Collaborative Multidisciplinary Workshop Report: Detection, Culture, Serology, and Antimicrobial Susceptibility Testing of *Chlamydia pneumoniae*

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Efforts toward Standardization of Tests for *Chlamydia pneumoniae*

The *C. pneumoniae* workshop group on Detection, Culture, Serology, and Antimicrobial Susceptibility Testing made a strong and unanimous recommendation for the standardization of *C. pneumoniae* diagnostic tests. With many laboratories entering this field and introducing their own versions of diagnostic methods, it is becoming impossible to compare results between laboratories because there is no understanding of the sensitivity and specificity of the different diagnostic tests. Thus, the committee recommends that emphasis be placed on collaborative multicenter trials to evaluate and attempt to standardize existing diagnostic procedures. We encourage the Centers for Disease Control and Prevention or the World Health Organization to recommend common methodologies for multicenter trials and to identify “reference standards” and “reference reagents” (materials that could be used in all laboratories) whose use would ensure comparability of laboratory findings.

In this same vein, the committee encouraged the development of commercial tests. These would allow for better comparability of results if the commercial tests were readily available and were used in different laboratories doing similar studies.

Laboratory Methods for Testing *C. pneumoniae*

**Culture.** The ability to grow *C. pneumoniae* varies from laboratory to laboratory. Some reports suggest very high recovery rates from selected specimens, while other laboratories cannot successfully culture the organism from what, in descriptive terms, appear to be the same types of clinical materials. Thus, there is some need to focus on optimal specimens for culture; for example, in respiratory disease cases, a nasopharyngeal swab, throat swab, sputum, or some other specimen sample best for culture? What cells offer the optimal system for culture? What is the best medium? What conditions should be used for centrifugation and at what times? How and when are blind passages performed? How are the organisms identified? What do laboratories do to avoid cross-contamination?

**Antimicrobial susceptibility.** The committee noted that there is a lack of standardized protocols for antimicrobial susceptibility testing of *C. pneumoniae*. There is not a standard process for selecting the appropriate cells to perform studies, and it is not known what end points are meaningful (e.g., minimum inhibitory and minimal cidal concentration).

**Polymerase chain reaction (PCR).** A number of studies have found PCR to be a more sensitive procedure than culture, but others have found it to be a relatively insensitive procedure overall due to low target DNA and the presence of large quantities of inhibitors and irrelevant DNA, which interfere with polymerization. The best protocols have yet to be established. Nested PCR may offer the best currently available technology. There is some suggestion that reverse transcription–PCR may...
be more meaningful in detecting replicating *C. pneumoniae* or that RNA targets may allow increased sensitivity. Methods of purifying DNA and removing inhibitors, appropriate primers, and enzyme sources all present variables that could be the subject of standardization efforts.

A stepwise approach for multicenter trials might proceed as follows. First, different PCR protocols and reagents would be evaluated using purified *C. pneumoniae* DNA to determine the sensitivity of the methods. DNA extraction procedures would be evaluated using infected cultures. The selected PCR and DNA purification protocols would then be evaluated with spiked clinical samples or samples from experimental models. Next, selected protocols would be evaluated with well-defined clinical samples from well-characterized patients. As a last step, there would be a multicenter application of the tests in diverse clinical settings to attempt to establish interpretation guidelines.

**Serology.** The microimmunofluorescence assay is the test that allowed identification of *C. pneumoniae* as a cause of disease and played a major role in seroepidemiology studies. It is clear, from a diagnostics viewpoint, that this is the best test for identification of acute infection. There is a need for an automated serologic test that will allow for higher-volume testing. This is another area for which standardization is needed. Efforts should be made to develop a standard panel of sera representing the types of patterns that are regularly seen so that laboratories can evaluate their internal results against a reference reagent panel.

Cross-reactions, largely due to antibody against the genus-specific lipopolysaccharide, can occur with other chlamydial infections. Some commercially available microimmunofluorescence tests use elementary bodies (EBs) stripped of lipopolysaccharide to make them more specific due to lower cross-reactivity with other *Chlamydia* and *Bartonella* species. A number of commercial EIAs based on peptide antigens or chlamydial outer membrane complex or using EBs treated to remove lipopolysaccharide have also been developed in an attempt to minimize such cross-reactions. These materials need further evaluation.

**Identification of Research Needs**

Four areas of future research were specifically addressed. The first is the selection of the best assay to determine a test of cure following antimicrobial therapy. In light of ongoing clinical trials, an assay to determine the efficacy of antibiotic therapy should be a high priority.

Given what is evolving concerning our understanding of the natural history of *C. pneumoniae* infection, it is clear that there is a need for a marker for chronic infection. Data indicating that IgA antibodies could be a marker for such infection were considered unconvincing. Thus, the second area identified by the committee involves identification of chronic infection, particularly with emphasis on the significance of immune complexes and antigen detection in circulation.

The third area of research identified by the committee is to determine a marker for viability of the microorganism. This test would be useful to implicate *C. pneumoniae* as a cause of chronic atherosclerotic disease and also would be useful to identify chronic infection. Several researchers have approached this problem by trying to detect mRNA for a variety of different genes. There should be a consensus as to which genes are appropriate targets for such studies and how reliable detection of mRNA will be for this purpose.

Determination of the burden of infection by *C. pneumoniae* was also thought to be an important research target. Quantitative PCR seems to be desirable, given the potential for long-term persistent infection and the possibility that there may be a relationship between infectious burden and disease.

**Recommendations**

On the basis of the workshop discussion, the panel made the following recommendations:

1. Further efforts must be made to standardize the methods to detect, culture, and assess the antimicrobial susceptibility of *C. pneumoniae*. Collaborative multicenter trials to evaluate the accuracy of current methods and new diagnostic kits were encouraged.

2. Further research should be done to assess the causal relationship of *C. pneumoniae* to atherosclerosis and other chronic infections. These include assays to determine the effect of antimicrobial therapy to cure infection, the identification of chronic persistent infection, tests to assess the viability of *C. pneumoniae* in tissue, and quantitative assays.

3. Ideas for future research projects included using fluorescence-activated cell sorter assays of infected peripheral blood mononuclear cells to select and enrich the population of cells for subsequent assays and applying microchip technology to identify human response genes regulated during the course of *C. pneumoniae* infection.