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Comparison of the performance of IFA, CFA, and ELISA assays for the serodiagnosis of acute Q fever by quality assessment

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ABSTRACT

The indirect immunofluorescence assay (IFA) is considered the reference method for diagnosing Q fever, but serology is also performed by complement fixation assay (CFA) or enzyme-linked immunosorbent assay (ELISA). However, comparability between these assays is not clear, and therefore a quality assessment was performed. A total of 25 serum samples from negative controls, Q fever patients, and a serial diluted high-positive sample were analyzed in 10 Dutch laboratories. Six laboratories performed CFA, 5 performed IFA, and 5 performed ELISAs. Three international reference laboratories from Australia, France, and the USA also participated in this study. Qualitative values between laboratories using the same methods were within close range, and all 3 methods correctly identified acute Q fever patients. The IFA, ELISA, and CFA are all suitable serodiagnostic assays to diagnose acute Q fever, but the IFA remains an important tool in the follow-up of patients and in identifying patients at risk for developing chronic Q fever.

1. Introduction

Q fever is a highly contagious zoonotic disease caused by *Coxiella burnetii* (*C. burnetii*), a Gram-negative obligate intracellular bacterium (Maurin and Raoult, 1999). Transmission occurs mainly through the inhalation of infected aerosols, and the inhalation of a single bacterium can lead to clinical disease (Tigertt et al., 1961). The most important reservoirs consist of goats and sheep, in which the infection is mostly asymptomatic except in the case of abortions or stillbirths (Aitken et al., 1987; Babudieri, 1959). The presentation of Q fever in humans is variable, from asymptomatic, acute, and chronic disease to post Q-fever fatigue syndrome. Patients with acute Q fever typically exhibit high fever, headache, cough, and atypical pneumonia. After infection, *C. burnetii* may persist in the body and subsequently lead to chronic Q fever, a serious condition which can lead to endocarditis or vascular infection (Botelho-Nevers et al., 2007; Brouqui et al., 1993).

Since 2007, the Netherlands has been faced with the largest outbreak of Q fever ever reported, with over 4000 notified cases (Delsing et al., 2010). During this outbreak, it has become clear that many questions remain unanswered regarding the best assay for the diagnosis of Q fever patients. Q fever is diagnosed principally by serology and antibody patterns which differentiate between acute, convalescent, and chronic Q fever. The smooth, full-length lipopolysaccharide (phase I, virulent) form of *C. burnetii* occurs in nature and is maintained in laboratories by passage through animal hosts.

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Antibodies against this form of the *Coxiella* bacteria are predominantly observed in chronic Q fever. Following several passages in cell culture, *C. burnetii* undergoes a phase transition known as phase II. Antibodies against this form are the first to be detected in acute Q fever patients (Fournier et al., 1998).

The reference method for serodiagnosing Q fever is the indirect immunofluorescence assay (IFA) (Fournier et al., 1998; Maurin and Raoult, 1999). In other laboratories serology is performed by using a complement fixation assay (CFA) or an enzyme-linked immunosorbent assay (ELISA). Differences in the performance of the serologic methods were previously investigated. In general, investigators reported that the CFA showed lower sensitivity compared to the IFA. The ELISA has been presented as a possible alternative method for serodiagnosing Q fever (Dupuis et al., 1985; Field et al., 2002; Péter et al., 1987; Slabá et al., 2005). However, the comparability between these assays used in the different laboratories was not clear. Therefore, in 2010, clinicians and scientists from 9 Dutch diagnostic laboratories united in a Q fever Consensus Council decided to perform a multicenter interlaboratory evaluation with a special focus on diagnosing acute Q fever. A quality assessment for various Q fever tests was performed by comparing IFA, CFA, and ELISA, using serum samples from both controls and patients from the outbreak in the Netherlands.

