Specific Interferon \(\gamma\) Detection for the Diagnosis of Previous Q Fever

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(See the Editorial Commentary by Graves on pages 1752–3.)

**Background.** Current practice for diagnosis of Q fever, caused by the intracellular pathogen *Coxiella burnetii*, relies mainly on serology and, in prevaccination assessment, on skin tests (STs), which both have drawbacks. In this study, *C. burnetii*–specific interferon \(\gamma\) (IFN-\(\gamma\)) production was used as a new diagnostic tool for previous Q fever, circumventing most of these drawbacks. Our aim was to compare this test to serology and ST.

**Methods.** One thousand five hundred twenty-five individuals from an endemic area with a risk for chronic Q fever were enrolled. IFN-\(\gamma\) production was measured after in vitro stimulation of whole blood with *C. burnetii* antigens. Various formats using different *C. burnetii* antigens were tested. Serology and ST were performed in all individuals.

**Results.** In all assay formats, *C. burnetii*–specific IFN-\(\gamma\) production was higher \((P < .0001)\) in seropositive or ST-positive subjects than in seronegative and ST-negative subjects. Whole blood incubated for 24 hours with *C. burnetii* Nine Mile showed optimal performance. After excluding subjects with equivocal serology and/or borderline ST results, IFN-\(\gamma\) production was 449 ± 82 pg/mL in the positive individuals \((n = 219)\) but only 21 ± 3 pg/mL in negative subjects \((n = 908)\). Using Bayesian analysis, sensitivity and specificity \((87.0\%\) and 90.2\%, respectively) were similar to the combination of serology and ST \((83.0\%\) and 95.6\%, respectively). Agreement with the combination of serology and ST was moderate \((84\%\) concordance; \(k = 0.542)\).

**Conclusions.** Specific IFN-\(\gamma\) detection is a novel diagnostic assay for previous *C. burnetii* infection and shows similar performance and practical advantages over serology and ST. Future studies to investigate the clinical value in practice are warranted.

**Keywords.** interferon \(\gamma\); Q fever; *Coxiella burnetii*; serology; skin test.

Acute Q fever, caused by *Coxiella burnetii*, is often not recognized because it may have an asymptomatic or a mild course [1]. Chronic life-threatening endovascular infection may, however, develop, particularly in patients with valvulopathy or aneurysmas [1, 2]. A correct diagnosis of *C. burnetii* infection is crucial for patient care, as well as for vaccination and epidemiological studies.

The standard method to detect Q fever is measurement of specific antibodies [3, 4]. However, assessment of T-lymphocyte immunity might be an additional and even superior method because defense against this intracellular pathogen mainly depends on cellular immunity, including interferon \(\gamma\) (IFN-\(\gamma\))–mediated macrophage activation [5–7]. Hence, a skin test (ST)
can be done to assess delayed-type hypersensitivity to intradermally injected C. burnetii. The ST is almost exclusively used for prevaccination screening. Its disadvantages are variability, lack of a well-defined cutoff, and dependence on trained personnel to perform a ST. Therefore, we developed an in vitro IFN-γ production assay, in which IFN-γ production in blood after exposure to C. burnetii antigens is measured, similar to the quantiferon test for tuberculosis [8, 9].

Between 2007 and 2010, the Netherlands experienced the largest outbreak of Q fever worldwide [10], with an estimated >40,000 infected individuals and >250 patients with chronic Q fever [11, 12]. In 2010, the health authorities decided to offer vaccination with the C. burnetii whole-cell vaccine Q-vax [13] to inhabitants of the endemic area at risk for chronic Q fever (ie, those with preexisting heart valve lesion, prosthetic valve, congenital heart anomalies, aortic aneurism, or vascular graft). Vaccine candidates were screened by serology and ST to exclude preexisting sensitization to C. burnetii antigens that may cause hypersensitivity reactions upon vaccination [14]. This prevaccination screening presented a unique opportunity to explore the applicability of our novel assay. We tested different formats of the assay and assessed its accuracy in diagnosing previous Q fever.

