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

M. de Vries-van der Horst, A.C. van der Zanden, A. Scholzen, A. Garritsen

#5029

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. IFNγ 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 IFNγ-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.

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

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

Results

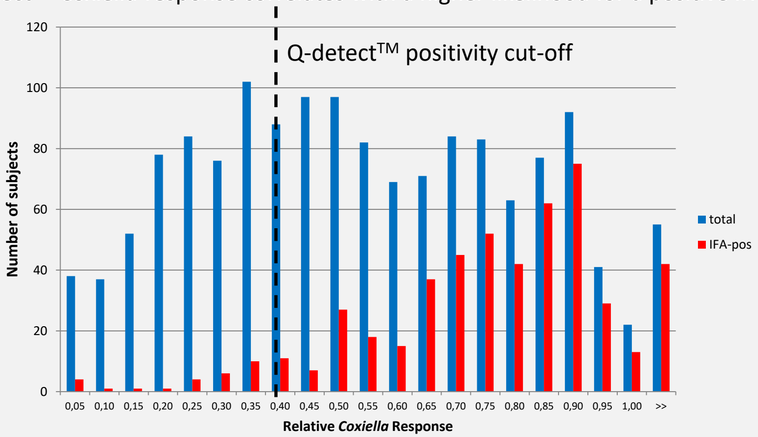
- 80 % more exposures were identified using Q-detect™ than IFA
- Q-detect™ identified 92% of IFA-positive samples

	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™

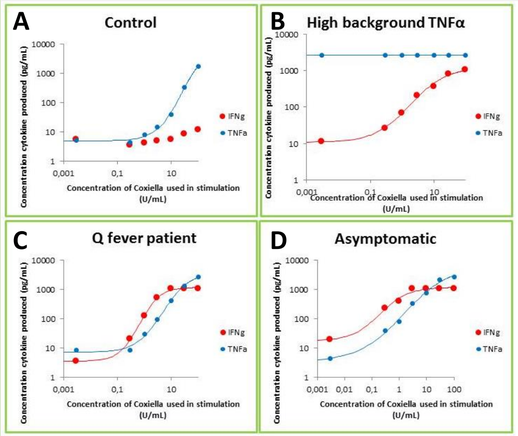
Strong Q-detect response = a higher chance of positive IFA

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.



IFNγ and TNFα are independently induced by *Coxiella*

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

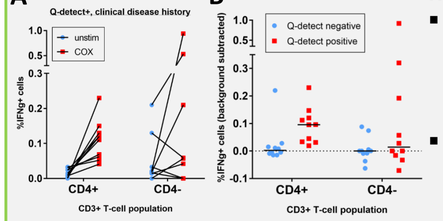


Results

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

Coxiella-induced IFNγ is primarily derived from CD4 T-cells

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



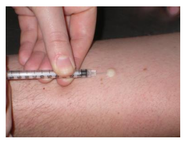
Results

- *Coxiella*-stimulated T-cells from Q-detect™-positive subjects show an elevated IFNγ production compared to unstimulated T-cells (A)
- CD4+ T cells are the primary source of IFNγ 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.