2. Material and methods

2.1. Participating laboratories and serologic methods used

A total of 13 laboratories participated (Table 1). The 10 Dutch participating laboratories were mostly located within the Q fever high-endemic area of the Netherlands (Fig. 1). Three national reference laboratories from outside the Netherlands also participated: the Australian Rickettsia Reference Laboratory (ARRL), the Assistance Publique Hopitaux de Marseille (APHM) in France, and the Centers for Disease Control and Prevention (CDC) in the USA. The 5 Dutch laboratories that performed IFA all used the same commercially available IFA (Focus Diagnostics, Cypress, CA, USA) using a dilution of 1:32 as the cut-off. The 3 participating reference laboratories all used an in-house IFA. Six laboratories performed a commercially available CFA, and 5 participants also performed ELISAs (Table 1). All samples were analyzed by the participants' own methodology. The Dutch laboratories were coded A to J.

Table 1
Summary of the serologic methods utilized by participating laboratories.

Laboratory	Used assay	Manufacturer	Antigenic phase	Isotype
A	CFA[a]	Virion/Serion, Würzburg, Germany	II and I	N/A
	ELISA	Inverness Medical Innovations, Waltham, MA, USA	II	IgM
B	CFA	Virion/Serion	II and I	N/A
C	CFA	Virion/Serion	II	N/A
D	CFA	Virion/Serion	II and I	N/A
E	IFA[a]	Focus	II and I	IgM and IgG
	ELISA	Virion/Serion	II and I	IgM and IgG
F	CFA[a]	Siemens	II	N/A
	IFA	Focus	II and I	IgM and IgG
G	IFA[a]	Focus	II and I	IgM and IgG
	ELISA	Virion/Serion	II and I	IgM and IgG
H	CFA[a]	Virion/Serion	II	N/A
	ELISA	Virion/Serion	II	IgM and IgG
I	ELISA[a]	Virion/Serion	II and I	IgM and IgG
	IFA	Focus	II and I	IgM and IgG
J	IFA	Focus	II and I	IgM and IgG
International reference laboratories				
ARRL	IFA	In-house	II and I	IgM and IgG
APHM	IFA	In-house	II and I	IgM, IgG, and IgA
CDC	IFA	In-house	II and I	Total Ig

CFA = Complement fixation assay; N/A = not applicable; ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence assay; ARRL = Australian Rickettsial Reference Laboratory; APHM = Assistance Publique Hopitaux de Marseille; CDC = Centers for Disease Control and Prevention.
[a] Principal serologic assay used.

2.2. Collection and validation of the serum samples

Sera from patients with typical Q fever symptoms (pneumonia, endocarditis) or control samples were contributed to the study by an expert from 4 different participating laboratories (laboratory A, C, E, and I). The Q fever cases were confirmed either by a positive polymerase chain reaction (PCR) or by seroconversion. In total, the proficiency panel consisted of 25 serum samples: 5 samples with an intended negative outcome, 6 samples from (acute) Q fever patients, and a serial diluted high-positive sample ($n = 8$) from a Q fever patient. The 5 negative control samples consisted of 4 samples derived from healthy blood donors and, in 1 case, a serum sample from a PCR-positive acute Q fever patient taken prior to seroconversion which was included as a sample with an intended negative serologic outcome. In addition, 2 samples from a nonacute past infection, 2 samples with a solitary IgM phase II reaction (false positive), and 2 samples with anticomplement activity (false positive in the CFA) were included in the panel.

3. Results

3.1. Results of the intended negative samples

For the diagnosis of Q fever, all assays reached a specificity of 95% to 100% in the samples with an intended negative outcome (Tables 2 and 3). Only 1 of 8 laboratories reported a low-positive IFA titer of 1:32 for IgG phase II in a sample from 1 blood donor. All results from the other laboratories that performed IFAs were reported as negative in both the IgM and IgG phase I and II assays. Of the 6 CFA participating laboratories, laboratory A reported a titer of 1:10 and laboratory B a titer of 1:20 in samples from 2 different blood donors for phase II. The results were, however, interpreted as negative and equivocal, respectively. Laboratory A also reported a titer of 1:10 in the phase I CFA assay in a blood donor interpreted as equivocal (Fig. 2A). All results from the laboratories that performed ELISAs were reported as negative in the IgM phase II ($n = 5$) and in the IgG phase I ($n = 3$) and II ($n = 4$).

