Comparison of Real-Time PCR and a Microimmunofluorescence Serological Assay for Detection of *Chlamydophila pneumoniae* Infection in an Outbreak Investigation

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We assessed the performance of a recently validated real-time PCR assay and a commercially available microimmunofluorescence serologic test for the detection of *Chlamydophila pneumoniae* infection during an outbreak. Evaluation of specimens from 137 individuals suggests that real-time PCR holds greater utility as a diagnostic tool for early *C. pneumoniae* detection.

Chlamydophila pneumoniae is one of the leading causes of community-acquired respiratory tract infections, accounting for approximately 10% of community-acquired pneumonia (CAP) cases and 5% of bronchitis and sinusitis cases in adults and children (18). Most respiratory infections caused by *C. pneumoniae* are asymptomatic or mild, although severe pneumonia can develop in elderly patients and those with co-existing cardiopulmonary diseases (2). Seroepidemiological studies have shown an antibody prevalence of 50 to 70%, suggesting a high frequency of previous infections, although these data may be misleading for reasons discussed below (12). Nearly all humans can expect to be infected with *C. pneumoniae* at least once during their lifetimes. Reinfections are common, and persistence of the agent in the host after primary infection is a potential risk for chronic infection (8, 9).

Reliable diagnosis of *C. pneumoniae* infection remains difficult due to the lack of standardized and commercially available diagnostic tests that are both sensitive and specific. Laboratory methods currently used for the diagnosis of acute *C. pneumoniae* infection include culture, immunohistochemical assays, serology, and PCR; the latter two are the most often applied (13). Although infections with *C. pneumoniae* can be identified by direct isolation of the agent, this procedure is laborious and time-consuming and often yields inconsistent results (15). The microimmunofluorescence (MIF) test is currently considered the "gold standard" for the serodiagnosis of *C. pneumoniae* infection, even though results are often subjective and require specialized training for interpretation (20). In addition, technical complexity, subjective endpoints, and the lack of standardized reagents result in significant intra- and interlaboratory variations in test performance (11). Moreover, the requirement of paired serum samples and the extended persistence of IgG antibody in some adult populations make this test retrospective in nature and unsuitable for timely diagnosis (6). MIF testing is further hampered by poor specificity due to cross-reaction with other chlamydial species and is unable to discriminate between past and persistent infections (3, 7, 17). Molecular analysis-based assays, such as real-time PCR, have recently been developed for the rapid and sensitive detection of *C. pneumoniae* (14, 22, 23). The overall diagnostic utility of PCR-based assays is currently unknown due to a lack of

specimen type, nucleic acid extraction method, and amplification protocol standardization and the unavailability of reliable commercial assays (2).

Outbreaks of *C. pneumoniae* occur in individuals living in surroundings where they are close to others, such as schools and military barracks (4, 16, 21). *C. pneumoniae* can also play a significant role in coinfections, as seen in a recent CAP outbreak due to *Streptococcus pneumoniae* on a military base (5). Recently, a *C. pneumoniae* CAP outbreak occurred in a prison with male inmates within the southern United States. A validated multiplex real-time PCR assay was used to identify *C. pneumoniae* as the causative agent of the CAP outbreak (22). After initial real-time PCR testing, a multipathogen PCR-based molecular detection assay was used to rule out coinfection with other pathogens (data not shown) (10). The aim of the present study was to evaluate the performance of a recently validated multiplex real-time PCR assay and a commercially available MIF serologic detection kit for their reliability in detecting *C. pneumoniae* during this recent outbreak.

Case subjects were defined as those having a fever (temperature of ≥38°C) and a cough that persisted for >3 days or clinical and/or X-ray-confirmed pneumonia. Asymptomatic individuals from shared prison facilities were randomly selected and assigned to a noncase group. Oropharyngeal (OP) and/or nasopharyngeal (NP) swab specimens and serum samples were obtained from symptomatic individuals (*n* = 38) and from individuals with no reported illness or symptoms (*n* = 99). Swabs were placed in 2 ml of Universal Transport Medium (BD) and transported frozen or cold (4 to 10°C) for molecular testing. Single serum samples were collected from each individual and transported frozen. Only individuals who provided both a swab and a serum sample were included in this study.

TABLE 1 Diagnostic sensitivities and specificities of real-time PCR and MIF assays for 38 case patients and 99 noncase individuals[a]

No. of case subjects	Sensitivity (%)				No. of noncase subjects	Specificity (%)			
	PCR	IgM MIF	IgG MIF	IgM + IgG MIF		PCR	IgM MIF	IgG MIF	IgM + IgG MIF
38	71 (55–83)	60 (43–75)	82 (65–92)	97 (87–100)	99	97 (91–99)	77 (67–84)	40 (30–51)	30 (22–40)

[a] Data are percentages (95% confidence intervals). The positive and negative predictive values of the assays are as follows: PCR, 90 and 90%; IgM + IgG MIF, 35 and 97%; IgM MIF, 50 and 82%; IgG MIF, 34 and 85%.

