

# Detection of Community Acquired Pneumonia Pathogens in Lower Respiratory Specimens Using the BD ProbeTec™ ET System

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## REVISED ABSTRACT

### OBJECTIVES:

- (1) To develop Strand Displacement Amplification (SDA) based assays for use on the BD ProbeTec™ ET System to detect three of the leading causes of Community Acquired Pneumonia (*Legionella pneumophila*, *Mycoplasma pneumoniae* and the *Chlamydiaceae* Family) from a single lower respiratory sample.
- (2) To assess the efficacy of a universal sample processing method for lower respiratory specimens including sputum and bronchial washings: a.) Evaluate the effect on the internal amplification control (IAC) b.) Determine analytical sensitivity in spiked processed specimens.

**METHODS:** Performance of the IAC for each of the three assays was evaluated using 119 lower respiratory specimens. All samples were processed according to a customized Qiagen® DNA extraction protocol and assayed using a universal buffer system and dried amplification reagents. To determine the analytical sensitivity for each assay, serial dilutions of the three organisms were seeded together into a negative lower respiratory specimen pool, then processed and assayed. Sample stability was determined by seeding a negative sputum pool with the three organisms and storing at different temperatures for various lengths of time. Where available, positive lower respiratory clinical samples were tested in blinded studies.

**RESULTS:** For the 119 lower respiratory samples examined, the IAC, an indicator of SDA reaction inhibition, showed indeterminate rates for the *L. pneumophila*, *C. Family* and *M. pneumoniae* assays of 1.8%, 5.4% and 7.6%, respectively. The analytical sensitivity using a processed lower respiratory specimen pool was determined to be 1092 colony forming units (cfu)/mL for *L. pneumophila*, 1002 elementary bodies (EBs)/mL for *C. pneumoniae* and 848 cells/mL for *M. pneumoniae*. All three organisms were shown to be stable in sputum for 6 hours at room temperature and at least 6 days when stored at 2 – 8°C or -20°C. The *L. pneumophila* assay detected all of five culture positive lower respiratory specimens in a blinded study with no false-positive results.

**CONCLUSION:** The BD ProbeTec ET Community Acquired Pneumonia assays offer the potential for highly sensitive detection of *L. pneumophila*, *M. pneumoniae* and the *Chlamydiaceae* Family from lower respiratory specimens. All three assays employ a common, rapid and straight-forward sample processing protocol that provides for efficient recovery of DNA and removal of potential assay inhibitors.

### INTRODUCTION

Community-Acquired Pneumonia (CAP) is diagnosed in approximately 4 million adults each year, 25% of whom require hospitalization.<sup>1</sup> Although *Streptococcus pneumoniae* remains the most common cause of CAP, *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Chlamydia pneumoniae* are being isolated with increasing frequency. These organisms are implicated in up to 50% of cases and frequently occur as co-pathogens in mixed infections that are associated with particularly high mortality.<sup>2</sup> A lack of distinctive clinical features, together with the absence of rapid and accurate laboratory tests, has contributed to the difficulty in diagnosing infections with these pathogens. Routine cultures of expectorated sputum are neither sensitive nor specific for the detection of the agents of atypical pneumonia due to delays in sample processing, overgrowth of cultures by contaminating normal flora and empirical administration of antibiotics. PCR is the most common method for detecting nucleic acids from CAP pathogens, but interpretation of results is often complicated by false-negative results due to inhibitors found in lower respiratory samples.

Here we describe the performance characteristics of three new assays for the detection of *L. pneumophila*, *M. pneumoniae* and the *Chlamydiaceae* Family (*Chlamydia pneumoniae*, *Chlamydia trachomatis* and *Chlamydia psittaci*) from lower respiratory specimens using the BD ProbeTec™ ET System. These tests are based upon Strand Displacement Amplification (SDA) of target DNA and real-time homogeneous detection utilizing a universal fluorescence energy transfer probe. All three assays employ a customized Qiagen® DNA extraction protocol that has been designed to reduce amplification inhibition associated with lower respiratory specimens. An internal control, co-amplified with target DNA, is also incorporated in each reaction to validate negative results.

After the sample processing step, workflow for these analytes is identical to other BD ProbeTec ET assay systems. All three assays employ a common buffer in conjunction with dried reagents to streamline workflow, maximize ease-of-use and reduce time-to-results. Here we demonstrate that the BD ProbeTec ET atypical pneumonia assays have the potential to provide a rapid, sensitive and specific means for diagnosis of pneumonia caused by *L. pneumophila*, *M. pneumoniae* and/or *Chlamydiaceae* Family using lower respiratory samples.