In the sample of a Q fever patient in an acute phase proven by a positive PCR but who previously tested serologically negative, only laboratory E reported low IFA titers to both IgG phase I and II of 1:32. The IgM phase II ELISA ratios in the PCR-positive acute patient sample were elevated to ratios of 0.31 to 0.48 compared to the negative blood donors (ratio 0.06 to 0.15). None of the assays yielded a positive response in the 2 samples that had anticomplement activity, and all CFA laboratories corrected the false-positive response detected in these samples.

3.2. Results of the intended positive samples from Q fever patients

All laboratories correctly identified Q fever patients with the algorithm of the methods that were applied (Table 2). IFA, ELISA, and CFA values between laboratories using the same methods were within close range, with usually no more than 2 dilution differences in the reported titer. However, there were differences in sensitivity between the methods (Table 3). All laboratories using the IFA yielded a positive response to IgG phase II and IgM phase I in Q fever patients. Only laboratory G missed a phase I IgG-positive response in 2 samples using the IFA (Fig. 2B). Two Q fever patients had low levels of IgM phase II antibodies as reported by 4 of the 8 participants using IFA. Laboratories using ELISA and CFA detected all patients with a positive

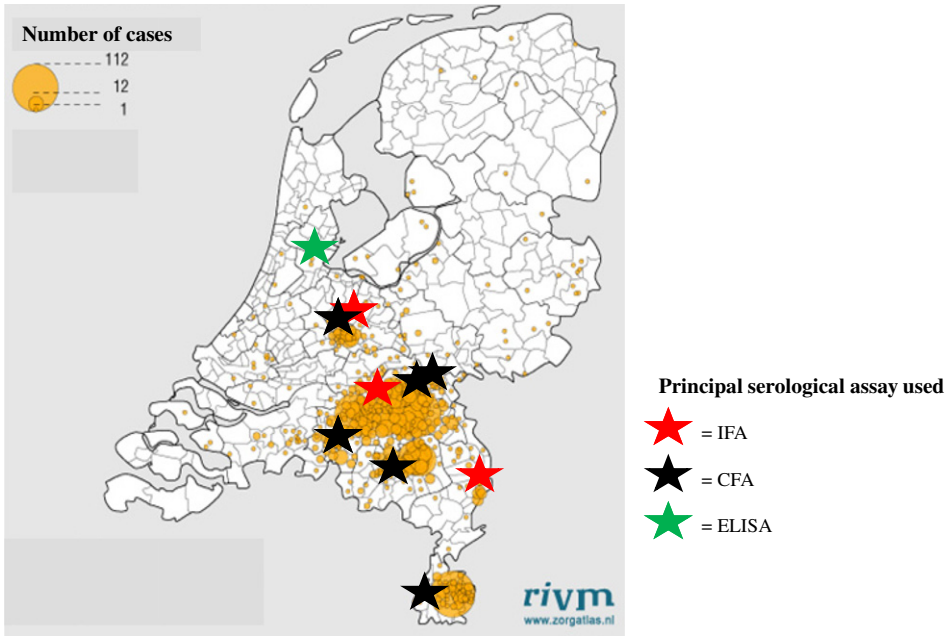

Fig. 1. Location of the 10 participating Dutch laboratories.

(IgG) phase II responses; however, 2 and 3 positive phase I patients were missed by the ELISA and CFA, respectively (Fig. 2B).

The IFA proved to be the most sensitive assay in detecting a past Q fever infection (Table 3). All laboratories using IFA detected both IgG phase I (1:32–1:256) and II (1:128–1:512) antibodies in samples in these patients (Fig. 2C). The ELISA yielded mostly intermediate IgG phase II responses, and 4 of 6 laboratories using CFA detected only low titers (1:10–1:20) against phase II. No IgM response was detected with any of the applied assays (Fig. 2C).

