Assessment of the Binax NOW Streptococcus pneumoniae Urinary Antigen Test in Children with Nasopharyngeal Pneumococcal Carriage

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We evaluated the Binax NOW Streptococcus pneumoniae urinary antigen assay by testing 210 healthy children aged 2–60 months living in urban slums of Quito, Ecuador. Healthy children with nasopharyngeal carriage of S. pneumoniae were significantly more likely to have positive urinary antigen test results than were children who were not carriers (30 of 138 vs. 3 of 71 children; $\chi^2 = 10.8; P < .001$). The rate of nasopharyngeal carriage of S. pneumoniae decreased with increasing age; the lowest rates were found in children with the worst nutritional status.

Acute respiratory infections kill ~4 million children annually [1]. Streptococcus pneumoniae is the leading bacterial pneumonia pathogen and a major cause of mortality [1], and infection with this pathogen is difficult to diagnose accurately in children. Antigen detection assays are an alternative to standard culture-based methods for diagnosis of pneumonia. During acute pneumococcal pneumonia, capsular and C-polysaccharide antigens are found in sputum, pleural fluid, blood, and urine [2, 3]. A rapid urinary pneumococcal antigen test (Binax NOW; Binax) that detects the C-polysaccharide antigen present in all S. pneumoniae [3] was approved by the US Food and Drug Administration in 1999 for use in diagnosis of pneumonia. It has a sensitivity of 78% in nonbacteremic cases and 82%–86% in bacteremic cases of community-acquired pneumococcal pneumonia, with a specificity of >95% in adults [4, 5].

Although this rapid urinary antigen test has excellent sensitivity and specificity in adults, a prospective study in China found that children with nasopharyngeal carriage of S. pneumoniae had high rates of positive test results, regardless of whether they had underlying pneumonia [6]. We evaluated the Binax NOW assay by testing healthy children living in urban slums of Quito, Ecuador, to determine the influence of pneumococcal nasopharyngeal carriage on the results of the test.

Methods. Healthy children aged 2–60 months were eligible for participation if their caregivers were willing to provide written informed consent. Children living in poor urban neighborhoods of Quito were selected for study because many potential risk factors for pneumococcal nasopharyngeal colonization were present in this population. Informed consent was obtained from the parents or other caretakers of the children participating in this study. The study protocol and the informed consent form were approved by the Human Investigation Review Committee at New England Medical Center–Tufts University School of Medicine (Boston) and the ethical committee of the Corporación Ecuatoriana de Biotecnología (Quito).

The birth dates of study subjects were obtained from vaccination records. Children were weighed, and a thorough physical examination was performed. Children with fever (aural temperature $>37.5^\circ$C) or physical findings suggestive of respiratory tract infections were excluded from the study. Clean-catch midstream urine specimens were collected in sterile containers with adhesive plastic urine bags, for infants, and in plastic flasks, for older children. Specimens were immediately tested in accordance with the manufacturer’s instructions. The Binax NOW test uses colloidal gold–labeled rabbit anti–C-polysaccharide antibodies immobilized on a nitrocellulose membrane. A colorimetric reaction occurs ≤15 min after application of a urine-containing swab, if the antigen is present. Manufacturer-provided positive and negative control tests were performed daily. All assays were interpreted by the same 2 doctors (F.S. and B.E.).

Within 15 min of urine collection, nasopharyngeal specimens were obtained with use of gently inserted calcium alginate swabs. Swabs were immediately inoculated onto trypticase soy agar (5% sheep’s blood) with 5 μg/mL gentamicin. Agar plates were transported in a candle extinction jar at ambient temperature to the microbiology laboratory and incubated at 37°C for 24–48 hr.

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in a candle extinction jar (5%-10% CO2) for 48 h. Pneumococci were identified by means of visual inspection for typical colony morphology, α-hemolysis, bile solubility testing, and susceptibility to ethylhydrocupreine (Optochin). Growth was classified as “light,” “moderate,” or “heavy.”

