JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1994, p. 903–905
0095-1137/94/$04.00+0

Vol. 32, No. 4

Diagnostic Utility of PCR-Enzyme Immunoassay, Culture, and Serology for Detection of *Chlamydia pneumoniae* in Symptomatic and Asymptomatic Patients

CHARLOTTE A. GAYDOS,[1] PATRICIA M. ROBLIN,[2] MARGARET R. HAMMERSCHLAG,[2] CHARLES L. HYMAN,[2] JOSEPH J. EIDEN,[1] JULIUS SCHACHTER,[3] AND THOMAS C. QUINN[1,4]*

Departments of Medicine and Pediatrics, The Johns Hopkins University School of Medicine, Baltimore,[1] and Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda,[4] Maryland; Departments of Medicine and Pediatrics, SUNY Health Science Center at Brooklyn, New York, New York,[2] and Department of Laboratory Medicine, University of California, San Francisco, California[3]

Received 8 September 1993/Returned for modification 30 November 1993/Accepted 27 December 1993

To assess the utility of PCR-enzyme immunoassay (EIA) for diagnosis of acute infection with *Chlamydia pneumoniae*, we compared tissue culture, PCR-EIA, direct fluorescent-antibody (DFA) stain, and serology in studies with 56 patients with respiratory symptoms and 80 asymptomatic persons. Thirty-five patients were positive by either culture or PCR-EIA, and 101 were negative by both assays. Thirty specimens from symptomatic patients and one from an asymptomatic patient were culture positive; 23 of these were also PCR-EIA positive. Of the eight culture-positive, PCR-EIA-negative specimens, five were DFA negative and three were DFA positive. Four additional specimens were culture negative and PCR-EIA positive; of these, three were DFA positive and one was DFA negative. When we used culture- and/or DFA-positive results as a reference or "gold standard," the sensitivity and specificity of PCR were 76.5 and 99.0%, respectively. When we used PCR- and/or DFA-positive results as the reference, the sensitivity of culture was 87.5%. On the basis of single acute serum specimens, only 8 of these 35 patients had diagnostic antibody titers. Of the asymptomatic patients, 75% had immunoglobulin G or immunoglobulin M antibody to *C. pneumoniae*; 15 (18.8%) of these had antibody levels considered to be diagnostic of acute infection. This multicenter study indicates that culture and/or PCR-EIA is more reliable for prompt diagnosis of *C. pneumoniae* infection than single-point serology alone.

Chlamydia pneumoniae is an important respiratory pathogen and has been implicated as a cause of up to 10% of cases of community-acquired pneumonia (5, 7). It has also been associated with asthma, bronchitis, pharyngitis, acute chest syndrome, and coronary heart disease (6, 8, 10). Serology for *C. pneumoniae* antibodies has been used for diagnosis in most of these studies (2, 7). Since serology based on paired sera is useful only retrospectively and culture may take 3 to 7 days for a result to be obtained, a rapid method such as PCR may be advantageous because it offers more timely treatment options. Previous studies have demonstrated that PCR can be used to detect *C. pneumoniae* (4). However, those initial studies did not determine the sensitivity or specificity of PCR by testing a large number of clinical specimens in parallel with cultures from symptomatic and asymptomatic individuals. We therefore initiated a study to determine the usefulness of PCR compared with culture for the rapid diagnosis of acute *C. pneumoniae* infections (4). In addition, this PCR has been adapted for detection of amplified DNA products by enzyme immunoassay (EIA), thereby offering a diagnostic tool for screening large numbers of clinical specimens (3).

MATERIALS AND METHODS

Nasopharyngeal specimens, collected with dacron-tipped wire shaft swabs, from a group of 42 pediatric and 14 adult patients with respiratory symptoms (n = 56) and a group of asymptomatic adult persons (n = 80) were cultured for *C. pneumoniae*. The symptomatic patients were randomly selected patients who were being evaluated for *C. pneumoniae* infection or were part of ongoing studies from 1989 to 1993 of the association of *C. pneumoniae* and asthma, pneumonia, or cystic fibrosis in children and adults from New York, N.Y. The asymptomatic persons were healthy adult health-care workers in Brooklyn without respiratory symptoms during the prior 3 months and without a known history of *C. pneumoniae* infection. Serological studies were performed at a different institution (the University of California), without knowledge of the culture results. Culture transport media (1.5 ml of 2-sucrose phosphate media) were stored at −70°C and were retrospectively analyzed by PCR-EIA at a third institution (The Johns Hopkins University), without culture or serology results being known.