3.3. Results of the solitary IgM phase II reactive samples

The solitary IgM-positive phase II response was detected by most laboratories using the IFA and the ELISA assays (Fig. 2D). Laboratory E reported an intermediate result for the phase II IgM IFA response, and one of the international reference laboratories interpreted the samples as nonreactive for Q fever. Low-level IFA titers were reported against IgM and IgG phase I (1:32–1:64) by some laboratories. The samples were all nonreactive in the CFA assays.

Table 2
Qualitative results of the intended negative and positive Q fever patient samples in the proficiency panel.

	Negative samples				Q fever				Past infection			
	IgM		IgG		IgM		IgG		IgM		IgG	
IFA	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
Laboratory												
E	0/7	0/7	1/7	1/7	4/6	6/6	6/6	6/6	0/2	0/2	2/2	2/2
F	0/7	0/7	0/7	0/7	4/6	6/6	6/6	6/6	0/2	0/2	2/2	2/2
G	0/7	0/7	1/7	0/7	6/6	6/6	6/6	4/6	0/2	0/2	2/2	2/2
I	0/7	0/7	0/7	0/7	5/6	6/6	6/6	6/6	0/2	0/2	2/2	2/2
J	0/7	0/7	0/7	0/7	6/6	6/6	6/6	6/6	0/2	0/2	2/2	2/2
ARRL	0/5	0/5	0/5	0/5	3/5	5/5	5/5	5/5	0/2	0/2	2/2	1/2
APHM	0/7	0/7	0/7	0/7	5/5	5/5	5/5	5/5	0/2	0/2	2/2	2/2
CDC[b]	0/7	0/7	0/7	0/7	ND	ND	5/5	4/5	ND	ND	2/2	0/2
% Positive	0%	0%	4%	2%	83%	100%	100%	93%	0%	0%	100%	81%
ELISA	Phase II		Phase II	Phase I	Phase II		Phase II	Phase I	Phase II		Phase II	Phase I
A	0/7		ND	ND	4/6		ND	ND	2/2		ND	ND
E	0/7		0/7	0/7	5/6		6/6	4/6	2/2		0/2	0/2
G	0/7		0/7	0/7	3/6		6/6	4/6	2/2		0/2	0/2
H	0/7		0/7	ND	4/6		6/6	ND	2/2		0/2	ND
I	0/7		0/7	0/7	6/6		6/6	4/6	2/2		1/2	0/2
% Positive	0%		0%	0%	60%		100%	67%	0%		13%	0%
CFA[a]	Phase II	Phase I			Phase II	Phase I			Phase II	Phase I		
A	1/7	1/7			6/6	3/6			0/2	1/2		
B	1/7	0/7			6/6	4/6			0/2	0/2		
C	0/7	ND			6/6	ND			2/2	ND		
D	0/7	0/7			6/6	4/6			2/2	0/2		
F	0/7	ND			6/6	ND			2/2	ND		
H	0/7	ND			6/6	ND			2/2	ND		
% Positive	5%	5%			100%	61%			67%	17%		

ND = Not done.
[a] For comparison, all reported CFA titers of ≥1:10 were scored as positive.
[b] Only total Ig was measured. Number of positive results/number of tested samples.

Table 3
Overall sensitivity and specificity of the used serologic methods using the disease state as a gold standard.

	Specificity				Sensitivity in acute Q fever				Sensitivity in past Q fever infection			
	IgM		IgG		IgM		IgG		IgM		IgG	
	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
IFA	100%	100%	96%	98%	83%	100%	100%	93%	0%	0%	100%	81%
ELISA	100%		100%	100%	60%		100%	67%	0%		13%	0%
CFA[a]			95%	95%			100%	61%			67%	17%

[a] Only total Ig was measured.

3.4. Results of a serial diluted high-positive sample

In the serial diluted samples, the IFA showed the highest sensitivity for both the phase I and II responses (Fig. 3). The ELISA and the CFA had the lowest sensitivity for phase II and I, respectively. Comparing the differences between the commercial and in-house IFA assays, we found that the results were highly comparable for phase II, with the exception that the APHM interpreted samples as non-responsive, whereas other laboratories detected IgG titers up to 1:128. The phase I results showed a slightly higher sensitivity in the methods used by the ARRL and the commercial assays, but the differences were small (Fig. 4).

