Molecular Detection of *Mycoplasma pneumoniae* From BAL Specimens Using Liquid Pap Medium

**Parker, M., Fuller, D., Buckner, B., Talbott, J., Blue-Hnidy, D., Davis, T.**

**Abstract**

**Background:** Identification of atypical pneumonia pathogens has been a difficult problem for clinical microbiology laboratories for many years due to the lack of sensitivity of both culture and serologic methods. Each year *Mycoplasma pneumoniae* is believed to infect 2 million people and cause an estimated 100,000 pneumonia-related hospitalizations. Bronchoalveolar (BAL) specimens are routinely cultured for microorganisms, to include *M. pneumoniae*, even though sensitivity is poor. ThinPrep (TP) Liquid Pap Medium (Cytyc Corp., Boxborough, MA) has long been an accepted method for preparing gynecological samples but can also be used for non-gynecologic specimens (such as BAL) to prepare slides for examination of cellularity and presence of microorganisms. Recently it has been established that TP can also be used as a collection medium from which pathogens can be recovered using DNA probe technology.

**Methods:** This study evaluated the use of two real-time PCR assays, *M. pneumoniae* Primer and Probe Set Analyte Specific Reagent (MpP) (Cepheid, Sunnyvale, CA) and the ProPneumo-1™ (PP-1) assay (Prodesse, Inc., Waukesha, WI) for the detection of *M. pneumoniae* in BAL specimens collected in TP. The MpP assay detects the P1 adhesion gene that is unique to *M. pneumoniae* and PP-1 targets 16S-23S rRNA genes for *M. pneumoniae*. A 2.5mL aliquot of BAL (both unseeded and seeded with *M. pneumoniae*) was inoculated into 5mL of TP solution and allowed to incubate for 2 hours. The BAL/TP mixture was then directly processed and tested with both the MpP and the PP-1 assays using the Cepheid SmartCycler® instrument (Sunnyvale, CA).

**Results:** Of the 141 specimens tested to date, both the MpP and the PP-1 assays show complete agreement with expected results. The MpP and PP-1 assays exhibit rapid (1.5 hours), accurate (100% sensitivity, 100% specificity), flexible (single sample or batch testing), and simple detection of *M. pneumoniae* in BAL specimens collected in TP.

**Conclusion:** The combination of TP collection and real time PCR assays offers an alternative and accurate method for identification of *M. pneumoniae*.

**Introduction:**

*Mycoplasma pneumoniae* (Mp) is a common cause of community-acquired pneumonia and is part of a group of organisms, including *Chlamydophila pneumoniae* (C. pneumoniae) and *Legionella* species, often causing “atypical pneumonia”. Analysis of 13 studies of community-acquired pneumonia showed 22.7% of infections were caused by Mp, while *C. pneumoniae* and *Legionella* species prevalence were 11.7% and 4.6%, respectively. Each year in the United States there are an estimated 2 million cases of Mp infection and 100,000 pneumonia-related hospitalizations. Upper respiratory tract involvement is present in 77% of Mp infections, 20% of infected patients are asymptomatic and 3% result in pneumonia.

Detection of Mp is difficult due to the fastidious nature of the organism. Special media is required for the recovery of this organism and it usually takes 2-4 weeks to grow. The specimen should first be incubated in SP4 with glucose broth. Hydrolysis of the glucose will cause an acidic shift, resulting in a color change of the broth, which usually takes 4 days or more. When the color changes, a blind subculture to SP4 agar should be done and allowed to incubate, checking every week for colonies up to 100µm using a stereomicroscope. Although culture is 100% specific, it is only 60% sensitive. Rapid detection of Mp antigen is also available for diagnostic use and includes direct immunofluorescence, immunoblotting, and antigen capture enzyme immunoassay. All of the antigen detection methods show low sensitivity and usually cross-react with other *mycoplasma* species that may be present as normal flora in the lower respiratory tract. Serological tests may cause false negative results because when a low number of organisms are present, they may evade the immune system.
Polymerase Chain Reaction (PCR) has several advantages over conventional culture and serology. First, PCR will yield results in one day and only requires one specimen. The specimen does not have to contain live, viable organisms and cultures that have been contaminated with other organisms can be used. RNA-based amplification techniques result in higher sensitivity due to the large amount of rRNA copies per mycoplasmal cell.

