

Experiences with detection of *Coxiella* exposure using Q-detect™, an IGRA for Q-fever

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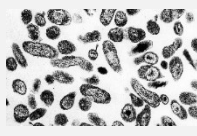


Introduction

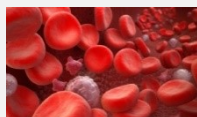
Q fever is a zoonosis that affects small ruminants and cattle. Q fever is caused by the obligatory intracellular bacterium *Coxiella burnetii*. This pathogen grows within the phagosome of macrophages. IFN γ is key in fusion of the phagosome with the lysosome resulting in elimination of bacteria (Ghigo et al., J Immunol. 169:4488, 2002). During the Dutch epidemic (2007-2011) a cellular immunity-based diagnostic test for exposure to *Coxiella* was set-up by Radboudumc (Nijmegen, NL) (Schoffelen et al., Clin Infect Dis. 56:1742, 2013) and further developed by Innatoss (Oss, NL). The patent-protected Q-detect™ test is a whole blood IFN γ -release assay (IGRA) registered in the Netherlands.

Q-detect™, an IGRA for Q-fever, is more effective in identifying exposure to *Coxiella*

***Coxiella* antigen** was produced by Wageningen Bioveterinary Research. Strain Cb2009-02629 was isolated from goat placenta and cultured under cell-free conditions. Antigen characterization was performed by Innatoss.



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Q-detect™ Whole blood was incubated in duplicate for 24 h at 37 °C with heat killed *Coxiella*. IFN γ , and when applicable TNF α , were measured by ELISA.

Data analysis: Log-transformed values were used for calculating the mean **IFN γ production**. The **relative *Coxiella* response** compared to the positive control was determined using the formula $((\log[\text{cox}]-\log[\text{neg}])/((\log[\text{pos}]-\log[\text{neg}]))$. The **cut-off** for Q-detect™ was determined as IFN γ production of 16 pg/ml above background combined with a minimum relative *Coxiella* response of 0,40 (compared to the positive control).

Results

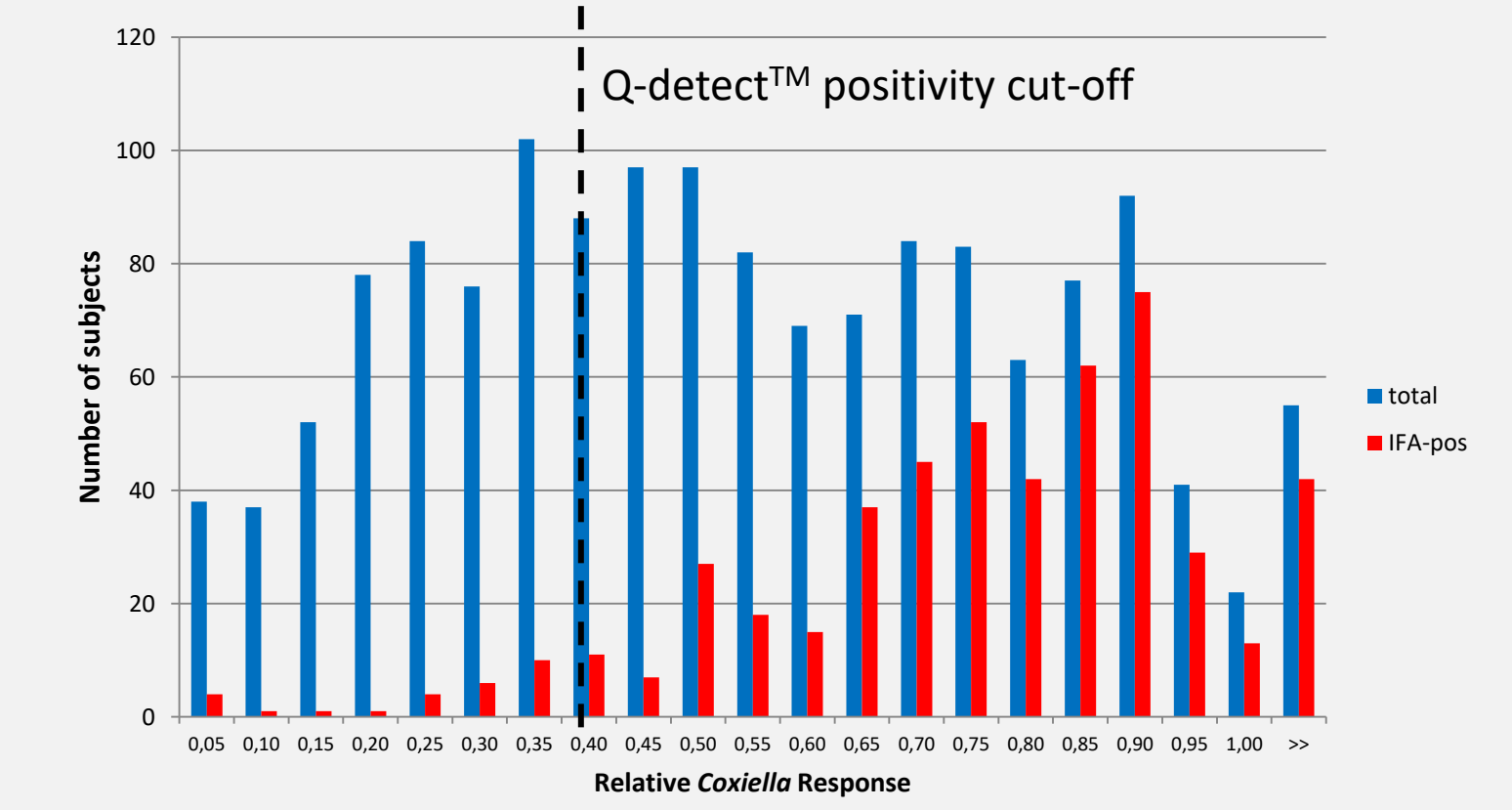
- 80 % more exposures were identified using Q-detect™ than IFA
- Q-detect™ identified 92% of IFA-positive samples

