Direct Serogrouping of *Streptococcus pneumoniae* Strains in Clinical Samples by Use of a Latex Agglutination Test

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Pneumotest-Latex (Statens Seruminstitut) was evaluated for direct serogrouping of *Streptococcus pneumoniae* strains in clinical samples from patients with invasive disease. The technique was accurate to its level of discrimination for 62 of 67 clinical samples (92.5%). Pneumotest-Latex would be a useful alternative for direct serogrouping of pneumococci in clinical samples.

The reference method for serotyping of *Streptococcus pneumoniae* is the Quellung reaction (1). Pneumotest-Latex (Statens Seruminstitut, Copenhagen, Denmark) is a simple latex agglutination procedure for partial serogrouping/serotyping of *S. pneumoniae* strains (10, 11). Strains are classified by Pneumotest-Latex into serotypes, serogroups, or pools of serogroups. Both the Quellung reaction and Pneumotest-Latex are intended for strains isolated in pure culture. However, for many patients with invasive pneumococcal disease (IPD), culture results are negative (7, 14). Although different PCR pro-

### TABLE 1. Primers, probes, and PCR conditions for serotyping of *S. pneumoniae*

<table>
<thead>
<tr>
<th>Serotype(s)</th>
<th>Primer or probe</th>
<th>Sequence(^a) (5′—3′)</th>
<th>Conc (μM)</th>
<th>Product size (bp)</th>
<th>Annealing temp/ elongation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1f</td>
<td>CTCTATAGAATGGAGTATATAAAAATATGGTTA</td>
<td>0.5</td>
<td>280</td>
<td>60°C/10 s</td>
</tr>
<tr>
<td></td>
<td>1r</td>
<td>CCAAAAGAAAAATACATACATACACAAATAATGGG</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-f</td>
<td>ATGGGTGTAGTTCTCTAGATTTGAAGATTAG</td>
<td>0.6</td>
<td>371</td>
<td>60°C/14 s</td>
</tr>
<tr>
<td></td>
<td>3-r</td>
<td>CTCTCGATTTGATTACCTGAGGATCG</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A/B/C</td>
<td>6A/B-f</td>
<td>AATTGAGATTTTATTTATTATTTATTTG</td>
<td>0.5</td>
<td>250</td>
<td>60°C/10 s</td>
</tr>
<tr>
<td></td>
<td>6A/B-r</td>
<td>TTGACGGGAGATAATTTATTTATTTATTTG</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14-f</td>
<td>CTTGCGAGGGTTGAGAATTCCCTTACTAC</td>
<td>0.5</td>
<td>208</td>
<td>54°C/9 s</td>
</tr>
<tr>
<td></td>
<td>14-r</td>
<td>GCCAAAATACTGACAAAGAGCTAGATATAGCC</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19A</td>
<td>19A-f</td>
<td>GTTAGTCTCTTTTTAGTTATTTTTGAGTGT</td>
<td>0.5</td>
<td>478</td>
<td>60°C/19 s</td>
</tr>
<tr>
<td></td>
<td>19A-r</td>
<td>GAGCAGTCAAATAAGATGACAGCATAGTAGTAG</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>19F-f</td>
<td>GTTAGTCTCTTTTTAGTTATTTTTGAGTGT</td>
<td>0.5</td>
<td>304</td>
<td>54°C/13 s</td>
</tr>
<tr>
<td></td>
<td>19F-r</td>
<td>GAGCAGTCAAATAAGATGACAGCATAGTAGTAG</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>SP_Ser5S</td>
<td>CTACTGGAATGCTGTGATGTGTGTTG</td>
<td>0.5</td>
<td>132</td>
<td>60°C(^b)</td>
</tr>
<tr>
<td></td>
<td>SP_Ser5A</td>
<td>CTGCGGATTAAAAAGATGATGCTT</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serotype 5 probe</td>
<td>FAM-TTGGAGCATGTGTGCTGTGATTGCC</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7F</td>
<td>SP_Ser7S</td>
<td>AGTCCAAATCTTTACAGAGACTCCA</td>
<td>0.5</td>
<td>111</td>
<td>60°C(^b)</td>
</tr>
<tr>
<td></td>
<td>SP_Ser7R</td>
<td>TTGCTACTATACCATAGAGATTTCC</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serotype 7F probe</td>
<td>FAM-TTGACATATCTTTATAGAGAACAGAACCBA-BQ</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) FAM, 6-carboxyfluorescein; BBQ, BlackBerry Quencher.  
\(^b\) In PCR with TaqMan probes, an elongation step is not necessary.