ThinPrep (TP) Liquid Pap Medium (Cytyc Corp., Boxborough, MA) is most commonly used as a method to screen for cervical cancer. The methanol based medium has been proven to be an excellent preservative in previous work with pathogens such as *Chlamydia trachomatis*, *Neissera gonorrhoea*, and Human Papillomavirus. The TP medium has been previously used for detection of pulmonary pathogens such as *Pneumocystis jiroveci*, *Histoplasma capsulatum*, *Aspergillus flavus*, and *Blastomyces dermatitidis* in BAL using immunologic methods. We evaluated detection of Mp in TP from BAL using real-time PCR.

Methods:

BAL specimens from immunocompromised patients were collected from in-patients at Wishard Memorial Hospital and University Hospital in Indianapolis, IN, USA. A 2.5mL aliquot of BAL (both unseeded and seeded with Mp) was inoculated into 5mL of TP solution and allowed to incubate at room temperature for at least 2 hours. A DNA extraction of the TP/BAL solution was performed using Roche High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Indianapolis, IN). The extracted DNA was then tested on the Cepheid SmartCycler® (Cepheid, Sunnyvale, CA) using both the *Mycoplasma pneumoniae* Primer and Probe Set Analyte Specific Reagent (Cepheid, Sunnyvale, CA) and the Pro-Pneumo-1™ assay (Prodesse, Inc., Waukesha, WI). Specimens found to be clinically positive for Mp were verified using IgG and IgM titer results from acute and convalescent serum samples.

Results:

Of the 141 BAL specimens tested to date, both the *M. pneumoniae* Primer and Probe Set Analyte Specific Reagent and the Pro-Pneumo-1™ assays show 100% sensitivity and 100% specificity. There were 25 unseeded BAL specimens, 15 seeded BAL specimens, 49 unseeded TP/BAL specimens, and 54 seeded TP/BAL specimens tested. (Table 1) During the study we had a positive result on one clinical specimen. This result was verified by using immunoglobulin titer results on acute and convalescent serum.

Table 1.
Agreement of the M. pneumoniae Primer and Probe Set Analyte Specific Reagent and the Pro-Pneumo-1 Assays using the Cepheid SmartCycler from Seeded and Unseeded BAL/TP Specimens

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>n</th>
<th>MpP+ PP-1+</th>
<th>MpP+ PP-1-</th>
<th>MpP- PP-1+</th>
<th>MpP- PP-1-</th>
<th>Positive Agrmt (%)</th>
<th>Negative Agrmt (%)</th>
<th>Overall Agrmt (%)</th>
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<tbody>
<tr>
<td>BAL unseeded</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>N/A</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BAL/TP unseeded</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>49</td>
<td>N/A</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BAL seeded</td>
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<td>15</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>N/A</td>
<td>100</td>
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</tr>
<tr>
<td>BAL/TP seeded</td>
<td>54</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>N/A</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

+ Positive, - Negative, N/A = Not Applicable, Agrmt = Agreement

Conclusion:

The combination of TP collection and real time PCR assays offers an alternative and accurate method for the identification of Mp (even in the presence of normal flora and other pathogens). The turnaroud time for conventional culture for Mp is 2-4 weeks while the turn around time using real time PCR is 1.5 hours. These assays are promising methods for detection of Mp. Additional testing on clinically positive specimens is planned.

These assays are:

1. Rapid (results in 1.5 hours)
2. Flexible (single sample or batch testing)
3. Accurate (100% sensitivity and specificity)

References:


