Laboratory diagnosis of Chlamydia pneumoniae infections

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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’antibiotérapi e 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’immuno dosage enzymatique et l’immuno dosage 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 sé ro logie 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...
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

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**TABLE 1**

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

**BAL** Bronchoalveolar lavage; **DFA** Direct fluorescent antibody assay; **N/P** Nasopharyngeal; **PCR** Polymerase chain reaction.
TABLE 2
Comparison of PCR procedures for the detection of *Chlamydia pneumoniae* (Cpn) or *Chlamydia psittaci* (Cps)

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

*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, bronchoscropy 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

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>MIF (2)</th>
<th>EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute infection</td>
<td>IgM titre ≥16 or fourfold rise in IgG titre</td>
<td>IgM titre ≥16 or fourfold rise in IgG titre</td>
<td>Cut-off varies according to manufacturer</td>
</tr>
<tr>
<td>Past infection</td>
<td>IgG titre 16-526</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen</td>
<td>Lipopolysaccharide</td>
<td>Elementary bodies</td>
<td>Recombinant lipopolysaccharide</td>
</tr>
<tr>
<td>Specificity</td>
<td>Genus-specific</td>
<td>Species-specific</td>
<td>Genus-specific</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>10-40%</td>
<td>60-80%</td>
<td>Insufficient evaluation</td>
</tr>
</tbody>
</table>

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. Ekanem 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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