### ACKNOWLEDGEMENTS

We would like to thank Dr. Victor Yu, Dr. Janet Stout (VA, Pittsburgh), Gordon Franklin, Karen Yanson, Daryl Shank, Christine Keys, Mark Hall and Paula Johnson (BD) for their contributions to this poster and Dr. Frank Krieg-Schneider and Dr. Markus Sprenger-Haussels of Qiagen® for their technical advice and assistance in the development of the Qiagen® custom protocol.

## METHODS

**DATA ANALYSIS.** All experiments were performed using the three CAP assays on the BD ProbeTec ET System. Data was analyzed using the Passes After Threshold (PAT) algorithm developed for these assays (Figure 3).

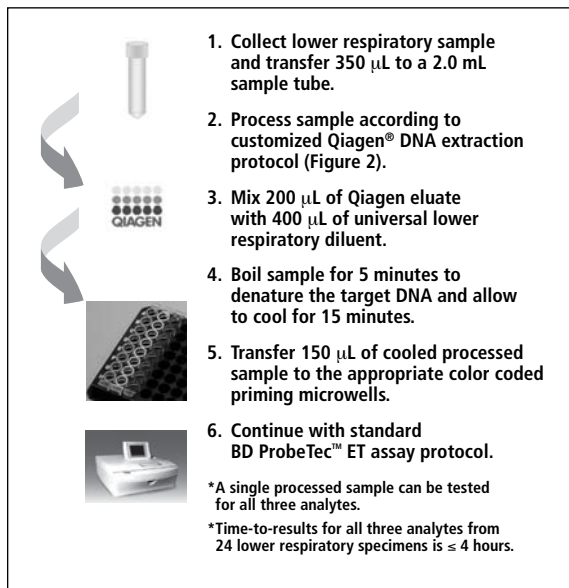
**SPECIMEN STABILITY.** A negative sputum pool was triple spiked with *C. pneumoniae*, *L. pneumophila* and *M. pneumoniae* at 140 EBs/rxn, 450 cfu/rxn and 2400 cells/rxn, respectively. The spiked pool was then stored at either room temperature, 2–8°C or -20°C for varying lengths of time. Specimens (n=8 per condition) were processed as described in Figure 1 and assayed for all three analytes (Figure 6).

**CHARACTERIZATION OF SAMPLE PROCESSING METHOD FOR REMOVAL OF SDA INHIBITORS.** To examine the performance of the Internal Amplification Control (IAC) and the efficiency of the DNA extraction protocol to remove inhibitors, 119 lower respiratory specimens were processed and assayed, as described in Figures 1 and 2. Results for each of the individual assays are summarized in Table 1.

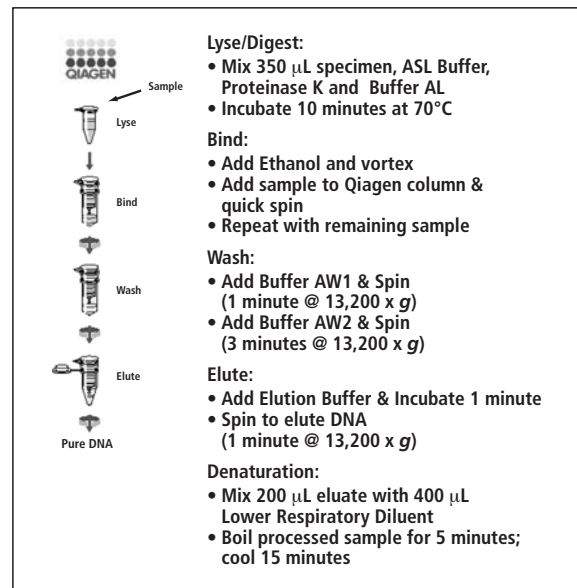
**ANALYTICAL SENSITIVITY WITH PROCESSED SPUTUM.** To determine the analytical sensitivity with processed spiked specimens, a negative sputum pool was subdivided and individual sub-pools seeded with *L. pneumophila*, *M. pneumoniae* and *C. pneumoniae*. Each spiked specimen was then processed and assayed for each organism as described in Figures 1 and 2. Sixteen replicates were analyzed at six different target levels (Figure 4). Negative samples consisted of an unseeded sputum pool.

**LOWER RESPIRATORY CLINICAL SAMPLES.** Five lower respiratory specimens that were culture positive for *L. pneumophila* were evaluated in the BD ProbeTec ET *L. pneumophila* assay. These samples were received from Dr. Victor Yu (VA Medical Center, Pittsburgh PA.) and were tested in a blinded panel that included known *L. pneumophila* culture-negative samples (Figure 5). Lower respiratory samples that were culture positive for *M. pneumoniae* or *Chlamydiaceae* Family were not available at the time of presentation. A summary of the analysis performed on the *L. pneumophila* positive clinical samples is given in Table 2.