We conservatively assumed a nasopharyngeal colonization rate of 50%, on the basis of data from other developing countries [7, 8]. We estimated the false-positive test result rate would increase from 10% to 20% for children with S. pneumoniae nasopharyngeal carriage. A sample size of 200 children was needed to provide a power level of 0.98 (α = 0.05) to detect this level of increase in the false-positive rate.

Data were analyzed with Epi Info, version 6.04c (Centers for Disease Control and Prevention), and SAS, version 8.0 (SAS). The proportion of children with pneumococcal nasopharyngeal carriage was determined for the entire group and then was stratified by age and weight-for-age Z (WAZ) score. Antigen test results were considered false positive for children who had cultures positive for nasopharyngeal S. pneumoniae carriage and had positive antigen test results. The proportion of children with and without carriage who had false-positive antigen test results was compared by use of the χ2 test. False-positive antigen test results were stratified by age and WAZ score. Logistic regression was performed to evaluate the association between nasopharyngeal carriage and age as well as to determine whether age or WAZ score might be predictors of false-positive results.

Results. A total of 210 children were enrolled; their mean age was 30.0 months (range, 2.66-60.5 months), and 52% were girls. One participant was excluded from analysis because no urine specimen could be obtained. Pneumococcal nasopharyngeal carriage was present in 138 (66%) of 209 children. A positive urinary antigen test result was obtained for 33 (15.8%) of 209 children. False-positive findings were more common in children with false-positive antigen test results were due to the detection of pneumococcal antigen that originates from pneumococci colonizing the upper airways. If this is indeed the case, then the assay may be too sensitive for some purposes, because these positive test results could be viewed as false-positive results when seeking evidence for invasive disease, such as pneumonia. However, for children who did not have nasopharyngeal carriage, the specificity of the pneumococcal urinary antigen test was 96%.

An analysis was performed to determine whether there was an association between age and colonization or false-positive antigen test results (table 1). Two trends were apparent. First, the rate of nasopharyngeal carriage was highest in infants and decreased with advancing age. Logistic regression analysis revealed an association between increasing age and a lower rate of nasopharyngeal carriage (P = .036). Second, the proportion of children with false-positive antigen test results diminished with increasing age; the highest rate was seen in infants (21%) and the lowest in children >3 years of age (11%). However, after controlling for nasopharyngeal carriage, there was no significant association between age and the rate of false-positive test results. For children with nasopharyngeal carriage, there was no association between age and the intensity of S. pneumoniae growth in culture (results not shown).

There was no association between nutritional status (as determined by WAZ score) and the rate of pneumococcal nasopharyngeal carriage (table 2). The smallest proportion of children with false-positive urinary antigen test results was found in the most malnourished stratum (children with a WAZ score of less than or equal to −2), even after correcting for the proportion of children who were nasopharyngeal carriers. By use of logistic regression analysis, there appeared to be a trend toward increasing rates of false-positive test results with increasing WAZ score, but this was not statistically significant. For the children with nasopharyngeal carriage, there was also no association between WAZ score and the intensity of S. pneumoniae growth in culture (data not shown).

Discussion. We found that rates of positive urinary antigen test results varied significantly according to the nasal colonization status of the patient. We suspect that these positive test results are due to the detection of pneumococcal antigen that originates from pneumococci colonizing the upper airways. If this is indeed the case, then the assay may be too sensitive for some purposes, because these positive test results could be viewed as false-positive results when seeking evidence for invasive disease, such as pneumonia. However, for children who did not have nasopharyngeal carriage, the specificity of the pneumococcal urinary antigen test was 96%.