Total nucleic acid extraction was performed on all NP/OP swab specimens following manufacturer's instructions for the Total NA Serum_Plasma_Blood protocol with a 200-μl sample volume and a 100-μl elution volume using MagNa Pure Compact nucleic acid isolation kit I (Roche Applied Science). A validated multiplex real-time PCR assay that tests for *C. pneumoniae* (CP-arg) along with *Mycoplasma pneumoniae* (MP181) and *Legionella* spp. (*ssrA*) was performed in duplicate on all extracted specimens as previously described (22). Serum samples were tested for reactivity to *C. pneumoniae* using the commercially available *Chlamydia* MIF IgG and IgM kits (FOCUS Diagnostics) following the manufacturer's instructions. As indicated by the manufacturer, a probable acute infection was defined by a single serum sample with an IgM titer of ≥1:10 and/or an IgG titer of ≥1:512. The diagnostic sensitivity, specificity, and positive predictive value were calculated for each of these laboratory tests using clinically defined cases as the gold standard (19).
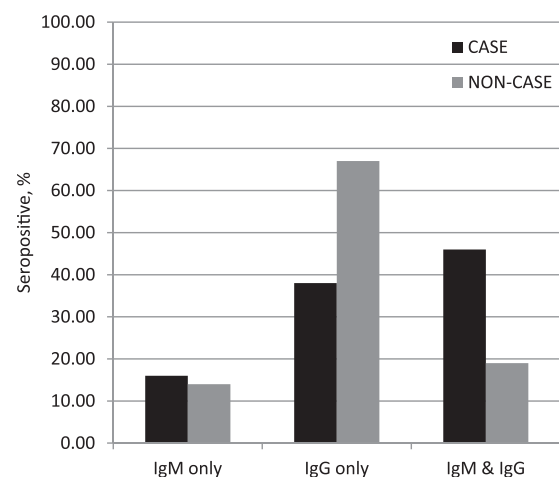
A total of 137 individuals (38 case and 99 noncase subjects) were tested for the presence of *C. pneumoniae* infection by real-time PCR and by MIF. Table 1 summarizes the sensitivity, specificity, and positive and negative predictive values of each diagnostic assay. Twenty-seven case patients (71%) had positive real-time PCR results, and 37 (97%) had positive results with the IgM + IgG MIF assay. The sensitivity and specificity of the IgM MIF assay were 60% and 77%, respectively, while the IgG MIF had a sensitivity of 82% and a specificity of 40%. While the IgM + IgG MIF assay appeared to be the most sensitive method studied, it had low specificity (30%). The sensitivity (71%) of the real-time PCR assay was lower than that of the IgM + IgG MIF assay (97%), but its specificity (97%) was much higher. Real-time PCR also had a much higher positive predictive value (90%) than the IgM + IgG MIF assay (35%). Table 2 summarizes the isotype distribution of PCR-positive case and noncase individuals. Twenty-seven case individuals were positive by PCR. Of these individuals, 2 (8%), 6 (22%), and 19 (70%) were positive for IgM only, IgG only, and IgM and IgG, respectively. Three individuals from the noncase group had positive PCR results. All three PCR-positive individuals also yielded positive MIF results (2 IgG only and 1 containing both IgM and IgG), suggesting an active but asymptomatic infection for these three individuals.

Figure 1 illustrates the isotype distribution among seropositive individuals from both the case and noncase groups that met the required titer indicative of a probable acute infection, as indicated by the manufacturer. Sixty-two percent of seropositive case individuals had IgM only (16%) or both IgG and IgM (46%) isotypes present, suggesting a primary infection, while 38% had IgG only, suggesting the presence of a recurring infection. In contrast, 67% of seropositive noncase individuals had only IgG present. This observation is consistent with the reported high prevalence of persistent IgG titers in some adults and highlights a significant limitation of the MIF assay. The remaining 33% of the seropositive noncase individuals comprised 14% positive for IgM only and 19% positive for both IgG and IgM, suggesting either background seropositivity or cross-reactivity, although a possible asymptomatic infection could be present as well.

In this particular outbreak, serology testing alone would likely have been misleading since the rate of seropositivity among noncase individuals was extremely high. Most studies recommend testing of paired serum samples. However, in an outbreak setting, only a single serum sample would be available for timely diagnostic testing and was therefore used for comparison in the present study. This limitation was minimized by testing serum specimens collected from the noncase group in order to establish the background seropositivity. Although real-time PCR is subject to some limitations as well, it has demonstrated greater utility for rapid and accurate detection of etiologies, especially in outbreak settings (1). Significant challenges continue to confound the accurate and dependable diagnosis of *C. pneumoniae* infection. Collectively, our analysis of these two commonly used tests for *C. pneumoniae* detection suggests that real-time PCR is a more useful diagnostic

TABLE 2 Isotype distribution of real-time PCR-positive case and noncase individuals

Group	Total no. PCR positive	No. (%) PCR positive for:		
		IgM only	IgG only	IgM + IgG
Case	27	2 (8)	6 (22)	19 (70)
Noncase	3	0	2 (67)	1 (33)

FIG 1 Isotype distribution of 37/38 case patients and 69/99 noncase individuals with positive serologic results as determined by the MIF assay. Seropositivity is defined as an IgM titer ≥1:10 and/or an IgG titer ≥1:512.

tool to confirm the etiology of *C. pneumoniae* in an outbreak setting.

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