BD ProbeTec ET Sample Processing Work Flow (Figure 1).



Custom Qiagen DNA Extraction Protocol (Figure 2)



## RESULTS

Table 1. Characterization of Sample Processing Method for Removal of SDA Inhibitors.

Chlamydiaceae Family Assay	
Sample Type	Unreportable Results
Sputum (n=97)	6
Bronchial Washings (n=22)	0
Overall Indeterminate Rate	5.40%
Legionella pneumophila Assay	
Sample Type	Unreportable Results
Sputum (n=97)	2
Bronchial Washings (n=22)	0
Overall Indeterminate Rate	1.80%
Mycoplasma pneumoniae Assay	
Sample Type	Unreportable Results
Sputum (n=101)	5
Bronchial Washings (n=18)	4
Overall Indeterminate Rate	7.60%

Table 2. *L. pneumophila* Positive Sample Profiles

Sample #	Specimen Type	Culture Result	Urinary Antigen Result	Legionella PAT Result	Culture Growth/Comments
2	Sputum	+	+ (RTN* = 8.6)	47	Few-colonies: Sero-group 1
3	Sputum	+	+ (RTN* = 42.8)	52	Heavy Growth: Sero-group 1
8	BAL	+	Not Done	52	No information
10	Sputum	+	+ (RTN* = 22)	52	Mod-heavy growth: Sero-group 1
13	Sputum	+	+ (RTN* = 23)	51	One colony: Sero-group 1

\* RTN = Ratio to Negative      PAT > 0 = positive

- Unreportable result (Indeterminate) = Target PAT ≤ 0 and Internal Amplification Control ≤ 0
- Four specimens were positive by the BD ProbeTec™ ET for *M. pneumoniae*. All four results were confirmed by PCR amplification of the 16S rRNA gene.

Figure 3. BD ProbeTec ET PAT Algorithm

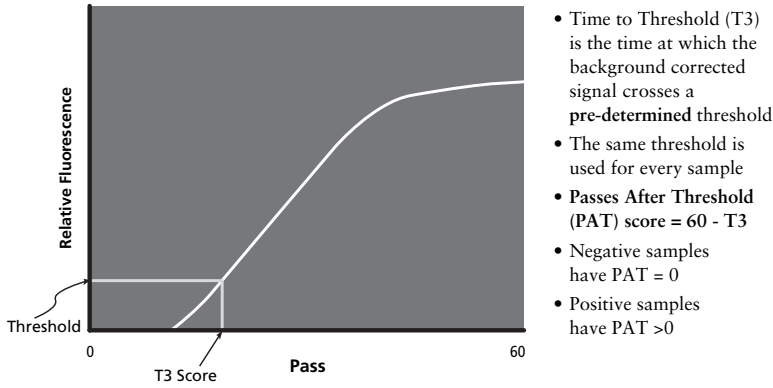
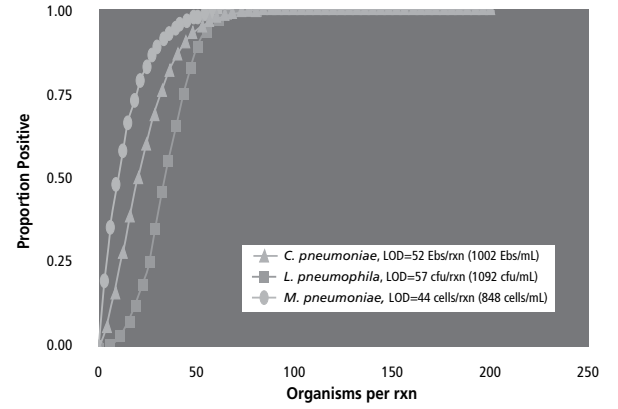
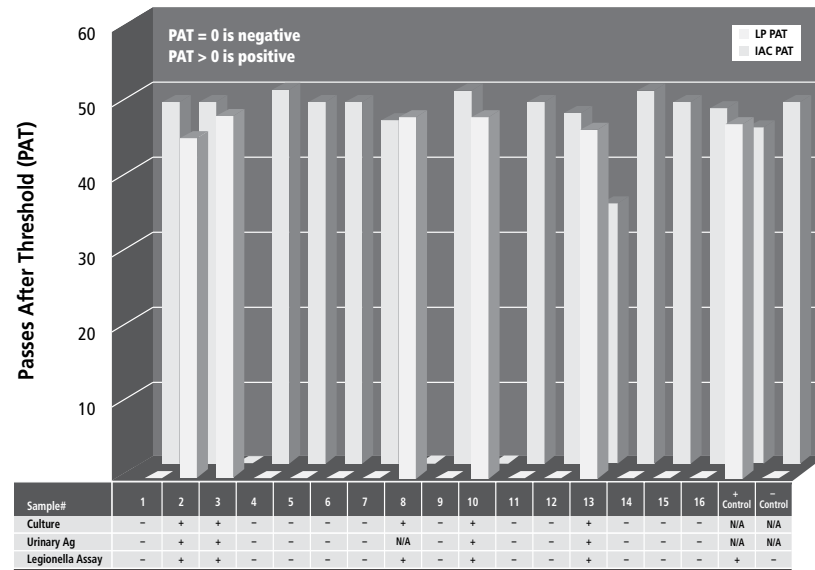