Cultures were performed by inoculating 200 μl of the specimen in chlamydia transport media into HEp-2 cells in 96-well microtiter plates, and negative cultures were passed once to fresh cells (9). Isolates were identified as chlamydiae with genus-specific fluorescein-conjugated monoclonal antisera (culture confirmation reagent; Sanofi Diagnostics Pasteur, Chaska, Minn.), and their identities were confirmed by using *C. pneumoniae*-specific monoclonal antibody (Washington Research Foundation, Seattle, Wash.). Isolates were also stained by anti-major outer membrane protein, *Chlamydia trachomatis*-specific monoclonal antisera (Syva, Palo Alto, Calif.) to identify *C. trachomatis*. Aliquots of the original culture transport medium were shipped on dry ice for analysis by PCR-EIA and direct fluorescent-antibody (DFA) staining. Two hundred microliters of the specimen was lysed by proteinase K-detergent treatment and dialyzed. Fifty microliters was then ampli-

* Corresponding author. Mailing address: Division of Infectious Diseases, The Johns Hopkins University School of Medicine, Ross Research Building 1159, 720 Rutland Ave., Baltimore, MD 21205-2196.

TABLE 1. IgG and IgM titers for specimens positive for *C. pneumoniae* by culture and PCR-EIA

Specimen	Titer[a] of:	
	IgG[b]	IgM[c]
H-17	1:16	–
H-18	–	–
H-19	–	–
H-20	1:64	–
H-21	1:128	1:64
H-22	1:32	–
H-23	1:128	1:32
H-25	1:128	1:32
T2213	1:256	1:64
T2223	1:16	–
T2452	1:1024	–
CF 12	1:1024	>1:256
P 12	–	–
CF 1	1:16	–
A 221	1:32	–
A 216	1:32	–
A 214	None[d]	
A 206	–	–
T 2251	1:32	–
086 (asymptomatic)	–	–
T 2244	1:256	–
A 155	1:8	–
A 147	1:16	1:16

[a] Negative (–) result indicates a titer of <1:16.
[b] Single IgG titers of ≥1:512 are considered to be diagnostic of acute infection.
[c] IgM antibody levels of ≥1:16 are considered to be diagnostic of acute infection with *C. pneumoniae*.
[d] No serum sample available.

fied in a microtiter format in a 9600 thermocycler (Perkin-Elmer Cetus) (3). The *C. pneumoniae*-specific PCR was based on primers from the 16S rRNA gene (4). PCR products were detected by an EIA following hybridization with a 270-bp biotinylated RNA probe to nested sequences within the amplicon (1, 3). The biotin-labeled DNA-RNA hybrid was captured in antibiotin-coated 96-well microtiter plates. Discrepant results of culture and PCR-EIA were analyzed by spinning the culture transport media and performing a DFA stain of the sediment for elementary bodies with Sanofi Diagnostics culture confirmation reagent. Sera were assayed by microimmunofluorescence for immunoglobulin G (IgG) and IgM antibodies (11).

RESULTS

Of 136 patients (56 symptomatic and 80 asymptomatic) tested, 35 were positive by either culture or PCR-EIA and 101 were negative. Thirty-one were tissue culture positive and 27 were PCR-EIA positive. Only one specimen positive by both assays was from an asymptomatic patient. There were 23 specimens positive by both tests (Table 1). There were 12 specimens with discrepant culture and PCR-EIA results; 3 were culture positive, PCR-EIA negative, and DFA positive. Five were culture positive, PCR-EIA negative, and DFA negative. Four specimens were culture negative and PCR-EIA positive, of which three were DFA positive (Table 2). Before discrepant results were resolved, the sensitivity and specificity of PCR-EIA were 74.2 and 96.2%, respectively. When we used DFA to resolve discrepant results, the revised sensitivity and specificity of culture were 87.5 and 97.1%, respectively, and those of PCR-EIA were 76.5 and 99.0%, respectively. If either culture or PCR-EIA positivity were accepted as true positivity, then tissue culture would have a sensitivity of 88.6% and a specificity of 100%. PCR-EIA would be 77.1% sensitive and 100% specific. One specimen was positive for *C. trachomatis* by culture and negative by the *C. pneumoniae*-specific PCR-EIA.

