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Usefulness of Pneumotest-Latex for Direct Serotyping of *Streptococcus pneumoniae* Isolates in Clinical Samples

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This study evaluated the usefulness of the Pneumotest-Latex assay for serotyping *Streptococcus pneumoniae* isolates directly in clinical samples. With an agreement of 88.1% with a PCR-based reference method, this test can be a useful tool for this study purpose, especially in clinical laboratories that do not have access to nucleic acid amplification technologies.

The Pneumotest-Latex assay (Statens Serum Institut, Copenhagen, Denmark) is a rapid latex agglutination test intended for serotyping or serogrouping *Streptococcus pneumoniae* isolates (1). It seems to be promising also for sample-based serotyping, as noted previously by Sanz et al. (2), who used this test for pneumococcal serotyping from incubated blood culture bottles. The aim of this study was to evaluate the usefulness of this test for serotyping *S. pneumoniae* isolates directly without any previous enrichment step using clinical samples from patients with invasive pneumococcal disease (IPD).

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Between January 2007 and May 2013, 4,290 clinical samples were sent to the National Reference Centre for Bacterial Meningitis (NRCBM) (Warsaw, Poland), with pneumococcal DNA detected in 165 samples (3.8%). In daily practice, the NRCBM receives culture-negative samples from normally sterile sites for PCR-based species identification. These samples were sent by local laboratories, with each having ≥250 μl volume, as required for molecular diagnostics on specimens from which no growth has been observed after 24 h of incubation. Our study encompassed only those samples with a volume sufficient to perform the Pneumotest-Latex assay. Finally, 64 clinical samples from 59 IPD patients with detected pneumococcal DNA were also studied on the following samples: blood, collected in EDTA or heparin ($n = 8$), serum ($n = 4$), cerebrospinal fluid (CSF) ($n = 40$), pleural fluid ($n = 4$), and 8 postmortem samples (2 liver, 2 kidney, 1 lung, 1 spleen, and 2 blood samples). Additionally, 18 clinical samples (blood, 7; serum, 1; CSF, 6; postmortem specimens, 4) from 17 patients with invasive meningococcal disease (IMD) were used as negative controls for serotyping.

The presence of pneumococcal DNA in the clinical samples was confirmed by PCR amplification of the *ply* (pneumolysin), *lytA* (autolysin), and *cps* (capsular polysaccharide) genes, as previously described (3–5). Clinical samples that were found to be positive for *S. pneumoniae* were collected for serotyping. Because the Pneumotest-Latex assay is intended for serotyping isolates in pure culture, it was necessary in our procedure to include additional sample preparation steps borrowed from the procedures of commercially available latex agglutination tests that directly detect pneumococcal antigens in body fluids (e.g., the Becton, Dickinson Directigen meningitis combo test and Bio-Rad Pastorex meningitis test). These procedures are based on a reaction with capsular polysaccharides similar to that in the Pneumotest-Latex assay (6–8).

Before the Pneumotest-Latex test, 200 μl of CSF was heated at 100°C for 3 min and centrifuged (9,660 × *g* for 5 to 10 min). For the other fluid samples, 400 μl was heated at 100°C for 5 min and then equally diluted in saline and centrifuged at 9,660 × *g* for 5 to 10 min. The postmortem tissue fragments were washed with 300 μl of saline and centrifuged (9,660 × *g* for 3 min). The supernatants were placed in new tubes, heated at 100°C for 5 min, and centrifuged (9,660 × *g* for 5 to 10 min). These supernatants were used in the Pneumotest-Latex assay, performed according to the manufacturer's instructions. For all samples, PCRs were run with 36 pairs of primers for serotype or serogroup determination, as previously described (3, 4, 9–15). *S. pneumoniae* serotypes 6A and 6B were identified by amplicon sequencing, as proposed by Pai et al. (9, 10), and further examined for serotypes 6C and 6D. The serotyping results from the PCR and sequencing analysis constituted the reference method. The percent agreement of the Pneumotest-Latex method was calculated versus the reference methods separately for every clinical sample type. The outcomes are summarized in Table 1.

The results of our study indicate that the Pneumotest-Latex assay may constitute a new diagnostic tool with good agreement (88.1%) compared to PCR, which can be used for direct *S. pneumoniae* serotyping/serogrouping in body fluid and tissue samples. The possible exceptions are serum samples, which performed poorly with the Pneumotest-Latex assay and PCR; there were 3 samples that were excluded due to negative results for both methods and only 1 sample that was positive by PCR (Table 1). However, we do not know if our negative results from both methods are really true negatives or if they arose from PCR limitations due to the inability to recognize all known serotypes. Therefore, we were unable to assess the utility and percent agreement for this clinical material (Table 1). Our difficulties concerning serum samples might be expected since the diagnostic value of the latex ag-

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TABLE 1 Serotyping results and agreement of the Pneumotest-Latex assay and PCR (reference method)

