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Performance of the Binax NOW *Streptococcus pneumoniae* Urinary Antigen Assay for Diagnosis of Pneumonia in Children with Underlying Pulmonary Diseases in the Absence of Acute Pneumococcal Infection

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The performance of the Binax NOW immunochromatographic test for detecting *Streptococcus pneumoniae* antigen in urine specimens from 103 children presenting underlying pulmonary diseases with no recent pneumococcal infection was assessed. Our data indicate that this assay is unlikely to be useful for discriminating between children with and without pneumococcal pneumonia.

Streptococcus pneumoniae is the leading bacterial agent causing community-acquired pneumonia among children in developed countries (9, 12). A rapid urinary pneumococcal antigen test (Binax NOW *S. pneumoniae* urinary antigen test; Binax, Portland, Maine) has been approved by the U.S. Food and Drug Administration for the diagnosis of pneumococcal pneumonia. The test appears to be highly specific and moderately sensitive for adults (3, 7, 11, 13); however, some concerns have been raised as to its specificity among children (2, 4), particularly in developing countries where nasopharyngeal colonization rates are high (1, 4, 5, 8). In this study, we assessed the performance of the Binax NOW assay among children presenting underlying pulmonary diseases with no recent pneumococcal infection, this being a subset of patients in whom rapid and accurate diagnosis of pneumococcal pneumonia is of particular clinical interest.

One hundred three children (54 males and 49 females) aged 1 to 15 years (mean, 5.8 years) with no recent history of respiratory tract infections were enrolled in the study. Children had been diagnosed previously with the following pulmonary diseases: recurrent pneumonia and atelectasis ($n = 38$), bronchial hyperreactivity and asthma ($n = 27$), cystic fibrosis ($n = 12$), recurrent upper respiratory infections ($n = 8$), bronchial tree malformations ($n = 8$), pulmonary bronchodysplasia ($n = 5$), and bronchiolitis obliterans ($n = 5$).

Fifty-six children had been vaccinated against pneumococcus before enrollment (37 with Prevenar and 19 with 23-valent vaccine).

Informed consent was obtained from the parents of the subjects. The study was approved by the Clinical Research Ethics Committee of the University Clinical Hospital.

Nasopharyngeal samples were obtained by calcium alginate swabbing and were cultured within 1 h of collection on nalidixic acid-supplemented Trypticase soy agar containing 5% sheep blood and brain heart infusion broth (BHI). The plates and the BHI were incubated at 37°C in 5% CO_2 for 24 h. The BHI was subcultured at 24 h onto nalidixic acid-supplemented Trypticase soy agar containing 5% sheep blood plates which were incubated as indicated above. *S. pneumoniae* was identified on the basis of colonial morphology, Gram stain characteristics, optochin sensitivity, and positive latex agglutination (Slidex Pneumo-Kit; bioMérieux SA). At least 10 colonies/plate were screened for pneumococcal identity. Clean-catch midstream urine specimens were obtained within 24 h of collection of the nasopharyngeal specimens and tested within 1 h of collection. Urine specimens were concentrated 25-fold by selective ultrafiltration (Minicon; Millipore Corp., Bedford, Mass.), boiled for 5 min, and centrifuged at $1,000 \times g$ for 15 min before testing in order to increase sensitivity (3, 7, 11) and prevent nonspecific reactions (2). Urine specimens yielding positive results were tested unconcentrated, as the manufacturer recommends.

The appearance of a visible band was considered a positive result. Readings were recorded as weakly positive or positive, depending on the intensity of the reactions compared with the control line. Since only strongly and moderately intense bands are likely to be associated with the presence of pneumococcal pneumonia in adults (2, 7), it was of interest to determine the intensity of bands in concentrated and nonconcentrated urine samples in children (for both colonized and noncolonized subjects) of our cohort.

Differences in the Binax NOW test results between groups were analyzed by the one-tailed Fisher exact test.

Fifteen out of 103 children (14.5%) were *S. pneumoniae* nasopharyngeal carriers.