**METHODS**

**Study Population and Sampling Procedure**

The study population consisted of vaccine candidates in the Dutch Q fever vaccination campaign from 21 January 2011 to 20 April 2011; the campaign coincided with a 30-fold decrease of new human Q fever cases in the spring compared with the peak in 2009, probably related to veterinary measures, including culling of all pregnant goats on infected farms. The target population consisted of individuals living in the highly endemic area based on surveillance data available. Voluntariness and application by general practitioners were required. One week before the planned vaccination, candidates were invited to the Municipal Health Service in 's-Hertogenbosch. Blood was collected for serology followed by the ST. All candidates were asked to participate in the study by donating 5 mL of heparinized blood for the IFN-γ assay. This sample was obtained simultaneously with the serological sample and processed within 12 hours, as previous experiments had shown that samples assessed at 2 or 12 hours did not affect the assay results. IFN-γ production was not relevant for the decision whether or not to vaccinate. The study was performed according to the guidelines of the local ethics committee, and written informed consent was obtained. Demographic details were collected anonymously.

**C. burnetii IFN-γ Production Assay**

IFN-γ production was measured after in vitro stimulation of whole blood incubated under 2 different conditions (A or B) described below. Also, 2 C. burnetii antigens were used separately for stimulation. In parallel, stimulation with phytohemagglutinin was performed as a positive control and incubation with no reagent as a negative control.

In format A, undiluted whole blood was incubated in a closed tube at 37°C for 24 hours. In format B, the blood was diluted 1:5 in medium (Roswell Park Memorial Institute 1640 Dutch modification supplemented with glutamax [2 mM], pyruvate [1 mM], and gentamicin [1 mg/mL]) and incubated in 24-wells plates at 37°C and 5% carbon dioxide for 48 hours.

The 2 C. burnetii antigens were the Q-vax vaccine [15] and the heat-inactivated laboratory strain C. burnetii Nine Mile RSA 493 (C. burnetii–NM) [16], kindly provided by Dr D. Frangoulidis (Bundeswehr Institute of Microbiology, Munich, Germany).

Q-vax vaccine contains formaldehyde-inactivated C. burnetii Henzerling strain phase 1 in 50 μg/mL. Previous dose–response experiments in Q fever patients showed optimal IFN-γ production in the range of 100–500 ng/mL. A single lot (No. 0980–07 201) was used and diluted to 100 ng/mL.

C. burnetii–NM phase 1 was cultured in a biosafety level 3 facility at the Central Veterinary Institute, using Buffalo Green Monkey cells in Earle’s Modified Eagle Medium supplemented with 10% fetal calf serum, 1% nonessential amino acids, and 1% L-glutamine. The concentration of C. burnetii DNA was determined by real-time polymerase chain reaction. The supernatant was centrifuged for 15 minutes at 1000×g and stored in aliquots at −80°C. Killing was done by heating for 30 minutes at 99°C. For stimulation C. burnetii–NM was used in an end concentration of 10⁷ bacteria/mL, previously shown to be optimal in Q fever patients. The same batch was used for all assays.

After incubation of blood samples with either C. burnetii antigens, phytohemagglutinin (PHA), or nil, cultures were centrifuged and supernatants were stored at −20°C.

IFN-γ was measured by enzyme-linked immunosorbent assay. Serological and ST results were unknown to those performing the assay. Net IFN-γ production was expressed as the concentration of IFN-γ in stimulated samples minus that in negative controls. If either IFN-γ production in the negative control exceeded 24 pg/mL (thrice the lower detection limit of the ELISA) or the IFN-γ production after PHA stimulation was <24 pg/mL without the C. burnetii–stimulated aliquots exceeding 24 pg/mL, the assay was considered inconclusive.