4. Discussion

All laboratories correctly identified Q fever patients in an acute phase using the different serologic methods—IFA, CFA, and ELISA. All 3 methods also showed a good specificity. Comparability between IFA, ELISA, and CFA values between laboratories using the same methods was also within close range, with usually no more than 2 dilutions

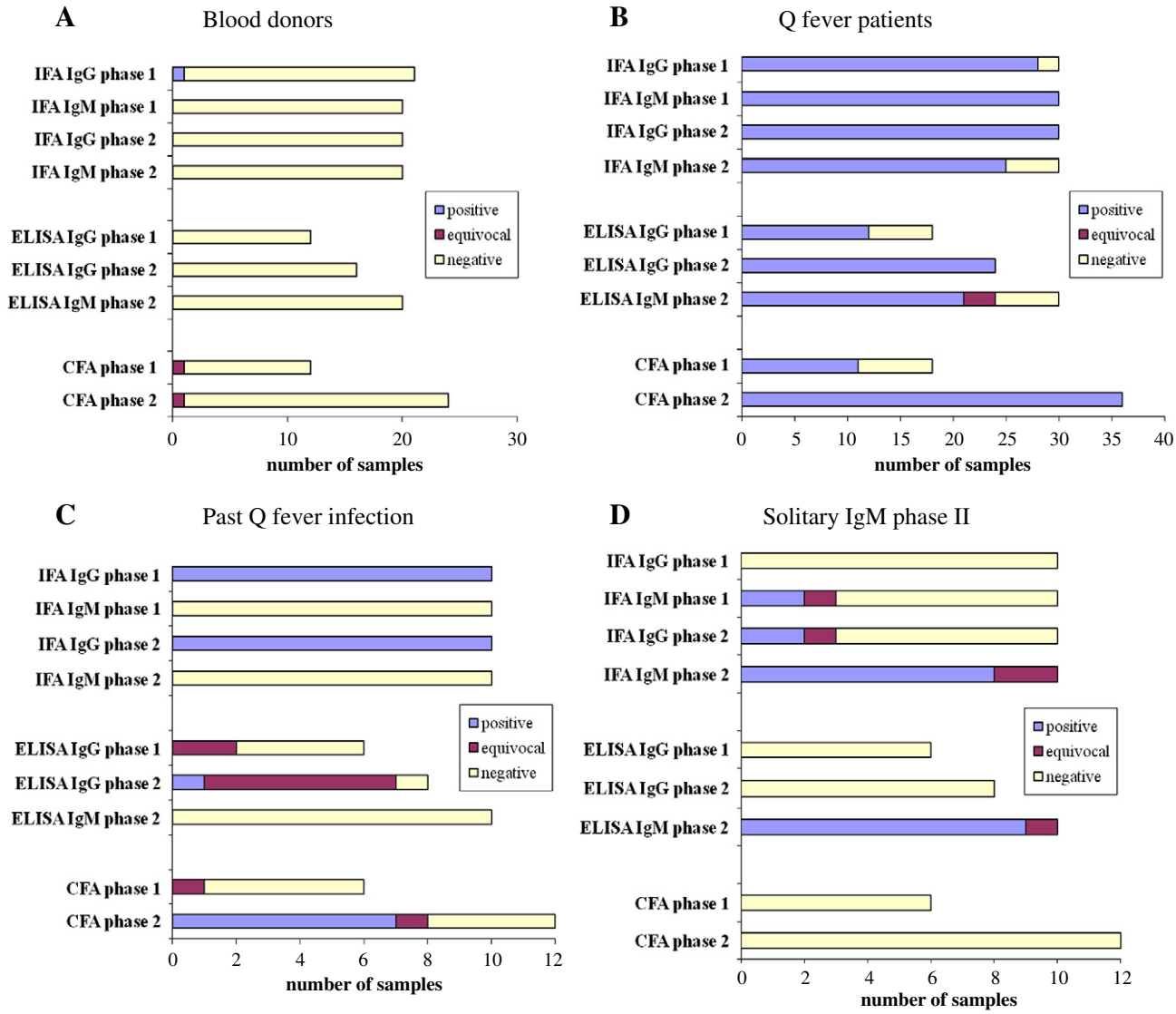

Fig. 2. Summary of the results with the different Q fever–specific serologic assays used in the Dutch laboratories.

