Polymerase Chain Reaction Techniques in the Diagnosis of Pneumonia

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Introduction

Approximately one half of the cases of pneumonia and lower respiratory infection are treated empirically, and no etiologic agent is identified (Bartlett et al., 1998). This represents approximately 250,000 cases of pneumonia annually in the United States for which treatment is not pathogen-directed (Centers for Disease Control and Prevention, 1997). Unfortunately, pneumonia is often treated empirically to prevent delays in the initiation of treatment and because an adequate diagnostic test is not available. This practice is partly responsible for the marked increase in penicillin-resistant Streptococcus pneumoniae in the last 10 years (Hofmann et al., 1995). These factors illustrate the need for rapid diagnostic tests to identify the etiologic agents of pneumonia. Currently, the laboratory test most likely to allow rapid diagnosis is polymerase chain reaction (PCR). Although PCR is routinely used in most medical laboratories for various procedures, this technique is not relied upon for diagnosis except for a few agents. This is partly due to the lack of studies that thoroughly evaluate PCR and compare the procedure to gold standard tests. PCR is particularly appealing as a potential diagnostic test for the “atypical” agents of pneumonia, namely, Chlamydia, Legionella, and Mycoplasma pneumoniae. Historically, a pneumonia patient’s illness has been categorized as typical or atypical to characterize the presentation of disease. This diagnostic approach generally led to the prescribing of β-lactam antimicrobial drugs for the treatment of typical pneumonia and macrolides for the treatment of atypical pneumonia. This practice is no longer encouraged and the Infectious Disease Society of America (IDSA) has recently published more specific guidelines for patient management (Bartlett et al., 1998). These organisms are extremely fastidious and may be considerably underdiagnosed. The specific difficulties in the diagnosis of each of the agents are discussed in the following sections.

Chlamydia pneumoniae

C. pneumoniae is now recognized as an important cause of respiratory infections including pneumonia, bronchitis, and sinusitis (Kou et al., 1995). More recently the bacterium has been associated with bronchial asthma and coronary artery disease based on serologic and PCR data. C. pneumoniae is implicated in 5% to 15% of cases of community-acquired pneumonia (CAP) although the prevalence is reported to vary from year to year (Marrie et al., 1989; Fang et al., 1990; Marston et al., 1997). Diagnosis of C. pneumoniae infection is usually
based on serologic evidence while PCR and culture are used less frequently. The recommended serologic test is a microimmunofluorescence (MIF) test (Kauppinin & Saikku, 1995). This assay has replaced the cumbersome complement fixation (CF) test, but MIF remains labor-intensive and is associated with problems in interpretation and specificity.

*C. pneumoniae* is an intracellular pathogen and must therefore be grown in cell culture. A nasopharyngeal swab is the preferred specimen for the isolation of these bacteria although they have been isolated from pharyngeal swabs and respiratory secretions (Kauppinin & Saikku, 1995). The procedure entails inoculation of a cell line (usually HEp-2 cells) with the patient specimen in a medium containing selective antibiotics to suppress overgrowth by normal flora and to inhibit HEp-2 cell protein synthesis (Wong et al., 1992). This procedure requires 3 to 7 days’ incubation and frequently requires that the material be subcultured (blind passes) multiple times. Positive cultures must be visualized by direct fluorescent antibody staining. Because these techniques can be cumbersome, only a limited number of research laboratories routinely culture for *C. pneumoniae*.

A number of PCR assays have been described for the detection of *C. pneumoniae*. This partly reflects the difficulty in diagnosing this infection by other techniques and a general lack of rapid, sensitive, or specific tests. Most of the published PCR assays target either 16S rRNA, the major outer membrane protein (MOMP), or a 437-bp target sequence of unknown function (Cambell et al., 1992; Gaydos et al., 1992; Sillis et al., 1992; Gaydos et al., 1993, 1994; Pruckl et al., 1995; Dalhoff & Maass, 1996; Khan & Potter, 1996; Wilson et al., 1996; Boman et al., 1997; Gnarpe et al., 1997; Messmer et al., 1997). The PCR assays vary in the use of nested primers and the method of amplicon detection. Gaydos and coworkers (1993) described a method to detect the PCR amplicon based on an enzyme-linked detection system (PCR-EIA). Modifications involving a nested, single-tube PCR assay have been subsequently published (Gaydos et al., 1994). Boman et al. (1997) described the use of nested, touchdown PCR to detect *C. pneumoniae* and *C. psittaci*. A 1994 comparison of culture, PCR-EIA, and single-point (acute) serology indicated that the sensitivity of PCR-EIA was 76.5% and the sensitivity of culture was 87.5% with both procedures far superior to single-point serology (Gaydos et al., 1994). Other reports on the sensitivity and specificity of PCR assays have varied widely, ranging as high as 100%. Unfortunately many of these studies have incorporated small populations or limited numbers of control patients, and a thorough evaluation of the PCR assays has not been completed. The difficulties and approaches to such evaluations will be discussed later in this chapter.

### Legionellae and *Legionella pneumophila*

*Legionella* species cause two forms of respiratory disease in humans—Legionnaires’ disease and Pontiac fever—that are collectively termed legionellosis (Helms et al., 1979). Legionnaires’ disease is a pneumonic infection and is implicated in 2% to 6% of CAP cases (Fine et al., 1996; Marston et al., 1997). Because the routine use of diagnostic tests is not advocated and the tests that are available are primarily specific for *L. pneumophila* serogroup 1, these figures may underestimate the number of legionellosis cases. *L. pneumophila* serogroup 1 is implicated in approximately 80% of legionellosis cases; however, this figure may be high since other species of legionellae are more fastidious and there are limited diagnostic reagents specific for these organisms (Marston et al., 1994).

Legionellae are facultative intracellular bacteria that multiply within freshwater protozoa in the environment and within phagocytic cells of humans (Fields, 1996). Although the bacteria are considered fastidious, they grow on a complex agar medium and are the least difficult atypical agents to isolate from clinical specimens. Legionellae are cultured on buffered charcoal-yeast extract agar enriched with α-ketoglutarate both with and without selective agents (Edelstein, 1981). Culture diagnosis remains the gold standard for legionellosis and is a sensitive procedure when appropriately performed early in the course of disease (Edelstein, 1993). The bacteria survive poorly in respiratory secretions, and immediate culture of these specimens is critical. The reported sensitivity of culture isolation from respiratory secretions ranges widely, from 20% to 80%, reflecting the perishable nature of