Figure 4. Analytical Sensitivity with Processed Sputum



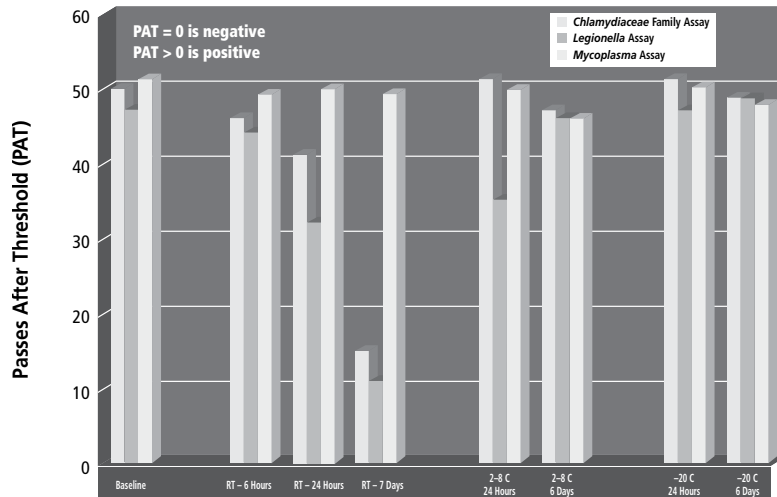
The LOD is defined as the number of targets that are amplified and detected with 95% probability.

Figure 5. Lower Respiratory Clinical Samples



- BD ProbeTec ET *L. pneumophila* assay results correlated with culture and urinary antigen analysis.

Figure 6. Specimen Stability



- All three organisms were stable for 6 hours at room temperature and at least 6 days when stored at 2 – 8°C or –20°C in a sputum matrix

## CONCLUSIONS

- Three homogeneous real-time SDA assays have been developed for the detection of *M. pneumoniae*, *L. pneumophila* and the *Chlamydiaceae* Family from lower respiratory specimens which incorporate the following features:
  - Internal Amplification Control to validate negative results
  - Simplified workflow process with rapid time-to-results
  - Ability to test for all three organisms from a single lower respiratory sample
- Organisms were shown to be stable in a sputum matrix for 6 hours at room temperature and at least 6 days at 2-8°C, or -20°C, thereby demonstrating flexibility and convenience in sample collection, transport and storage.
- The customized Qiagen DNA extraction protocol efficiently removes amplification inhibitors from lower respiratory specimens and captures target nucleic acids.

Assay	LOD (organisms/mL)	LOD (organism/rxn)	Indeterminate Rate (N=119)
<i>Chlamydiaceae</i> Family	1002	52	5.4%
<i>Legionella pneumophila</i>	1092	57	1.8%
<i>Mycoplasma pneumoniae</i>	848	44	7.6%

- BD ProbeTec ET *L. pneumophila* assay results correlated with culture and urinary antigen analysis.
- The BD ProbeTec ET pneumonia tests provide sensitive, specific and rapid alternatives to conventional microbiological approaches to aid in the diagnosis of pneumonia caused by *M. pneumoniae*, *L. pneumophila* and/or the *Chlamydiaceae* Family.

## REFERENCES

1. Battleman DS, Callahan M, Thaler HT. Rapid antibiotic delivery and appropriate antibiotic selection reduce length of hospital stay of patients with community-acquired pneumonia: link between quality of care and resource utilization. *Arch Intern Med* 2002 Mar 25; 162(6): 682-688.
2. Gleason PP. The emerging role of atypical pathogens in community-acquired pneumonia. *Pharmacotherapy* 2002 Jan; 22(1 Pt 2): 2S-11S.