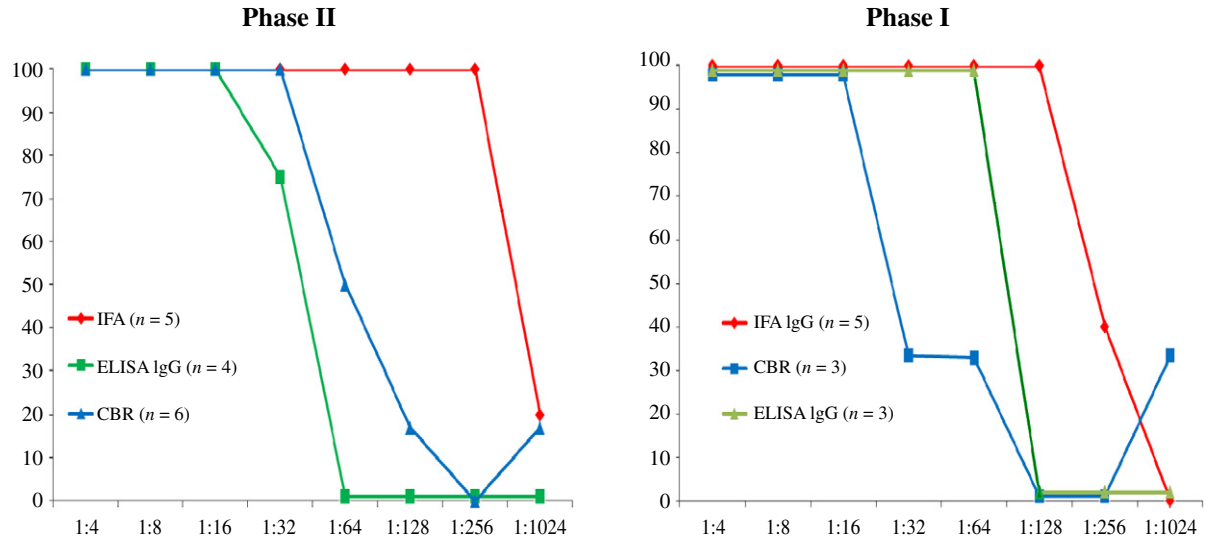

Fig. 3. Summary of the IFA, ELISA, and CFA results in samples from diluted phase II and I IgG-positive patient serum as tested by the Dutch laboratories.

between the reported titer. This high level of agreement was most likely positively influenced by the use of assays derived from the same manufacturer, and, in some cases, even the same batch numbers were used. However, different cut-off levels for the CFA were used in various laboratories (≥1:10 and ≥1:20), which can possibly lead to miscommunication and an underestimation of positive CFA results.

As expected from previous reports in the literature (Dupuis et al., 1985; Field et al., 2002; Péter et al., 1987; Slabá et al., 2005), some quantitative differences between the 3 different assays were observed, with the IFA being the most sensitive method. The higher sensitivity of the IFA was apparent in samples from both Q fever patients and the serially diluted samples. These differences were most pronounced in identifying past Q fever infection and detecting phase I responses. These results indicate that the IFA remains an important tool in the follow-up of Q fever patients and for identifying patients at risk for developing chronic Q fever. Although low antibody titers can indicate an initial immune response, the clinical value of low IFA titers is not always clear and the results may indicate false-positive responses. It is known that, in approximately 1% of serology results of patients suspected of acute Q fever, a solitary positive IgM phase II is detected by IFA and ELISA that cannot be confirmed by PCR or seroconversion to IgG in consecutive samples indicating a false-positive reaction. One advantage of the CFA is that it does not detect these false-positive IgM phase II responses. However, low titers detected by CFA can indicate both an acute and a past infection since the assay does not distinguish between IgM and IgG antibodies. A true acute Q fever case therefore can only be correctly identified in both of these circumstances by serologic follow-up and additional PCR testing (Fournier and Raoult, 2003; Schneeberger et al., 2010).