Concentrated urine samples were assayed first (Table 1). Ten (66.6%) of 15 pneumococcal nasopharyngeal carriers and 29 (32.9%) of 88 noncarriers tested positive. Nonconcentrated urine samples were subsequently analyzed (Table 1). Eight pneumococcal carriers (53.3%) and 15 noncarriers (17.0%)

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TABLE 1. Performance of the Binax NOW *Streptococcus pneumoniae* urinary antigen test in children with underlying pulmonary diseases

Study group (no. of children)	Binax NOW result with urine that is:						*P* value for tests with urine that is:[b]	
	Concentrated			Nonconcentrated			Concentrated	Nonconcentrated
	+[c]	Weakly +	−	+	Weakly +	−		
Carriers (15)[a]	10	1	5	8	5	7		
Noncarriers (88)	29	3	59	15	9	73		
Both groups							0.019	0.004

[a] Nasopharyngeal colonization by pneumococci.
[b] Statistical significance for *P* values of <0.05.
[c] Positive (+) columns include both strongly and weakly reacting specimens.

tested positive. Thus, a positive urinary antigen assay was significantly more common in *S. pneumoniae* carriers than in noncarriers.

Concentrated urine samples testing weakly positive became negative when tested unconcentrated, suggesting that these urine samples contained either small amounts of pneumococcal C polysaccharide or variable quantities of C polysaccharide pneumococcal-like sugars (6). Nine of the 16 children (2 pneumococcal carriers) whose urine samples turned negative when retested unconcentrated had been vaccinated with Prevenar (a mean of 121 days prior to urine testing).

We next investigated whether a positive urinary antigen test was an occasional or rather a persistent finding among noncarriers. Sequential urine specimens ($n = 27$), collected over a mean of 60 days (range, 39 to 270 days), were available from 11 children who had tested positive (nonconcentrated urine samples). Samples were assayed without prior concentration. Seven of the 11 children had at least 2 positive samples (range, 2 to 5) during follow-up (21 positive samples overall, of which 8 reacted weakly). None of these children became colonized by *S. pneumoniae* during follow-up.

Our data show a high rate of positive urinary antigen test results in children with underlying pulmonary diseases with no acute pneumococcal infections, a result which has been reported previously for other population groups (1, 2, 4, 5, 8). In agreement with Domínguez et al. (2), we found that concentrating urine prior to testing notably reduces the specificity of the test when applied to the diagnosis of pneumococcal pneumonia; however, positive reporting only for strongly reacting nonconcentrated urines would increase the specificity of the test, particularly among noncarriers.

S. pneumoniae present in the nasopharynx is the most likely source of antigen in pneumococcal carriers testing positive with the assay. Positive results in noncarriers might be due to undetected low-level pneumococcal colonization, colonization by *Streptococcus mitis*, which has been shown to harbor pneumococcal C polysaccharide-like antigens (6) or antigen excretion after pneumococcal vaccination (10). In this context, vaccination records of children from our cohort not carrying pneumococci in the nasopharynx were consulted. Data are summarized in Table 2. Seventeen of 29 children (58.6%) who tested positive (all strongly reactive) by the urinary antigen assay (concentrated urine samples) had been vaccinated with Prevenar (heptavalent vaccine) a mean of 7.1 months before the collection of specimens (5 had been vaccinated approximately 1 month before testing), whereas only 12 out of 59 (22.0%) children with a negative urinary antigen assay had received the vaccine ($P = 0.0005$), a mean of 6.8 months earlier. The median ages of children of both groups did not differ significantly. Thus, it is likely that some of the positive urinary antigen tests recorded in noncarriers might have been due to recent vaccination with Prevenar, though we are unable to prove this. In summary, our data indicate that the Binax NOW *Streptococcus pneumoniae* urinary antigen test is not likely to be useful for discriminating between children with and without pneumococcal pneumonia.

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TABLE 2. Performance of the Binax NOW *Streptococcus pneumoniae* urinary antigen test in nonpneumococcal nasopharyngeal carriers with underlying pulmonary diseases either vaccinated or not with Prevenar

Study group (no. of children)[a]	Binax Now assay result with urine that is:						*P* value for tests with urine that is:[b]	
	Concentrated			Nonconcentrated			Concentrated	Nonconcentrated
	+[c]	Weakly +	−	+	Weakly +	−		
Vaccinated (29)	17	0	12	10	6	19		
Nonvaccinated (59)	12	3	47	7	6	52		
Both groups							0.0005	0.014

[a] Nonpneumococcal nasopharyngeal carriers.
[b] Statistical significance for *P* values of <0.05.
[c] Positive (+) columns include both strongly and weakly reacting specimens.

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