**Serology and Skin Testing**

Antibodies against C. burnetii were determined in serum by indirect immunofluorescence measuring immunoglobulin M and immunoglobulin G against C. burnetii–NM phase 1 and 2. Seropositivity was defined as anti-C. burnetii titers ≥1:32. A solitary weak reactivity of immunoglobulin G against phase 2
antigens below the cutoff level of 1:32 was defined as equivocal because this was considered possible false positive or cross-reacting. Seropositivity led to exclusion from vaccination.

Q-vax skin tests [15] were performed by professionals. In short, the local response to 0.1 mL intradermally injected Q-vax ST (containing the same C. burnetii antigens as Q-vax vaccine, but in a lower concentration) in the forearm was measured after 7 days. Only participants with negative or equivocal serological results were invited for ST reading. The ST results were classified as positive (induration ≥5 mm), borderline (induration 1–4 mm or any swelling and/or redness), or negative (no induration, swelling, or redness). Positive and borderline results led to exclusion from vaccination.

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 5 and SAS version 9.2. Mann–Whitney U tests were performed to test statistical significance of IFN-γ production differences between groups. Association between inconclusiveness of specific IFN-γ production and demographics was assessed with a generalized linear model with identity link function [17]. Receiver operating characteristics (ROC) curves were created to assess accuracy (area under the curve [AUC]) of the different IFN-γ assay formats. Spearman’s r was calculated to report correlation. Subsequently, a Bayesian model [18], in which no test is considered to be the gold standard, was used to determine the sensitivity and specificity of the IFN-γ assay of the best performing format. Using this method [18], adapted for several cutoffs, the ROC curve was estimated. The proportionate agreement and the Cohen’s κ were calculated to assess agreement [19]. The McNemar test was used to compare the proportion of positive results between tests. The level of significance was set at $P \leq .05$.

**RESULTS**

During the prevaccination visit, 1786 individuals were screened. No subject had signs of acute Q fever (no

<table>
<thead>
<tr>
<th></th>
<th>All Individuals in Prevaccination Screening (n = 1786)</th>
<th>Study Population (n = 1525)</th>
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<tbody>
<tr>
<td>Mean age* ± SD, years</td>
<td>63.5 ± 14.7</td>
<td>64.0 ± 14.2</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>668 (37.4)</td>
<td>558 (36.6)</td>
</tr>
<tr>
<td>Exclusion of vaccination based on:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive serology, %</td>
<td>181 (10.1)</td>
<td>158 (10.4)</td>
</tr>
<tr>
<td>Positive/borderline ST, %</td>
<td>210 (11.8)</td>
<td>190 (12.5)</td>
</tr>
<tr>
<td>Serology indicating chronic Q fever infection</td>
<td>12 (0.7)</td>
<td>9 (0.6)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; ST, skin test.

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**Figure 1.** Flow diagrams of individuals included in the specific interferon γ (IFN-γ) production measurement. The numbers of individuals are shown separately for the IFN-γ assay in format A and format B. Format A: IFN-γ production assay in undiluted whole blood incubated 24 hours; format B: IFN-γ production assay in 1:5 diluted blood incubated 48 hours. Abbreviations: IFN-γ, interferon γ; ST, skin test.
immunoglobulin M level compatible with acute Q fever), whereas 12 had a serological profile compatible with chronic Q fever and were referred for further analysis [20]. Blood samples from 1525 individuals were obtained for IFN-γ production. Assay format A was performed on all 1525 samples; format B was discontinued after 1306 samples. Table 1 shows the characteristics of the vaccine candidates.

Results of the IFN-γ measurements in format A and B were conclusive for 1278 (83.8%) and 1223 (93.6%) individuals, respectively. After excluding individuals with equivocal serology or borderline ST results, 1127 (73.9%) and 1087 (83.2%) individuals were left with a conclusive outcome for all 3 tests (Figure 1).