The 3 different in-house IFAs were also qualitatively comparable to the commercial IFA assay with some minor quantitative differences. The observed differences indicate that each laboratory should establish the cut-off values of their IFA independently, and reported cut-off levels by other laboratories cannot be copied as suggested by other investigators (Dupont et al., 1994; Healy et al., 2011; Villumsen et al., 2009). The in-house IFA used at the ARRL generally showed the highest endpoint titers. In this laboratory, the antigens for both phase I and II are grown in-house and the slides are made fresh monthly. This could positively affect sensitivity, especially for the phase I assay. The APHM applies an elegant method of prescreening samples with a total immunoglobulin conjugate, eliminating possible low reactive false-positive reactions, such as false-positive IgM phase II responses.

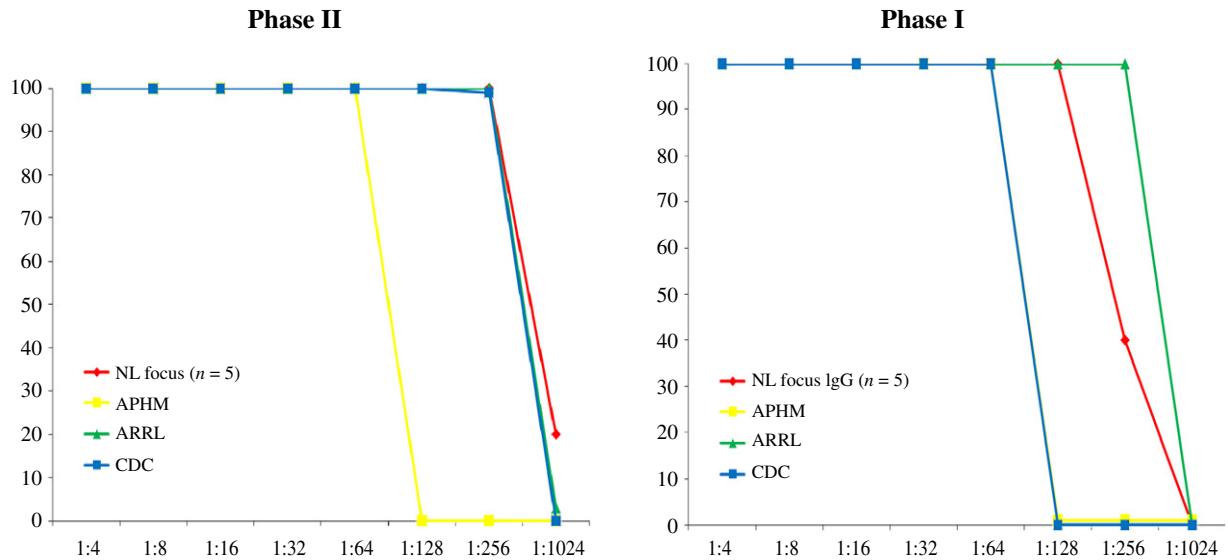

Fig. 4. Summary of diluted phase II and I IgG Q fever–positive patient serum results between the 3 international reference laboratories and Dutch laboratories (FOCUS).

This algorithm most likely also explains the lower sensitivity detected in the samples of the serial diluted positive Q fever patient.

In summary, IFA, ELISA, and CFA are all suitable serodiagnostic assays for diagnosing acute Q fever. ELISAs can be an alternative for screening large sample numbers. The IFA appears to be the method of choice when high sensitivity is required, especially against phase I (e.g., identifying chronic Q fever). However, these observations were based on a limited number of samples and need to be supported by a larger study which will allow statistical analysis to confirm our results.

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