	QD-pos	QD-neg	QD-inc	Total
IFA +	461	41	11	513
IFA -	455	532	11	998
Total	916	573	22	1511

Table 1. 1511 adults in the village of Herpen, in the epicenter of the Dutch epidemic, were tested for *Coxiella* serology by immunofluorescent assay (IFA) using a cut-off of 1:64 (FocusDx) and cellular responses by Q-detect™

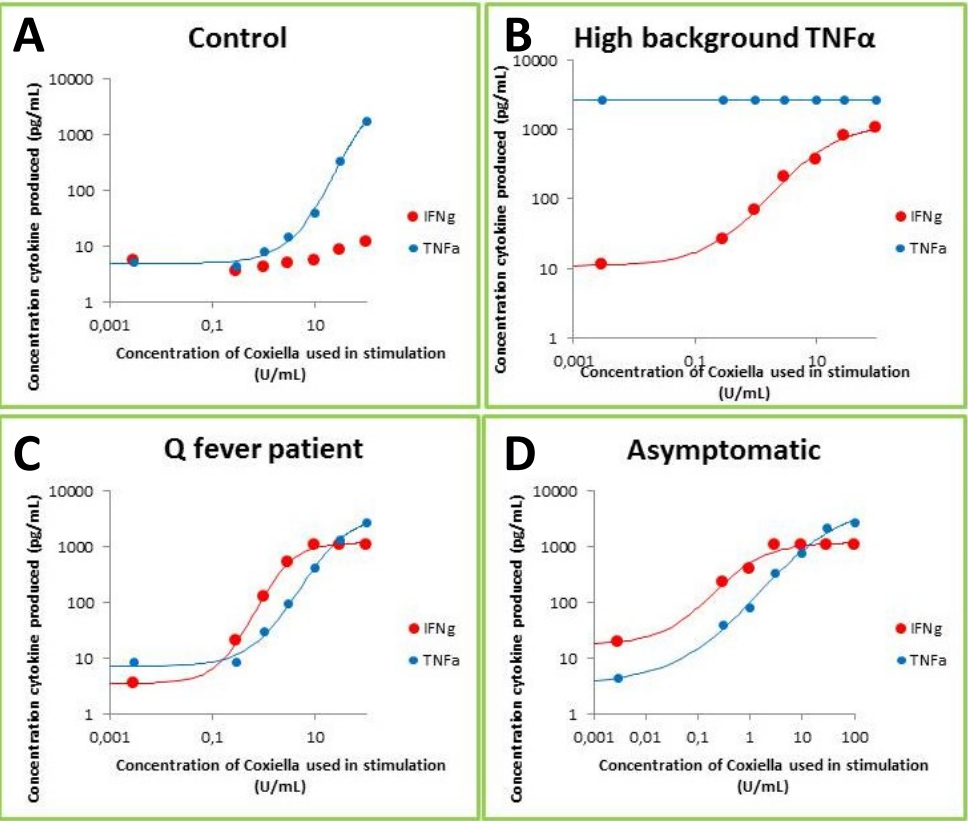
Strong Q-detect response = a higher chance of positive IFA

To understand the relationship between IFA and Q-detect, subjects were categorized according to the **relative Q-detect™ response**. For each group the total and IFA-positive number of subjects was determined. A high relative Q-detect™ *Coxiella* response correlates with a higher likelihood for a positive IFA.