Assay format A showed inconclusive results in 247 (16.2%) individuals; format B had only 83 (6.4%) inconclusive results. Inconclusiveness in format A was associated with age and sex (P = .02 and P = .0008 respectively; no interaction, P = .40), being most frequent in female subjects aged >80 years (29%; 95% confidence interval [CI], 21–38) and least frequent in male subjects aged <80 years (13%; 95% CI, 11–15). Only 41 of the 247 inconclusive results in format A were due to high background IFN-γ production in the control sample; these were judged as technical errors or low-grade inflammation. Two hundred six results were inconclusive because of absent PHA response in the absence of a clear antigen-specific response (judged as anergy). However, 157 of 206 (76%) had an adequate PHA response in the corresponding sample in format B.

Subjects with conclusive results for all 3 tests were divided, based on the combined outcome of serology and ST, in seropositive or ST-positive (previous C. burnetii exposure) or seronegative and ST-negative (no C. burnetii exposure) groups. In the 1127 subjects with conclusive results in format A, 218 were seropositive or ST-positive, and 908 were seronegative and ST-negative. In the 1087 subjects with conclusive results in format B, 190 were seropositive or ST-positive, and 897 were seronegative and ST-negative. The IFN-γ production (mean ± standard error) was compared between these groups in both formats separately (Figure 2A and 2B). PHA-induced IFN-γ production did not distinguish between individuals with previous and no C. burnetii exposure. However, C. burnetii antigen-specific IFN-γ production was significantly higher in subjects with previous exposure. IFN-γ production in assay format A stimulated with C. burnetii–NM distinguished best between seropositive or ST-positive individuals and negative individuals; IFN-γ production was 449 ± 82 pg/mL in the sero- or ST-positive individuals and 21 ± 3 pg/mL in the seronegative or ST-negative individuals, respectively.

The ROC curves (Figure 3), combining serology and ST as (surrogate) gold standard, revealed the highest AUC for format A with C. burnetii–NM (AUC, 0.8452). The correlation between C. burnetii–NM– and Q-vax–induced IFN-γ production in format A and between the C. burnetii–NM–induced IFN-γ production in format A and format B are shown in a scatter plot (Figure 4). The Spearman’s rank correlations were 0.5410 and 0.637, respectively (Figure 2).

**Comparison With Serology and Skin Testing**

We focused on format A using C. burnetii–NM for further analysis. Figure 5 shows the data of 1278 individuals with conclusive results in format A, stratified according to the outcome.
of serology and ST, including equivocal serology and borderline ST results (see also Supplementary Table 1, showing the other IFN-γ assay formats). Specific IFN-γ (mean ± standard error) production was calculated in each group. This was highest in positive serology outcome, followed by positive ST outcome in combination with equivocal or negative serology. IFN-γ production was lowest in seronegative and ST-negative subjects. In borderline ST outcome, IFN-γ production was not significantly different between those with equivocal and negative serological findings (P = .07).

Using Bayesians statistics, we determined sensitivity and specificity of the IFN-γ assay at predefined cutoff values from 16 to 80 pg/mL, equivalent to 2–10 times the lower detection limit for the enzyme-linked immunosorbent assay. For the 1127 individuals with conclusive results for the IFN-γ assay, serology and ST were analyzed. A noninformative prior distribution was used for all parameters. The prior prevalence of C. burnetii infection was given a flat prior distribution over the range 0–0.5.

The prior sensitivity and specificity of the tests were given a flat prior distribution over the range 0–1. The data of the performance of the IFN-γ measurement at different cutoffs are shown in Table 2. The resulting ROC curve for the IFN-γ assay (Figure 6) shows an accuracy (AUC) of 0.9239 (95% credible interval [CrI], 0.8466–0.9756). Choosing an optimal cutoff value of 32 pg/mL, sensitivity and specificity of the IFN-γ assay were 87.0% and 90.2% (95% CrI, 72.2–98.8 and 86.0–94.5, respectively). The sensitivities and specificities of combined serological and ST, as calculated in this analysis, were 83.0% and 95.6% (95% CrI, 70.1–98.7 and 93.3–99.4, respectively). The prevalence of past Q fever was estimated in this analysis to be 19.3% (95% CrI, 14.9–25.0). In comparison, available data on blood donors in the high-incidence area in 2009, based on serological screening alone, show a C. burnetii immunoglobulin G prevalence of 12.2% [21].

