Q-blot: a highly sensitive immunoblot for detection of *Coxiella burnetii* infections

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Background

Q fever

- Neglected zoonotic disease with **world-wide prevalence** (1); primarily spread by small ruminants (goat, sheep) and cattle in parturition season
- Caused by the obligate intracellular bacterium *Coxiella burnetii*
- **Highly environmentally stable and contagious; airborne transmission** (potential biothreat agent)
- **Long-term complications** common (chronic fatigue, persistent infection = chronic Q fever)
- Exposure during **at-risk occupational and domestic activities** (farming, slaughterhouse work, military deployment, rural tourism) (1, 2)

Epidemiology

- **Europe**: 824 confirmed notified cases in 2015 (1.9 cases per million); case number in Germany and France increasing since 2013 (3)
- **Netherlands**: 2007-2010 outbreak with >4000 notified cases and estimated >40,000 infections (4)
- **US**: 2000-2012 0.38 notified cases per million inhabitants per year (5)
- **Africa**: studies indicate substantial and underestimated contribution to "fever & infectious endocarditis of unknown origin" (1,6)

Diagnosis/surveillance

- **Serology** most common method to test for exposure/infection if suspected (passive surveillance)
- In-house developed tests commonly used (no uniform procedure)
- **Indirect immunofluorescence assay** (IFA)
  - Reference method
  - Regarded most sensitive (1)
  - Time-consuming, non-automated, subject to inter-observer variability
- Other commercially available serological tests: ELISA and complement fixation test (CFT)

Objective, Rationale & Approach

Objective

Develop an immunoblot that is more sensitive than IFA and allows fast, automatable, objective detection of exposure to *Coxiella burnetii*

Need for a more sensitive serological Q fever assay

- 2014 field study identified 33,8% of inhabitants in Dutch outbreak village Herpen as IFA-positive (7)
- Parallel assessment by cellular interferon gamma release assay (IGRA, Q-detect™) identified 92% of IFA-positive samples, and 80 % more exposures than IFA (8, 9, unpublished data)
- Question: Can a more sensitive serological assay identify *Coxiella*-specific IgG responses in IGRA-positive, IFA-negative individuals?

Approach & Initial results

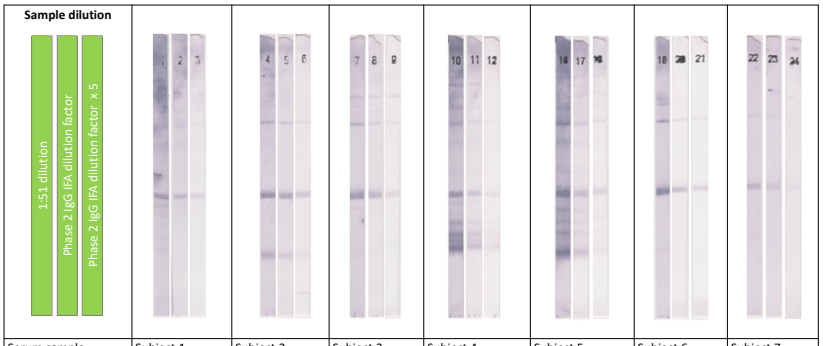
- Immunoblot developed by Innatoss Laboratories B.V. using the same proprietary *Coxiella* antigen as Q-detect™ (strain Cb2009-02629 cultured by Wageningen Bioveterinary Research under cell-free conditions)
- First generation Q-blot identified 28/41 (68%) of Q-detect™ IGRA-positive, IFA-negative serum samples from convalescent subclinically/clinically *Coxiella burnetii* infected individuals as sero-positive
- Q-blot was further optimized and tested to address the following question:

Is Q-blot more sensitive than the FocusDX IFA in detecting anti-*Coxiella* IgG?

Q-blot has at least 5-fold higher sensitivity than IFA

Results

- Q-blot **signal of hallmark band correlates with phase 2 IgG IFA titers** (similar signal across samples at the same dilution as IFA)
- Q-blot shows (lower) **second hallmark band** for all samples with a **phase 1 IgG** titer ≥ 1:128
- **Q-blot is more sensitive than IFA** (still detects anti-*Coxiella* IgG at a 5-fold lower serum dilution than IFA)
- **Q-blot is easy to handle**; assay time (manual): ~2 hours 15 minutes; Hands-on time: ~20 minutes



Serum sample	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Q fever history	Acute infection 2009, Recovered	Occupational exposure, QFS patient	Chronic Q fever since 2017	Possible Chronic Q fever since 2008	Occupational exposure	Acute infection 2017, Recovering	Acute infection 2008, QFS patient
IFA phase 1 IgG titer	< 1:32	1:128	1:32	1:512	1:1024	1:64	< 1:32
IFA phase 2 IgG titer	1:512	1:256	1:512	1:1024	1:2048	1:4096	1:32

Figure 1: Sensitivity of Q-blot for detection of Coxiella burnetii specific IgG with at least 5-fold higher sensitivity than IFA.
Heat-killed Coxiella antigen was separated using gel electrophoresis followed by Western blotting onto PVDF membranes. Strips were cut using an Accutran Strip Cutter. Incubation with seven different patient sera and detection with polyclonal anti-human-IgG labeled with alkaline phosphatase was performed manually using standard immunoblotting procedures. For each patient sample, 3 strips from the same experiment are shown, with serum samples being diluted (1) (at a fixed dilution factor of 1:51, (2) the dilution factor of the phase 2 IgG IFA titer or (3) 5-fold higher than the phase 2 IgG IFA titer of the respective sample. Phase 1 IgG IFA titers were determined at the Jeroen Bosch Hospital, Den Bosch, NL using the FocusDX IFA. IFA = Indirect immunofluorescence assay; QFS = Q fever fatigue syndrome

Ongoing work

- **Conversion** from research-only-use in house assay **to commercial product**
  - Development of **technically stable product**
  - Test **lot-to-lot variation** using different *Coxiella* antigen batches
  - Establishment of **automated** protocol
- **Clinical validation against IFA and Q-detect™ IGRA** to determine sensitivity and specificity using an in-house collection of sera from
  - Convalescent individuals with past acute or asymptomatic Q fever
  - Chronic Q fever patients
  - Q fever fatigue syndrome patients

Outlook

Clinical utility in Q fever surveillance

- Sensitive, fast and easy to use assay
- Suitable for remote locations where implementation of the Q-detect™ IGRA is less feasible
- Suitable for (semi) automated screening of large serum collections

Looking for collaborations and customers!

References

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