Laboratory diagnosis of *Chlamydia pneumoniae* infections

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RW PEELING. Laboratory diagnosis of *Chlamydia pneumoniae* infections. Can J Infect Dis 1995;6(4):198-203. *Chlamydia pneumoniae* is an important cause of respiratory illness. There is a need for accurate and rapid laboratory diagnostic methods that will lead to improved patient care, appropriate use of antimicrobial therapy and a better understanding of the epidemiology of this emerging pathogen. Culture is highly specific but is technically demanding, expensive, has a long turnaround time and its sensitivity is highly dependent on transport conditions. Antigen detection tests such as enzyme immunoassay and direct fluorescent antibody assay, and molecular detection methods such as the polymerase chain reaction assay, may provide a rapid diagnosis without the requirement for stringent transport conditions. The results of these tests should be interpreted with caution until more thorough evaluation is available. Serology remains the method of choice. The limitations of different serological methods for the laboratory diagnosis of *C. pneumoniae* are discussed.

Key Words: *Chlamydia pneumoniae*, Laboratory diagnosis

Épreuves de laboratoire dans le diagnostic des infections à *Chlamydia pneumoniae*

RÉSUMÉ : *Chlamydia pneumoniae* est une importante cause de maladie respiratoire. Il faut se doter de méthodes diagnostiques de laboratoire précises et rapides qui permettront d'améliorer les soins aux patients, d'orienter l'antibiothérapie et de mieux comprendre l'épidémiologie de ce pathogène émergent. La culture est très spécifique mais elle est techniquement exigeante, coûteuse, elle nécessite du temps et son degré de sensibilité dépend fortement des conditions de transport. Des épreuves de dépistage antigénique, comme l'immunodosage enzymatique et l'immunodosage par fluorescence directe, ainsi que des méthodes de dépistage moléculaire, comme les épreuves de réactions en chaîne des polymérases, peuvent offrir un diagnostic rapide sans exiger de conditions de transport précises. Le résultat de ces épreuves doit être interprété avec précaution tant qu'une évaluation plus complète n'est pas disponible. La sérologie demeure la méthode de choix. Les limites de différentes méthodes sérologiques appliquées à l'analyse de *C. pneumoniae* en laboratoire diagnostique sont présentées ici.

CHLAMYDIA PNEUMONIAE IS A COMMON CAUSE OF RESPIRATORY illness and accounts for 10 to 20% of community acquired pneumonia cases (1-3). Recent seroepidemiological studies have linked *C. pneumoniae* infection with atherosclerosis, asthma and acute exacerbations of chronic obstructive pulmonary disease (4-6). Infection often appears as a mild, self-limiting illness but outbreaks of *C. pneumoniae* infection have been reported within families, in schools, military barracks and small communities (7-12). Infection in preschool-

aged children are uncommon (13). Most individuals acquire their primary infection of *C. pneumoniae* between five and 14 years of age. *C. pneumoniae* immunoglobulin (Ig) G antibodies may persist for months to years, with less decline in titre after reinfection than in primary infection and in adults compared with children (13). Seroprevalence studies conducted worldwide show that 50 to 70% of adults have IgG antibodies

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TABLE 1
Laboratory diagnosis of *Chlamydia pneumoniae* infections

Feature	Culture	DFA	PCR	Serology
Detection	Infectious organism	Antigen	DNA	Antibodies
Specimen/site	Throat swab N/P swab BAL Sputum	Throat swab N/P swab BAL	Throat swab N/P swab BAL Sputum	Blood
Sensitivity	50%	20-60%	10-100 organisms	60-80%
Specificity	100%	70-95%	95-100%	90-100%
Maximum time to laboratory	2 days	7 days	7 days	2 days
Specimen collection and transport	Collect swabs in chlamydia transport media; Transit time: <48 h, refrigerate, transport on cold packs; >48 h, freeze, transport on dry ice	Fix smear in acetone; transport at room temperature	Vortex swab in PCR buffer or saline for 10-15 s; discard swab; transport on cold packs or frozen	Separate blood; transport sera on cold packs or at room temperature
Report available	2-6 days	Within 24 h	48-72 h	Within 48 h
Stat testing	No	Yes	No	No
Reading of results	Subjective	Subjective	Objective	Subjective
Comments	Performance highly dependent on transport and laboratory expertise	Reading requires technical expertise	Limited performance evaluation	Sensitivity may be variable in children and the elderly

BAL Bronchoalveolar lavage; DFA Direct fluorescent antibody assay; N/P Nasopharyngeal; PCR Polymerase chain reaction

to *C pneumoniae*, suggesting that reinfection is common throughout adulthood.