Agreement between the dichotomized IFN-γ assay (with cutoff of 32 pg/mL) and the combination of the other 2 tests

![Figure 3](https://academic.oup.com/cid/article-abstract/56/12/1742/401457)
is shown in Table 3. Overall agreement was 84% (949 of 1127) with $\kappa = 0.542$ ($P < .0001$), which is considered moderate [22]. The proportion of individuals with past or present C. burnetii infection identified by the IFN-\(\gamma\) assay, but not by serology or ST, was significantly higher (118 of 178 = 0.66) than the proportion identified by serology and

**Figure 4.** Scatter plots showing the correlation in interferon \(\gamma\) (IFN-\(\gamma\)) production between Q-vax and Coxiella burnetii–Nine Mile (NM) stimulation within IFN-\(\gamma\) assay format A (upper plot) and the correlation in IFN-\(\gamma\) production induced by C. burnetii–NM between assay format B and A (lower plot). The Spearman’s rank correlations were 0.5410 and 0.637, respectively ($P < .0001$). Every dot represents 1 subject, so far as dots are not overlapping. Abbreviations: IFN-\(\gamma\), interferon \(\gamma\); NM, Nine Mile.
Figure 5. Distribution of serology and skin test outcome and interferon γ (IFN-γ) production in the study population with conclusive IFN-γ production results in assay format A (n = 1278). The upper boxes indicate the number (%) of individuals. The lowest boxes indicate the specific IFN-γ production (mean ± standard error; pg/mL) in whole blood incubated for 24 hours and stimulated with Coxiella burnetii Nine Mile. Abbreviation: IFN-γ, interferon γ.

Table 2. Sensitivity and Specificity With Concomitant 95% Credible Interval of the Specific Interferon γ Production at Cutoff Values in the Range of 16–80 pg/mL.

<table>
<thead>
<tr>
<th>Cutoff Value for Specific IFN-γ Production (pg/mL)</th>
<th>Sensitivity, %</th>
<th>95% Crl, %</th>
<th>Specificity, %</th>
<th>95% Crl, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>91.8</td>
<td>78.4–99.7</td>
<td>82.4</td>
<td>78.1–86.7</td>
</tr>
<tr>
<td>24</td>
<td>87.9</td>
<td>73.7–98.6</td>
<td>87.1</td>
<td>82.8–91.5</td>
</tr>
<tr>
<td>32</td>
<td>87.0</td>
<td>72.2–98.8</td>
<td>90.2</td>
<td>86.0–94.5</td>
</tr>
<tr>
<td>40</td>
<td>82.3</td>
<td>67.8–94.9</td>
<td>92.5</td>
<td>88.4–96.7</td>
</tr>
<tr>
<td>48</td>
<td>79.2</td>
<td>65.0–92.4</td>
<td>94.0</td>
<td>90.4–98.0</td>
</tr>
<tr>
<td>56</td>
<td>78.3</td>
<td>63.9–91.4</td>
<td>94.9</td>
<td>91.3–98.7</td>
</tr>
<tr>
<td>64</td>
<td>76.3</td>
<td>62.1–89.3</td>
<td>95.9</td>
<td>92.4–99.4</td>
</tr>
<tr>
<td>72</td>
<td>73.0</td>
<td>58.9–85.4</td>
<td>96.1</td>
<td>92.7–99.5</td>
</tr>
<tr>
<td>80</td>
<td>70.3</td>
<td>56.3–83.1</td>
<td>96.9</td>
<td>93.6–99.8</td>
</tr>
</tbody>
</table>

A Bayesian model was used with flat priors for prevalence and test parameters, including interferon γ (IFN-γ) production and the combination of serology and skin test. The IFN-γ production measurement was performed in 24 hours incubated whole blood, stimulated with Coxiella burnetii Nine Mile. Only individuals with conclusive results for all 3 tests were included (n = 1127).