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tocols have been applied for direct serotyping of *S. pneumoniae* (8, 12), the technical requirements of these assays make them difficult to use in most laboratories. Direct antigen detection in clinical samples constitutes an alternative for the diagnosis of IPD (9). The utility of some latex agglutination techniques for direct antigenic detection of *S. pneumoniae* in normally sterile clinical samples (NSCS) is related to the capsular serotype (4). The aim of the present study was to evaluate Pneumotest-Latex for direct serogrouping of *S. pneumoniae* strains in NSCS. We studied 67 NSCS (positive for *S. pneumoniae* by culture or PCR) from IPD patients. *S. pneumoniae* was isolated from cultures of 47 samples (44 blood samples and 3 cerebrospinal fluid [CSF] samples). For 20 culture-negative pleural fluids, *S. pneumoniae*-specific PCR assays using the pneumococcal targets pneumolysin and autolysin (encoded by *ply* and *lytA*, respectively) were positive (3). Twenty-four blood samples and the 20 pleural fluids were inoculated into Bactec bottles and incubated in the Bactec 9240 system (Becton Dickinson Microbiology Systems, Cockeysville, MD). The remaining 20 blood samples were inoculated into BacT/Alert vials and incubated in the BacT/Alert system (bioMérieux, Marcy l’Etoile, France). The three CSF samples were cultured using standard methods.

The 47 *S. pneumoniae* isolates were serogrouped/serotyped using Pneumotest-Latex. Overnight pure cultures of *S. pneumoniae* in Todd-Hewitt broth were mixed with the Pneumotest-Latex suspension antiserum panels (A-I to P-T) (11). The serotype, serogroup, or pool of serogroups was assigned according to the chessboard provided by the Pneumotest-Latex manufacturer (10). Specific serotypes were confirmed by the Quellung reaction using commercial factor antisera (Statens Seruminstitut, Copenhagen, Denmark).

We adapted a classic PCR (8) to identify serotypes 1, 3, 6A/B, 14, 19A, and 19F of *S. pneumoniae* to a real-time PCR using the LightCycler SYBR green format followed by melting-curve analysis. Four new primers and two TaqMan probes were designed (Table 1) to detect serotypes 5 and 7F. All primers and probes were tested against the Quellung reaction for their specificity and cross-reactivity. No other serotypes were studied by PCR. The 20 culture-negative pleural fluid samples were analyzed by this method.

For direct sample agglutination serogrouping, blood culture bottles were inoculated with blood, CSF, and pleural fluid samples, 10 $\mu$L of the supernatant from each NSCS culture was mixed with 10 $\mu$L of each latex reagent, and the results were read. Serogrouping results for the isolated strains and results from PCR analyses of NSCS were considered to be reference criteria. Table 2 shows the distribution of the results from the reference and direct agglutination methods.

**Table 2. Distribution of results from reference and agglutination methods**

<table>
<thead>
<tr>
<th>Serogroup determined by latex agglutination test applied directly to clinical sample(s) (no. of samples)</th>
<th>Type of clinical sample (no. of samples)</th>
<th>Serotype(s) of isolated strain(s) (no. of strains)</th>
<th>Serotype determined by PCR analysis of clinical sample(s) (no. of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (13)</td>
<td>Blood (6)</td>
<td>1 (6)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>3 (6)</td>
<td>Pleural fluid (7)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>5 (3)</td>
<td>Blood (3)</td>
<td>5 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>6 (3)</td>
<td>Pleural fluid (1)</td>
<td>6B (1)</td>
<td>6A/B/C* (2)</td>
</tr>
<tr>
<td>7 (4)</td>
<td>Blood (3)</td>
<td>7F (3)</td>
<td>7F (1)</td>
</tr>
<tr>
<td>8 (5)</td>
<td>Blood (5)</td>
<td>8 (5)</td>
<td>9N (1), 9V (2)</td>
</tr>
<tr>
<td>9 (3)</td>
<td>Blood (3)</td>
<td>9N (1), 9V (2)</td>
<td></td>
</tr>
<tr>
<td>10 (1)</td>
<td>Blood (1)</td>
<td>10A (1)</td>
<td></td>
</tr>
<tr>
<td>11 (1)</td>
<td>Blood (1)</td>
<td>11A (1)</td>
<td></td>
</tr>
<tr>
<td>12 (2)</td>
<td>Blood (2)</td>
<td>12F (2)</td>
<td></td>
</tr>
<tr>
<td>14 (2)</td>
<td>Pleural fluid (1)</td>
<td>14 (1)</td>
<td></td>
</tr>
<tr>
<td>15 (3)</td>
<td>Blood (2)</td>
<td>15B (2)</td>
<td>15C (1)</td>
</tr>
<tr>
<td>19 (11)</td>
<td>Blood (7)</td>
<td>19A (7)</td>
<td>19A (4)</td>
</tr>
<tr>
<td>20 (1)</td>
<td>Pleural fluid (4)</td>
<td>20 (1)</td>
<td></td>
</tr>
<tr>
<td>21 (1)</td>
<td>Blood (1)</td>
<td>21 (1)</td>
<td></td>
</tr>
<tr>
<td>22 (1)</td>
<td>Blood (1)</td>
<td>22F (1)</td>
<td></td>
</tr>
<tr>
<td>23 (3)</td>
<td>Blood (1)</td>
<td>23B (1)</td>
<td></td>
</tr>
<tr>
<td>23 (3)</td>
<td>Pleural fluid (1)</td>
<td>23B (1)</td>
<td></td>
</tr>
<tr>
<td>33 (1)</td>
<td>CSF (1)</td>
<td>23B (1)</td>
<td></td>
</tr>
<tr>
<td>13 or 28$^c$ (1)</td>
<td>Blood (1)</td>
<td>33$^c$ (1)</td>
<td></td>
</tr>
<tr>
<td>24, 31, or 40$^c$ (1)</td>
<td>Blood (1)</td>
<td>13 (1)</td>
<td></td>
</tr>
<tr>
<td>25, 38, 43, 44, 45, or 48$^c$ (2)</td>
<td>Blood (2)</td>
<td>24F (1)</td>
<td></td>
</tr>
<tr>
<td>25, 38, 43, 44, 45, or 48$^c$ (2)</td>
<td>Blood (2)</td>
<td>25A (2)</td>
<td></td>
</tr>
</tbody>
</table>