The clinical presentation of respiratory illness caused by *C pneumoniae* is often indistinguishable from that of viral or mycoplasma etiology. Since curative antimicrobial therapy is available for *C pneumoniae* infection, specific laboratory diagnosis may be useful for patient management. In particular, where respiratory illness is associated with a recent history of bird or animal contact, it is important to rule out *Chlamydia psittaci* infection, which may require public health action. In outbreak situations, a specific laboratory diagnosis is important for early and appropriate intervention to prevent further spread of infection. As *C pneumoniae* becomes recognized as an important emerging pathogen, reagents for laboratory diagnosis are now more widely available. An understanding of the performance as well as limitations of different types of laboratory tests available for the diagnosis of *C pneumoniae* is essential for the proper interpretation of laboratory results. Table 1 shows the attributes and limitations of the diagnostic tests available for the laboratory diagnosis of *C pneumoniae* infection.

CULTURE

The isolation of *C pneumoniae* in cell culture is technically more demanding than that of *Chlamydia trachomatis* and often requires multiple passages over a period of weeks in cell culture to show a positive result (14). Studies have shown that HEp-2 and HL cells are more sensitive for *C pneumoniae* than are HeLa or McCoy cells, which are traditionally used for the culture of *C trachomatis* (15-17). The use of serum-free media has been reported to improve isolation rates (18). The sensitivity of culture is estimated to be 50% compared with serology. The specificity is assumed to be 100% because

monoclonal antibodies specific for *C pneumoniae* are now commercially available for the identification of *C pneumoniae* inclusions in cell culture (19). Failure to isolate the pathogen may be due to several reasons such as inadequate specimen sampling, specimen toxicity in cell culture or failure to preserve specimen viability during transportation of the specimen to the laboratory. Viability of the organism is rapidly lost through freezing and thawing. Specimens from the throat and the nasopharynx may have a lower yield of the organism than deep-seated specimens such as sputum or bronchoalveolar lavage (BAL). Isolation from the nasopharynx of healthy individuals has been reported but the rate of asymptomatic carriage in a normal population is unknown (20,21). Hence, a positive isolation of *C pneumoniae* from a nonsterile site should be interpreted with caution.

ANTIGEN DETECTION TESTS

Antigen detection tests include direct fluorescent antibody assays (DFA) and enzyme immunoassays (EIA). These tests have the advantage of rapid turnaround time as well as ambient temperature transport. Specimens taken for antigen detection tests are stable at room temperature for up to a week. For DFA, smears are usually made on site from nasopharyngeal or throat swabs and fixed with acetone or methanol before staining. The use of rayon swabs to collect specimens for DFA should be avoided because rayon fibres left on the smear will autofluoresce and interfere with reading. Smears can be also be made from BAL specimens but they may need to be diluted with saline to minimize nonspecific background staining. Methanol was reported to destroy the antigenic reactivity of *C pneumoniae* (22). Montalban et al (19) evaluated the use of several *C pneumoniae*-specific fluorescein-conjugated monoclonal antibodies for staining

TABLE 2
Comparison of PCR procedures for the detection of *Chlamydia pneumoniae* (Cpn) or *Chlamydia psittaci* (Cps)