Abbreviations: Crl, credible interval; IFN-γ, interferon γ.

Table 3. Relationship Between the Interferon γ Production Outcome and the Combination of Serology and Skin Test Outcome

<table>
<thead>
<tr>
<th>IFN-γ Production Measurement Outcomea</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>159</td>
<td>118</td>
<td>277</td>
</tr>
<tr>
<td>Negative</td>
<td>60</td>
<td>790</td>
<td>850</td>
</tr>
<tr>
<td>Total</td>
<td>219</td>
<td>908</td>
<td>1127</td>
</tr>
</tbody>
</table>

The interferon γ (IFN-γ) production measurement was performed in whole blood incubated 24 hours, stimulated with Coxiella burnetii Nine Mile. Only individuals with conclusive results for all 3 tests were included (n = 1127). Overall agreement was 84% (949 of 1127), κ = 0.542 (P < .0001).

Abbreviations: IFN-γ, interferon γ; ST, skin test.

a IFN-γ production measurement was considered positive at value >32 pg/mL and considered negative at value ≤32 pg/mL.
b Combination of serology and ST was considered positive if either one was positive and considered negative if both were negative.
Q fever in individuals with equivocal serology and borderline ST results.

**DISCUSSION**

In a sample of >1500 individuals at risk for chronic Q fever, we demonstrated that an assay that measures antigen-specific IFN-γ production in vitro is a valuable tool in the diagnosis of Q fever. The accuracy of this test is similar to the traditional serology and ST. The test is easy to perform: no advanced personnel or equipment are required for incubation, and read-out and the data are available the next day. It is patient friendly by excluding the need for in vivo testing, which, in sensitized individuals, can be troublesome.

The IFN-γ production assay was analyzed in individuals with a specific risk for development of chronic Q fever and considerable *C. burnetii* exposure. The assay was carried out in 2 formats with different incubation periods and 2 different *C. burnetii* antigens. The results of the 4 assay conditions were consistent, showing higher IFN-γ production in individuals with positive serology or ST response, whereas PHA stimulation was not specific. Thus, *C. burnetii*-specific IFN-γ production seems to be a useful diagnostic aid in determining previous Q fever infection.

The IFN-γ production in undiluted blood exposed to *C. burnetii*-NM for 24 hours showed the highest AUC, using serology and ST as the putative gold standard. This format performed better than in 1:5 diluted blood. This may be because of higher concentrations of serum factors, the 5× higher inoculum, and the shorter incubation in the undiluted assay. Stimulation with *C. burnetii*-NM performed better than Q-vax. In the ROC curves, Q-vax stimulation reached maximal sensitivity of 53%. The scatter plot data support this finding by showing no Q-vax–induced IFN-γ in many samples with *C. burnetii*-NM–induced IFN-γ production. The sensitivity of the assay may be strain dependent; the Henzerling strain of Q-vax may be less potent than *C. burnetii*-NM in inducing IFN-γ in the Dutch setting. Formaldehyde in Q-vax may have led to a greater loss of antigen than heat inactivation. Lastly, 0.49 µM thiomersal in Q-vax may lead to a T helper 2 cell response, thereby reducing IFN-γ production [23].

A major advantage of the IFN-γ production assay is its internal positive and negative control. Consequently, 16.2% of the findings in format A were inconclusive. These were mainly because of low PHA response, suggesting anergy. However, in 76%, PHA did elicit an IFN-γ response, albeit in format B. In the commercial IFN-γ production assays for tuberculosis, indeterminate results are rarely addressed, but low mitogen responses may be as high as 21% [24]. Although inconclusive observations are a limitation of our assay, false-negative or

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**Figure 6.** Receiver operating characteristic curve of the best performing interferon γ (IFN-γ) production assay at cutoff values ranging from 16 to 80 pg/mL using a Bayesian model without taking serology and skin test (ST) as gold standard. The IFN-γ production measurement was performed in whole blood incubated 24 hours and stimulated with *Coxiella burnetii* Nine Mile. Only individuals with conclusive results for all 3 tests were included (n = 1127). The area under the curve with concomitant 95% credible interval indicates the accuracy of the IFN-γ production measurement. Abbreviations: AUC, area under the curve; CI, credible interval.