Clinical sample type (no. of samples)	ST or SG result (no. of samples) from clinical samples[a]		Latex/PCR % agreement (no./total no.)
	Pneumotest-Latex assay	PCR	
CSF (40)	1 (1)	1 (1)	92.3 (36/39)
	3 (2)	3 (2)	
	4 (1)	4 (1)	
	6 (3)	6A (2), 6B (1)	
	7 (1)	7F (1)	
	9 (5)	9A/V (3), 9N/L (2)	
	10 (1)	10A (1)	
	11 (2)	11A (2)	
	12 (5)	12F (5)	
	14 (2)	14 (2)	
	18 (4)	18C (4)	
	19 (2)	19A (1), 19F (1)	
	23 (4)	23F (4)	
	33 (1)	33F (1)	
	NV (5)	**10A**, **14**, **23F**, 34, 37 (1 each)[b]	
	NT (1)	ND (1) excluded[c]	
Whole blood (8)	1 (1)	1 (1)	62.5 (5/8)[d]
	6 (1)	6A (1)	
	8 (1)	8 (1)	
	9 (1)	9A/V (1)	
	19 (1)	19A (1)	
	NV (2)	**14** (1), **23F** (1)	
	NT (1)	**6A** (1)	
Serum (4)	NT (4)	**18C** (1), ND (3) excluded[c]	NA[e]
Pleural fluid (4)	1 (1)	1 (1)	100 (4/4)
	3 (1)	3 (1)	
	19 (2)	19A (2)	
Postmortem (8)	4 (3)	4 (3)	87.5 (7/8)
	6 (1)	6A (1)	
	15 (3)	15C (3)	
	NT (1)	**19F** (1)	
All (64)	ST (13)	ST (54)	88.1 (52/59)
	SG (37)	SG (6)	
	NV (7)	ND (4)[c]	
	NT (7)		

[a] ST, serotype; SG, serogroup; NV, nonvaccine serogroups/serotypes; NT, not typeable by the Pneumotest-Latex test; ND, not determined finally by PCR, but the following serotypes were excluded: 1, 2, 3, 4, 5, 6A/B, 7C, 7F, 8, 9A/V, 9N/L, 10A, 11A, 12A, 12F, 13, 14, 15A, 15B/C, 16F, 17F, 18C, 19A, 19F, 20, 22F, 23A, 23B, 23F, 24A/F, 31, 33F, 34, 35 B, 35F, and 38.

[b] Bold type indicates inconsistent serotyping results between the Pneumotest-Latex assay and PCR.

[c] Samples were excluded from assessment, since results were negative by the Pneumotest-Latex assay and PCR or only one sample was available for calculation.

[d] When blood samples collected postmortem were included in the calculation of the percent agreement, the result was 70.0% (7/10).

[e] NA, no assessment.

glutination results obtained from serum is controversial. Accordingly, some definitions of IPD do not accept positive latex test results from serum as confirmation of the presence of the disease (16–19).

All 18 samples from the IMD cases were found to be negative by the Pneumotest-Latex assay and PCR serotyping. Concordant results were obtained for multiple samples from one patient. Good agreement of the latex method was observed for the pleural fluid and CSF specimens tested (100% and 92.3%, respectively), which is in accordance with the results observed in previous studies (20–22). One of the most important observations of our study, despite the small number of samples tested, was the high agreement (87.5%) of the test for postmortem materials. Therefore, this direct serotyping technique may allow an association of mortality cause with *S. pneumoniae* serotypes/serogroups, especially in culture-negative cases. The lowest percent agreement, 62.5%, was obtained with whole blood but increased to 70.0% if the blood samples collected postmortem were included in the calculation. The lower results for the whole blood samples are not surprising, because that type of clinical sample is not recommended for latex agglutination testing due to the observation of nonspecific reactions (7, 8). The limitation of the Pneumotest-Latex assay is that the test can fully detect only 8 *S. pneumoniae* serotypes, 1, 2, 3, 4, 5, 8, 14, and 20, and cannot distinguish between certain specific types, e.g., 9A and 9V or 6A and 6B. For the majority of cases, only the information about serogroup or a pool of specific serogroups was gained. Generally, according to its level of discrimination, the Pneumotest-Latex assay typed 88.1% of the clinical samples, including 22.0% of the samples with final serotype designations. Our results differ from those of a previous study by Sanz et al. (2), who obtained a higher serotype/serogroup detection level for the test (92.5%) when 67 positive blood culture bottles were tested. This discrepancy may easily be explained by the fact that Sanz et al. (2) performed the additional culture step in a liquid medium, which resulted in a higher concentration of bacteria and increased sensitivity of the method (23–25). Based on our study and the experience of other users with the Pneumotest-Latex assay (M. Janulaitiene, National Public Health Surveillance Laboratory, Lithuania, personal communication), it is known that the culture conditions can strongly influence the results of isolate serotyping, as observed for serotype 3 and serogroup 6 (our unpublished data). Despite sufficient growth in Todd-Hewitt broth, these isolates strongly reacted with R or Q pooled serum only, giving clearly visible reactions, but they did not react with initial pool serum (A or B). Such irregularity was not observed for isolates grown on blood agar.

Another difference which might influence the sensitivity of the method is the quality of the clinical samples that were used. Sanz et al. (2) inoculated blood culture bottles with fresh clinical samples. In our study, the clinical samples were transported from the laboratory to the NRCBM at ambient temperature (postmortem samples at 2 to 8°C) within the time span of a few hours, up to 24 h, and were stored at −20°C before performing the Pneumotest-Latex assay.

To summarize, according to its level of discrimination, the Pneumotest-Latex assay may be a useful tool for serotyping/serogrouping *S. pneumoniae* isolates directly from clinical samples of patients with IPD. This is an easy and rapid test, especially for clinical microbiology laboratories that do not have access to nucleic acid amplification technologies.

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