Features	Campbell et al (26)	Tong & Sillis (27)	Gaydos et al (28,30)	Rasmussen et al (29)
Target sequence	Unknown gene, Cpn specific	ompA gene, Cpn/Cps specific	16S RNA gene, Cpn specific	ompA gene, genus-specific
Target size (bp)	437	333 (Cpn/Cps) 207 (Cpn only)	463	145
Primers	20 mer	20 mer/21 mer 21 mer/21 mer	21 mer/18 mer	24 mer/26 mer
PCR procedure + detection	PCR + agarose gel electrophoresis	Nested stepdown PCR + gel electrophoresis	PCR + enzyme immunoassay	PCR + restriction enzyme digestion
Cycling conditions	94°C 1 min 55°C 1 min 72°C 1 min	94°C 1 min 65-55°C 1 min 72°C 1 min	94°C 1 min 55°C 1 min 72°C 1 min	94°C 1 min 55°C 1 min 72°C 1 min
Number of cycles	40	40 cycles with stepwise decrease in annealing temperature; 30 cycles	30	35
Time required	<24 h	<24 h	<24 h	<6 h
Reported sensitivity	<1 inclusion	5-10 EBs	4 inclusions	<10 EBs
Confirmation of specificity	20 mer probe	Nested amplification	270 bp rRNA	21 mer probe

EB Elementary bodies; PCR Polymerase chain reaction

inclusions in cell culture and found that the choice of methanol or acetone as fixatives depended on the antibody used. Results of smears can be available in 30 mins if antibodies used for staining are directly conjugated with fluorescein. The sensitivity of DFA is estimated to be 20 to 60% (23). A limitation of DFA is that the reading of the smear is subjective. Hence, its specificity is highly dependent on the expertise of the technologist.

EIAs that are currently marketed for *C trachomatis* antigen detection have been used for the detection of *C pneumoniae* from swabs (24,25). This is possible because EIA technology is based on the capture of the genus-specific chlamydial lipopolysaccharide (LPS). The sensitivity of these EIAs is reported to be comparable with that of DFA but, being LPS-based assays, they lack specificity. Sputum and BAL specimens require mucolytic digestion before being processed for EIAs (24).

NUCLEIC ACID-BASED TESTS

Polymerase chain reaction (PCR) techniques have been developed for the detection of *C pneumoniae* DNA. A comparison of the published procedures for the laboratory detection of *C pneumoniae* is shown in Table 2 (26-31). Unlike *C trachomatis*, *C pneumoniae* does not possess a plasmid. Hence plasmid-based PCR kits for *C trachomatis* cannot be used to detect *C pneumoniae*. Sputum, bronchoscopy specimens, throat washings or swabs, and nasopharyngeal swabs are suitable specimens for PCR assays. Because DNA is stable in transport, specimens for PCR can be transported at room temperature if they cannot be sent to the laboratory immediately. Traces of DNA may be present up to three weeks after antibiotic treatment.

We evaluated the user friendliness and performance of four published PCR protocols using 10-fold titrations of *C pneumoniae* strain TW-183 (32). The sensitivity of all the protocols was found to be comparable with detection limits of 10

to 100 elementary bodies. The protocols of Campbell et al (26) and Gaydos et al (28,30) were the most sensitive and simplest to perform because the primers are *C pneumoniae*-specific and the specificity of the amplified product can be confirmed by probes internal to the target sequence. The protocol developed by Tong and Sillis (27) amplifies a target sequence conserved between *C pneumoniae* and *C psittaci* and hence has the advantage of being able to detect DNA from either pathogen in a single assay. A nested PCR procedure is then used to differentiate between the *C pneumoniae* and *C psittaci* amplicons. The protocol of Rasmussen et al (29) amplifies a genus-specific target sequence, followed by species differentiation using restriction enzyme digestion. In collaboration with the Provincial Laboratory for Southern Alberta, we evaluated 312 throat swabs collected in 2-SP buffer as part of a viral watch program (32). Three specimens were culture-positive for *C pneumoniae* in HeLa 229 cells. When the protocol of Campbell et al was used, three swabs were PCR-positive, of which two were culture-positive. Further evaluations are ongoing. At present, these PCR methods are research techniques. Multiplex PCR assays containing primers specific for a panel of respiratory pathogens are being developed.