**Figure 7.** Interferon γ (IFN-γ) production (pg/mL) according to skin test results in individuals with equivocal serology. The individuals with conclusive results in the IFN-γ production assay in format A stimulated with *Coxiella burnetii* Nine Mile with equivocal serology are included (n = 101). The dotted line indicates the cutoff of 32 pg/mL. Abbreviation: IFN-γ, interferon-γ.
false-positive results are identified, whereas serology and ST lack internal controls.

Because an imperfect gold standard does not allow a realistic estimation of the accuracy of a novel test, we used a Bayesian model (without taking serology and ST as surrogate gold standards). Bayesian models allow the evaluation of the true accuracy of tests and do not require that any test or combination of tests be perfect [25, 26]. Each unknown parameter in the model should have a prior distribution, based on previous findings. However, data on prevalence of Q fever exposure in the Netherlands are based on serology alone. Moreover, little is known about sensitivity and specificity of serology and STs in Q fever diagnosis. Therefore, we chose to use noninformative priors, which resulted in broad 95% CrI of posterior parameters. With these rough estimates, we found a good performance of the IFN-γ test, similar to the combination of serology and ST.

Specific IFN-γ testing provides data different from serology and ST when used in practice. The discordant results were mainly because of positive IFN-γ production in seronegative and ST-negative individuals. Obviously, the absence of a gold standard makes it impossible to assess definitely whether the IFN-γ assay was more sensitive or less specific than the combination of serology and ST. Bayesian analysis seems to indicate both: somewhat higher sensitivity (87.0% vs 83.0%) and somewhat lower specificity (90.2% vs 95.6%). Moreover, previous studies on immunity to C. burnetii after Q fever vaccination, using both lymphocyte proliferation assays and IFN-γ based assays, reveal a higher sensitivity of measurement of cellular immunity than serology [27, 28].

The IFN-γ assay might replace ST in the prevaccination setting because both tests measure cellular immunity to C. burnetii. The IFN-γ assay has important operational advantages: the data are available in <2 days, and no follow-up visit is required. Furthermore, the assay can be used repeatedly, whereas ST may affect subsequent (serological) tests by boosting C. burnetii-specific immunity [29]. The IFN-γ assay also offers advantages over serology because it is a controlled test with a continuous parameter as read-out, not using titers. In contrast to immunofluorescence serology, there is no inter-reader variability. Moreover, it bypasses the ongoing discussion on phase 1/phase 2 antibodies and optimal cutoff [4, 30, 31]. We are hesitant to state that the IFN-γ production assay could replace serology; we rather see it as being complimentary, with added value in cases with equivocal serology. The additional value in active Q fever disease, both the acute and chronic form, is currently being addressed.

### Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

#### Acknowledgments

T. Sp., M. v. D., M. G. N., L. A. B. J., and J. W. M. v. d. M. designed the study. T. Sc. performed the IFN-γ production assay, together with A. A. T. H., H. C. R., and C. J. W. were involved in the prevaccination screening and collecting the data on serology and skin test results. H. I. J. R. provided the C. burnetii Nine Mile antigens. T. Sc., A. d. H., T. Sp., and M. v. D. performed the data analyses. All authors interpreted the data. T. Sc., T. Sp., and M. v. D. wrote the first draft, and all authors helped to revise the manuscript.

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#### Potential conflicts of interest

A patent application has been submitted by M. v. D., J. W. M. v. d. M., M. G. N., and L. A. B. J. for this C. burnetii-specific IFN-γ production assay to diagnose Q fever and registered by the number PCT/NL 2011/005064. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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