TABLE 2. Comparison of culture, PCR-EIA, and DFA for detection of *C. pneumoniae* in nasopharyngeal specimens

Test and result	No. of samples with result by:					
	Culture		Culture and/or DFA		PCR-EIA and/or DFA	
	Positive	Negative	Positive	Negative	Positive	Negative
PCR-EIA						
Positive	23	4	26	1		
Negative	8	101	8	101		
Culture						
Positive					28	3
Negative					4	101

On the basis of a single serum specimen from each of these 35 patients, 8 (22.9%) had titers considered to be diagnostic of acute infection (Tables 1 and 3). For one patient no serum sample was available. Of the 23 individuals who were both culture and PCR-EIA positive, 12 (52.2%) demonstrated levels of IgG antibody to *C. pneumoniae* of ≥1:32, but only 6 of these patients also had IgM antibody levels of ≥1:16 and one additional patient had only a high IgG antibody level (Table 1). Therefore, only 7 (30.4%) of these 23 patients met diagnostic criteria for a serological diagnosis of active infection based on a single serum sample. However, since paired specimens are usually used to determine acute infection by serodiagnosis, more of these individuals might have had diagnostic titers if convalescent sera had been available.

Only one of the asymptomatic patients was both culture and PCR-EIA positive. However, 60 (75%) of 80 were antibody positive (IgG level of ≥1:32) by microimmunofluorescence. Fifteen (18.8%) of these asymptomatic patients met diagnostic criteria for acute infection, as indicated by Grayston et al. (5), by demonstrating IgM antibody levels of ≥1:16 (n = 10) or IgG levels of ≥1:512 (n = 5) in serology.

TABLE 3. Discrepant results of culture, PCR-EIA, and DFA

Specimen	Test result by:			Titer[a] of:	
	Culture (no. of inclusions/well)	PCR-EIA	DFA	IgG[b]	IgM[c]
ASK 20	+ (2, second passage)	–	+	1:8	–
ASK 29	+ (10, second passage)	–	+	–	–
T2224	+ (1, second passage)	–	–	–	–
T2229	+ (>100)	–	–	1:128	1:16
ASK 18	+ (35)	–	–	1:32	–
T2382	–	+	+	1:32	–
T2444	+ (14)	–	+	–	–
CF3	–	+	+	–	–
087	–	+	–	–	–
A192	+ (10)	–	–	1:32	–
T2259	+ (5)	–	+	1:256	–
085	–	+	+	1:256	–

[a] Negative (–) result indicates a titer of <1:16.
[b] Single IgG titers of ≥1:512 are considered to be diagnostic of acute infection.
[c] IgM antibody levels of ≥1:16 are considered to be diagnostic of acute infection with *C. pneumoniae*.

DISCUSSION

These results indicate that PCR-EIA of respiratory specimens can reliably detect the presence of *C. pneumoniae* with relatively high sensitivity and specificity. In addition, this assay had previously detected *C. pneumoniae* in respiratory specimens from a nonhuman primate model of infection (4). In this study of 35 patients positive by either PCR or culture, 23 were positive by both tests. There were 12 specimens that gave discrepant results for culture and PCR-EIA. Three specimens were culture and DFA positive but PCR-EIA negative, and they may have contained inhibitors to the polymerase enzyme. One of these specimens, for which additional transport media were available, became repeatedly PCR-EIA positive when diluted 1:10, supporting the possibility of the presence of an inhibitor. The specimens were also tested with *C. trachomatis* primers and were negative (1). Five specimens were culture positive but PCR-EIA and DFA negative. A possible explanation for this discrepancy could be that there was too small an aliquoted sample for adequate DFA testing. Conversely, falsely negative PCR-EIA may be due to the presence of inhibitory substances or to the loss of amplifiable DNA due to nucleases in the sample. Three samples which were falsely negative by PCR-EIA were continually PCR-EIA negative after dilution to 1:10, and all became PCR-EIA positive when a dilute positive control was added.

A false-positive PCR result may have been obtained for the one specimen (087) which was culture and DFA negative but PCR-EIA positive, especially since the specimen was PCR negative four times upon repeat testing. The three PCR-EIA- and DFA-positive specimens which were culture negative probably were specimens with which PCR-EIA was more sensitive than culture. The use of DFA testing of culture transport media appeared to be a valuable way to resolve discrepancies between culture and PCR-EIA results, but it is difficult to define a perfect gold standard. However, when we used the results of two tests as a gold standard, the sensitivity of culture was better than that of PCR. Specificities were similar.