SEROLOGY

The complement fixation (CF) test has traditionally been used for the serodiagnosis of respiratory chlamydial infections. The chlamydial antigen involved in CF is the genus-specific LPS. Therefore, the CF test cannot be used to distinguish the antibody response resulting from *C trachomatis*, *C psittaci* or *C pneumoniae* infections. The microimmunofluorescence (MIF) assay developed by Wang et al (33) is used to detect species-specific antibodies and is the gold standard for chlamydia serology today. Antibody cross-reactivity among chlamydia species observed in MIF may be due to the presence of antibodies against genus-specific antigens such as

TABLE 3
Criteria for positivity in the serodiagnosis of *Chlamydia pneumoniae* infections

	CF	MIF (2)	EIA
Acute infection	IgM titre ≥ 16 or fourfold rise in IgG titre	IgM titre ≥ 16 or IgG titre ≥ 512 or fourfold rise in IgG titre	Cut-off varies according to manufacturer
Past infection		IgG titre 16-256	
Antigen	Lipopolysaccharide	Elementary bodies	Recombinant lipopolysaccharide
Specificity	Genus-specific	Species-specific	Genus-specific
Sensitivity	10-40%	60-80%	Insufficient evaluation

CF Complement fixation; EIA Enzyme immunoassay; Ig Immunoglobulin; MIF Microimmunofluorescence

the LPS or due to impurities in the antigen preparation (23,34,35). In sera from adults with antibodies against *C trachomatis* and *C pneumoniae*, it is likely that the patient has a history of infection with both organisms. Because acute *C pneumoniae* infections generally induce higher levels of IgG antibody that are rarely seen in infections with other chlamydia species, low level cross-reactive antibodies are unlikely to present a problem for the serodiagnosis of *C pneumoniae* infections. Kits for EIA that use extracted or recombinant chlamydial LPS and for MIF are commercially available. Although less sensitive than the MIF assay and lacking in specificity, both the CF test and the EIAs are much less technically demanding than the MIF, are amenable to batching and have objective end-points. Another advantage of these tests is that LPS antibodies are produced early in infection. Thus, a diagnosis may be reached by the CF test or EIA in paired sera taken a week apart compared with three weeks or more for MIF (36,37).

There are also antibody detection kits where the antigens are cells infected with a lymphogranuloma strain of *C trachomatis* fixed onto a glass slide. Because antigenic relatedness between *C pneumoniae* and lymphogranuloma has not been determined, antibody titres from these kits should be interpreted with caution.

The criteria for seropositivity for past and acute infection with *C pneumoniae* are shown in Table 3 (2). In collaboration with Saskatchewan Health, we used these seropositivity criteria for MIF to evaluate the usefulness of the CF test in the diagnosis of *C pneumoniae* infections. Of 103 sera, the CF test was positive for four of seven (57%) sera positive for IgM antibodies by MIF against *C pneumoniae*, but only detected two of 16 (13%) sera positive for IgG antibodies (38). Since IgM antibodies are rarely produced in reinfections with *C pneumoniae*, the CF test appears to be of limited use in the diagnosis of reinfections in adults. Although the numbers for our evaluation were small, these results were later confirmed in a much larger study. Ekman et al (36) compared the performance of the CF, LPS-based EIA and MIF tests for the serodiagnosis of *C pneumoniae* and *C psittaci* infections in an elderly population and found that the CF test has a sensitivity of 10.3% compared with 87.9% and 72.4% for MIF and EIA, respectively (36). IgM antibodies were only detected in 11.3% of cases. It is not clear why there was such disparity between the CF test and the EIA, both of which are LPS-based tests.

We reviewed the results of 2247 sera referred to the National Laboratory for Sexually Transmitted Diseases for *C*

pneumoniae serology in 1994. Of 163 (7.8%) patients whose sera met the criteria for acute infection, 27 (16%) were diagnosed based on IgM antibody titre alone, four (2%) based on a fourfold rise in IgG antibody titre, and 132 (82%) on a combination of IgM antibody titre greater than 16 and IgG antibody titre of 512 or greater (unpublished data). The low percentage of serodiagnosis by fourfold rise in IgG antibody titre may reflect the low number of paired sera ($n=166$) sent for testing, insufficient time between acute and convalescent sera, or delay in patients seeking care, especially if their symptoms were mild. The age of the those tested, the frequency of reinfection and delay in seeking care may be responsible for the low percentage of diagnosis by IgM antibodies. Serodiagnosis in the elderly may be compromised by the presence of rheumatoid factor and immune senescence (39). The serological response in children appears variable and needs further evaluation (23).

CONCLUSION

C pneumoniae is an important cause of respiratory infections. There is a need for more accurate and rapid laboratory diagnostic methods that would improve patient care through the appropriate use of antimicrobial therapy and advance our understanding of the epidemiology of these infections.

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