IFN γ and TNF α are independently induced by *Coxiella*

To ascertain that the IFN γ response is due to specific stimulation rather than general activation of innate immunity, dose-response curves for IFN γ (adaptive) and TNF α (innate) were generated in close to 150 subjects.

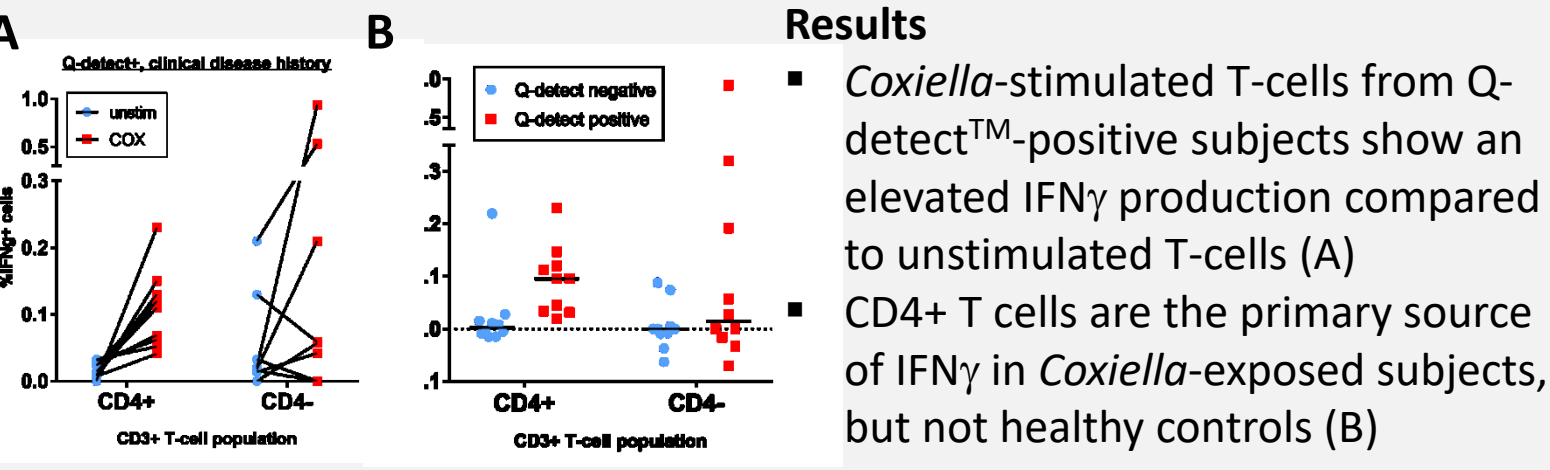


Results

- Healthy controls (A) respond to *Coxiella* with TNF α production
- High background TNF α (B) did not interfere with the IFN γ response to *Coxiella*
- Coxiella*-exposed subjects with (C) or without (D) disease showed a dose-dependent increase in IFN γ as well as TNF α

Coxiella-induced IFN γ is primarily derived from CD4 T-cells

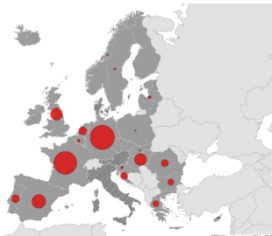
To address the question whether IFN γ production was indeed derived from adaptive T cells, flow cytometry was used as an alternative readout to ELISA following whole blood *Coxiella* stimulation.



Results


- Coxiella*-stimulated T-cells from Q-detect™-positive subjects show an elevated IFN γ production compared to unstimulated T-cells (A)
- CD4+ T cells are the primary source of IFN γ in *Coxiella*-exposed subjects, but not healthy controls (B)

Q-detect™ – clinical utility in Q fever and QVS



Severe fatigue but no antibodies

Confirm IgG phase 2 IFA below 1:256



- Fit for purpose**
- Public Health: source identification in low-endemic regions
 - Confirmation of low-positive IFA
 - Exclusion of past Q fever in patients with chronic fatigue.
 - Replacing T cell-driven skin test in pre-vaccination screening

Looking for customers



- Position to be determined**
- Acute Q fever
 - Differentiation between latent and active disease
 - Determining immune status
 - Monitoring treatment efficiency

Looking for collaborations! new outbreaks, cohorts of chronic patients

Conclusion

Q-detect™ is a valuable addition to the diagnostics toolkit of the infectious disease specialist. The test can be used to identify *Coxiella* exposure, followed by more extensive testing when positive.