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*Strains were not classified at the serotype level.*

*Serotypes 1, 3, 5, 6A/B, 7F, 14, 19A, and 19F were excluded.*

*Strain(s) was not classified at the serogroup level by the latex agglutination method.*
lung reaction) and in pleural fluid samples with negative cul-
ture results (by PCR). Only one isolate was identified by the
serogroup only (serogroup 33). PCR assays could not differ-
tiate the serotypes (6A/B/C) in two pleural fluid samples and
could not identify any serotype (serotypes 1, 3, 5, 6A/B, 7F, 14,
19A, and 19F were excluded) in a third pleural fluid sample.

Direct latex serogrouping of strains from NSCS was accurate
to its level of discrimination (serotype or serogroup) in 62 of 67
cases (92.5%). In 30 cases (44.8%), the serotype (1, 3, 5, 8, 14,
or 20) and serogroup agreed and, therefore, the latex method
allowed the greatest possible discrimination among strains
from NSCS. In 32 cases (47.8%), direct agglutination distin-
guished strains only to the level of the serogroup (6, 7, 9, 10, 11,
12, 15, 19, 22, 23, or 33). Although there was not disagreement
between serogroups determined by the reference and latex
methods, the latex test did not allow the maximum discrimi-
nation (to the serotype level) in these cases. PCR serotyping
was optimized to detect the most common serotypes of \(S.\) pneu-
moniae isolated from our environment. However, in one
case (1.5%), the serotype was not detectable by the PCR as-
says, and although the serogroup identified by direct latex
agglutination (serogroup 23) agreed with the PCR result (not
serotype 1, 3, 5, 6A/B, 7F, 14, 19A, or 19F), the result was
considered incorrect. In four cases (6.0%), direct latex agglu-
tination assigned the strains to pools of specific serogroups (13
and 28; 24, 31, and 40; and 25, 38, 43, 44, 45, 46, and 48). Even
though serotypes identified for isolated strains (serotypes 13,
24F, and 25A) were consistent with the serogroups pooled by
direct latex agglutination, these results were considered incor-
correct.

The Quellung reaction is time-consuming and requires ex-
erience (10). Therefore, development of molecular serotyping
technology is crucial for the surveillance of IPD (7, 12). How-
ever, molecular serotyping methods are not yet widely
available (14). Serogrouping of \(S.\) pneumoniae strains by ag-
glutination assays is considered cost-effective (6). If performed
directly on NSCS, it may improve the diagnosis of IPD (2).
According to the level of discrimination, direct agglutination
serogrouping of strains in NSCS by Pneumotest-Latex agreed
with the results from the reference methods (strain and PCR
typing) for most of the serogroups tested. The capsular reac-
tion has been applied previously for simultaneous detection
and typing of pneumococci in enrichment broth inoculated
with nasopharyngeal swabs (5), and the direct use of Pneumot-
est-Latex is applicable to serotype identification for colonized
 carriers (13). In the present study, groups of NSCS considered
to be incorrectly identified by Pneumotest-Latex did not rep-
tresent technical failures of agglutination. One case was not
included in the PCR analysis, and strains in four samples could
not be discriminated by the latex method. Although the num-
ber of samples was low and serotype representation was in-
complete, the results obtained in this study suggest that Pneu-
motest-Latex is a simple alternative for direct serogrouping of
\(S.\) pneumoniae strains in NSCS. It provides preliminary results
and could be used for screening before PCR serotyping when
culture results are negative.

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mos Community of Madrid, Spain, for submitting invasive isolates to
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improvement of real-time PCR assays targeting \(lxAT,\) \(ph,
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