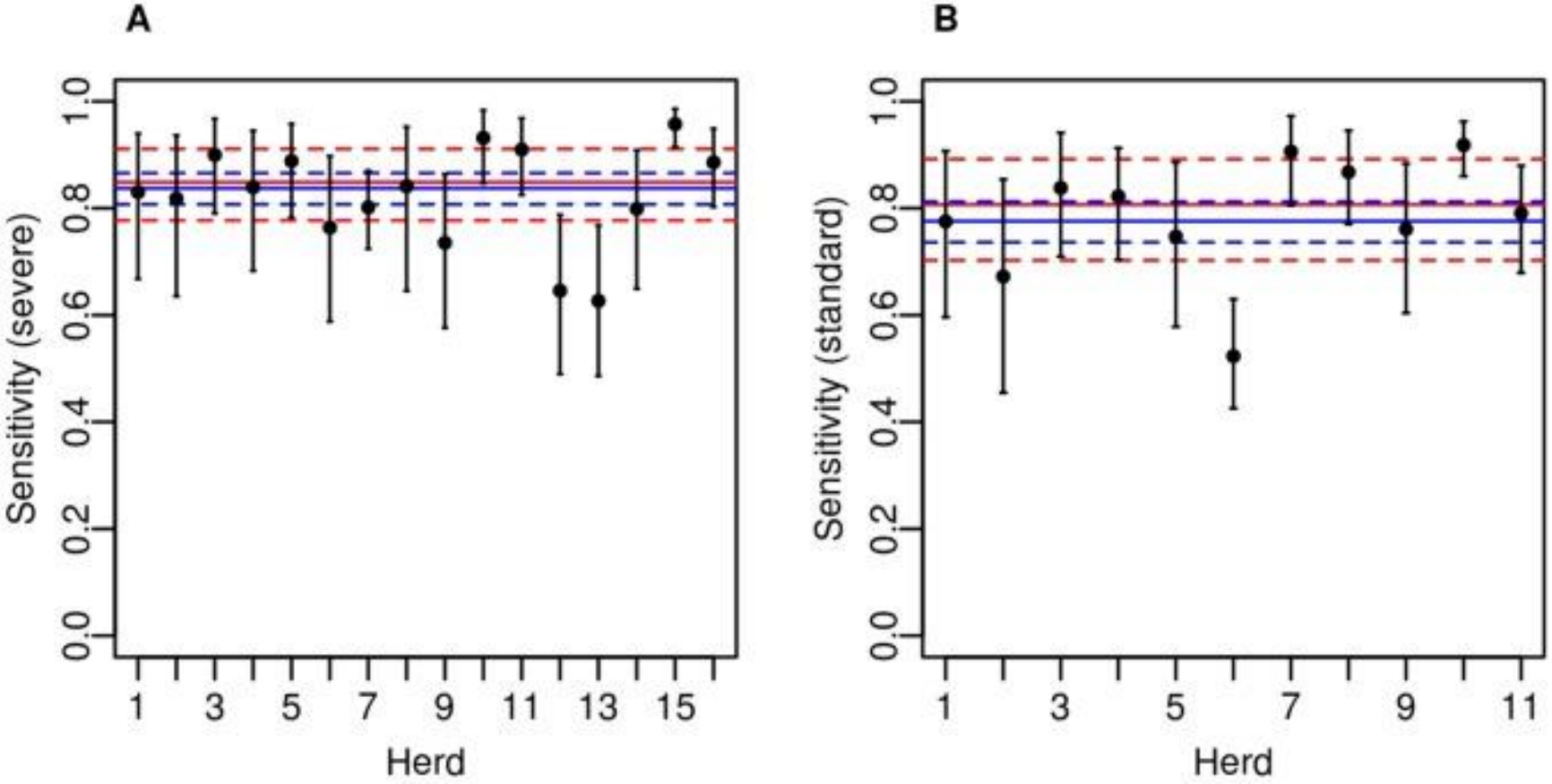
Potential causes of false negative results to tuberculin/IFNγ tests

- Overwhelming or generalised infection with *M. bovis* (anergy)
- Co-infection with Johnes disease
- Vaccination against Johnes disease
- Co-infection with (or pre-exposure to) environmental mycobacteria
- Concurrent infection with pathogens that depress the immune system, e.g. BVDV, liver fluke

Modified from de la Rua Domenech *et al* (2006), Res. Vet. Sci. 81: 190–210

Karolemeas *et al* (2012)

Estimation of the Relative Sensitivity of the Comparative Tuberculin Skin Test in Tuberculous Cattle Herds Subjected to Depopulation PLOS One 7: e43217



- Undetected infected cattle may remain in some breakdown herds that are officially released from movement restrictions.
- Almost a quarter of all breakdowns that are de-restricted recur in the same herd within 12 months, and 38% recur within 24 months [29].

Is there any evidence that the Enferplex TB test detects infected animals missed by CMI tests?

Cattle with lesions and/or acid fast bacteria and/or *M. bovis* culture+	Enferplex TB test	
	Number positive/tested	%
Inconclusive skin test	29/34	85.3
Negative skin test	24/26	92.3
Total	53/60	88.3%

Whelan *et al* (2011) J Vet Diagn Invest 23:499-503

MV DIAGNOSTICS

synergy FARM HEALTH

enfer SCIENTIFIC

Is there any evidence that the Enferplex TB test detects infected animals missed by CMI tests?