The findings of this study, however, raise a concern about the sensitivity and specificity of serology for diagnosing active infection with *C. pneumoniae*. Although many (75%) of the asymptomatic persons had antibody to *C. pneumoniae*, it is surprising that 15 (18.8%) of the asymptomatic persons had antibody levels considered to be diagnostic of acute infection with *C. pneumoniae* (5). The finding of diagnostic antibody titers among asymptomatic patients who were culture and PCR negative suggests that the criteria developed by Grayston et al. for adults may not be appropriate for this population. Conversely, of the 56 symptomatic patients, 30 (53.6%) had IgG antibody levels of $\geq$1:32, 3 had IgG antibody levels of $\geq$1:512, and 15 (26.8%) had IgM antibody levels of $\geq$1:16. Thus, 18 (32.1%) had antibody levels considered to be diagnostic of acute infection with *C. pneumoniae* on the basis of a single serum specimen, but only 7 of these patients had a positive culture and/or a positive PCR-EIA result. The lack of diagnostic antibody titers among 28 patients who were either PCR or culture positive indicates a lack of sensitivity with a single serologic specimen. If convalescent sera had been obtained, these serologic results might have been improved. However, treatment decisions are often based on a single specimen, and convalescent serum is then only useful retrospectively. In addition, the criteria for the serologic diagnosis of acute infection as developed by Grayston et al. were based on adult samples (5). The serologic responses may be different in children.

In summary, these data indicate that culture positivity and/or PCR-EIA positivity may be more reliable for the diagnosis of acute *C. pneumoniae* infection than positivity by serology, which may be nonspecific and insensitive for treatment considerations. This retrospective study of a large number of culture-positive specimens also indicates that PCR-EIA can detect *C. pneumoniae* in clinical respiratory specimens and that the specificity of PCR-EIA is excellent, although its sensitivity may be improved. However, additional prospective studies are needed to resolve the diagnostic differences between serology, culture, and PCR-EIA.

ACKNOWLEDGMENTS

We thank L. E. Welsh, M. Gelling, and L. Ramos for technical help, as well as Patricia Buist for editorial assistance.

REFERENCES

1. **Bobo, L., F. Coutlee, R. H. Yolken, T. Quinn, and R. P. Viscidi.** 1990. Diagnosis of *Chlamydia trachomatis* cervical infection by detection of amplified DNA with an enzyme immunoassay. J. Clin. Microbiol. **28:**1968–1973.
2. **Fang, G. D., M. Fine, J. Orloff, et al.** 1990. New and emerging etiologies for community-acquired pneumonia with implications for therapy: a prospective multicenter study of 395 cases. Medicine (Baltimore) **69:**307–316.
3. **Gaydos, C. A., C. L. Fowler, V. J. Gill, J. J. Eiden, and T. C. Quinn.** 1993. Detection of *Chlamydia pneumoniae* by PCR-EIA in an immunocompromised population. Clin. Infect. Dis. **17:**718–723.
4. **Gaydos, C. A., T. C. Quinn, and J. J. Eiden.** 1992. Identification of *Chlamydia pneumoniae* by DNA amplification of the 16S rRNA gene. J. Clin. Microbiol. **30:**796–800.
5. **Grayston, J. T., L. A. Campbell, C. C. Kuo, et al.** 1990. A new respiratory tract pathogen: *Chlamydia pneumoniae*, strain TWAR. J. Infect. Dis. **161:**618–625.
6. **Hahn, D. L., R. W. Dodge, and R. Golubjatnikou.** 1991. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA **266:**225–230.
7. **Marrie, T. J., J. T. Grayston, S. P. Wang, and C. C. Kuo.** 1987. Pneumonia associated with the TWAR strain of Chlamydia. Ann. Intern. Med. **106:**507–511.
8. **Miller, S. T., M. R. Hammerschlag, K. Chirgwin, et al.** 1991. Role of *Chlamydia pneumoniae* in acute chest syndrome of sickle cell disease. J. Pediatr. **118:**30–33.
9. **Roblin, P. M., W. Dumornay, and M. R. Hammerschlag.** 1992. Use of HEp-2 cells for improved isolation and passage of *Chlamydia pneumoniae*. J. Clin. Microbiol. **30:**1968–1971.
10. **Saikku, P., M. Leinonen, L. Tenkanen, et al.** 1992. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki heart study. Ann. Intern. Med. **116:**273–278.
11. **Wang, S.-P., C.-C. Kuo, and J. T. Grayston.** 1979. Formalinized *Chlamydia trachomatis* organisms as antigen in the micro-immunofluorescence test. J. Clin. Microbiol. **10:**259–261.