AHVLA SE3263 Study

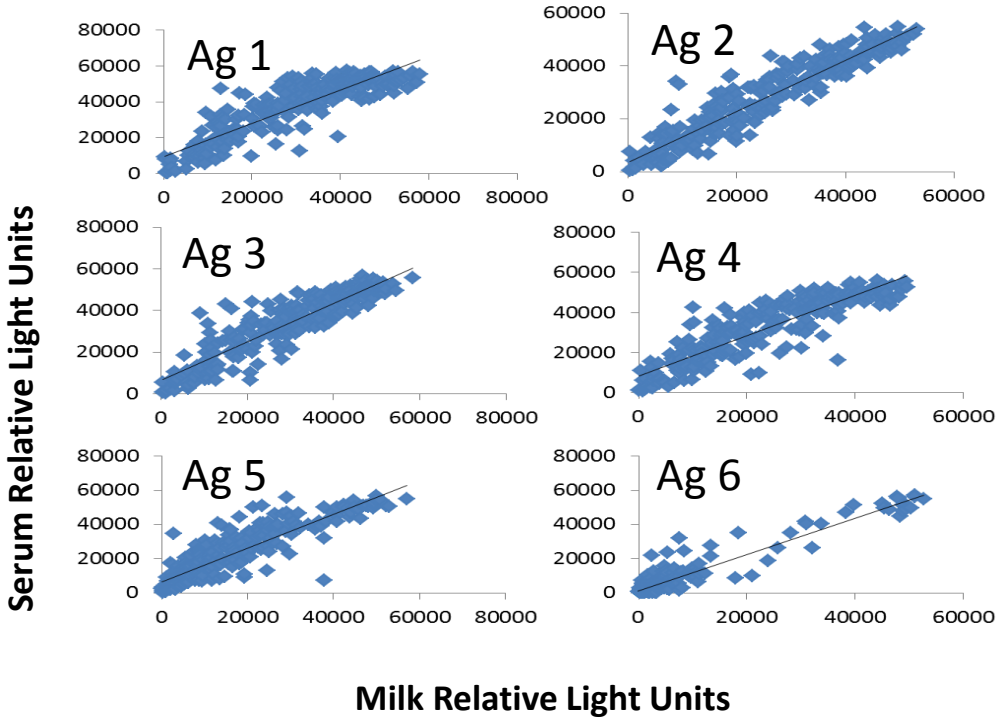
- Cattle from 555 TB breakdown herds after removal of skin test reactors
- Total = 29,612 samples tested by IFNγ test
  - 1399 IFNγ positive (3%)
- 1012 IFNγ negatives selected for Enferplex testing
  - 114/1012 Enferplex positive (11.1%)
  - ?/29,612

SE3263 Validation of new serology tests for bovine tuberculosis in cattle
Rhodes & Vordermeier, 2012 AHVLA Report

Goat TB outbreak

- 500 dairy goats, all vaccinated against *M. paratuberculosis,* purchased 200 goats from one source in July 2013, clinical problems seen in Sept 2013, TB diagnosed with visible lesions and *M. bovis* cultured.
- Skin tested in Dec 2013, 150 reactors, most lesioned and majority from purchased herd
- Skin tested March 2014 (> 500 animals) and 14 reactors disclosed – all slaughtered.
- Nine days later tested by Enferplex, 339 positive in serum
- 5 out of 9 goats sent for PM (8 skin negative; 1 skin positive) had visible lesions.
- 78 skin-negative goats became reactors in May 2014 – 38/39 tested previously were antibody-positive

Correlation between goat serum and milk TB antibody levels



Spearman's correlation	Antigen					
	1	2	3	4	5	6
Coefficient rho	0.875	0.942	0.913	0.901	0.894	0.787
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Enferplex TB test in Badgers

Badgers (Ireland)

- Sensitivity 56.7%
- Specificity 96.9%

Current Situation

- Looking at the potential for use of blood spots and or saliva as testing substrate
- Use it in LFD format as pen side diagnostic?
- Needs funding and development
- Working with Woodchester Park
- Irish trial ongoing, 1000-2000 badgers from vacc non-vacc areas

The Way Forward – trial Enferplex TB under GB conditions

- **Pre-validation trial to optimise antigens and set cutoffs for UK condition**
  - 10 TB-free and 10 TB-infected (5 new breakdowns, 5 chronic) dairy herds
  - All from HRA, herd size 100-200 cows per herd
  - Sample blood and milk at SICTT disclosing test and 10-30 days later
  - Needs Ministerial approval – the request has been made

- **Validation trial**
  - Criteria to be agreed in advance with DEFRA
  - Model on BAS trial, 50 true positives and 300 true negatives

If the above are successful and show commercial viability Sure Farm will offer private testing for breakdown herds and other situations

SUMMARY and CONCLUSIONS

- **Enferplex test can detect infected animals missed by skin and IFNγ tests in cattle, goats and other species.**

- **Enferplex could be used in combination with SICCT for:**
  - **Clearing infection from breakdown herds more effectively, especially where infection is persistent or there is co-infection with Johnes, BVDV, Fluke**
  - **Pre-purchase / movement testing**
  - **Informing on need for herd clearance**
  - **Resolution of inconclusive reactors**
  - **Detecting epidemiologically important animals - shedders and those that may become shedders and pose the greatest risk**

- **The high correlation between serum and milk antibody results suggests that milk could be used for sero-diagnosis**