<table>
<thead>
<tr>
<th>Age, months</th>
<th>No. of children (n = 209)</th>
<th>Percentage of children With nasopharyngeal S. pneumoniae carriage</th>
<th>With positive test results</th>
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</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>28</td>
<td>82.1</td>
<td>21.4</td>
</tr>
<tr>
<td>≥12 and &lt;24</td>
<td>46</td>
<td>60.9</td>
<td>17.4</td>
</tr>
<tr>
<td>≥24 and &lt;36</td>
<td>60</td>
<td>75.0</td>
<td>18.3</td>
</tr>
<tr>
<td>≥36</td>
<td>75</td>
<td>56.0</td>
<td>10.7</td>
</tr>
</tbody>
</table>
A study from Beijing, China, found that 54% of control children with dermatitis or diarrhea and pneumococcal nasopharyngeal carriage and 21% of control children without carriage had false-positive urinary antigen assay results [6]. Another study from The Gambia found that 87% of children had nasopharyngeal carriage of *S. pneumoniae*, and 55% of these tested positive for pneumococcus antigen with use of the Binax NOW test [8]. In contrast, the rate of false-positive pneumococcal antigen test results encountered in our study was lower. We found that 21.7% of children with pneumococcal carriage and only 4.2% of noncarriers had false-positive test results. Children from the Chinese study were of the same age range as those in our study [6], whereas the Gambian children were slightly older [8], which suggests that age-related differences in false-positive test results are unlikely to account for this finding. Variability in test interpretation is another possible explanation for the difference in the rate of false positive results. We had 2 people read all test results, to reduce potential variability in interpretation. Last, it is possible that the rate of detection of pneumococci in the 3 studies varied and that misclassification of children as noncarriers when they were true carriers elevated the rate of false-positive results among children who were noncarriers.

Although our study was not designed or adequately powered to address this question, we found that malnourished children had a trend toward fewer false-positive results, despite having nasopharyngeal colonization rates that were similar to the other WAZ score strata. Healthy children may mount more potent immune responses to colonizing pneumococci than do malnourished children. As a result, there may be more destruction of pneumococci and greater release of cell-wall antigens, thus leading to higher levels of antigen in the serum and urine.

One potential shortcoming of our study was the failure to identify children with recent but resolving acute respiratory infections. Because pneumococcal antigens are shed for weeks after pneumonia resolves [3], recent but resolved infections might explain the false-positive urinary antigen test results for children who did not have carriage. Alternatively, false-positive results might be due to cross-reacting antigens from other species of streptococci, such as *Streptococcus mitis* and *Streptococcus oralis* [9]. The isolation of *S. viridans* from 1 of the 3 children with a positive urinary antigen test provides a tentative explanation for this case. Alternatively, the level of nasopharyngeal colonization with *S. pneumoniae* might have been below the level that could be detected by culture.

On the basis of our results and the results of the studies from China and The Gambia [6, 8], it appears that the Binax NOW *S. pneumoniae* urinary antigen test should be used with caution for the detection of pneumococcal pneumonia or bacteremia in young children, especially in developing countries where nasopharyngeal colonization rates are high. However, the test may be useful for the diagnosis of pneumococcal pneumonia and bacteremia in populations with low rates of pneumococcal nasopharyngeal carriage, such as young children in developed countries, among whom low rates of nasopharyngeal carriage are common.

## Acknowledgment

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## References


### Table 2. Children with *Streptococcus pneumoniae* nasopharyngeal carriage and/or positive Binax NOW *S. pneumoniae* urinary antigen test results, stratified by weight-for-age Z (WAZ) score.

<table>
<thead>
<tr>
<th>WAZ score</th>
<th>Proportion (%) of children</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>With positive urinary antigen test results</td>
<td>With nasopharyngeal carriage of pneumococci</td>
</tr>
<tr>
<td>Less than or equal to −2 (malnourished)</td>
<td>2/30 (6.7)</td>
<td>18/30 (60)</td>
</tr>
<tr>
<td>Greater than −2 and less than or equal to −1 (mildly malnourished)</td>
<td>12/75 (16.0)</td>
<td>54/75 (72)</td>
</tr>
<tr>
<td>Greater than −1 and &lt;0</td>
<td>14/68 (20.6)</td>
<td>43/68 (63.2)</td>
</tr>
<tr>
<td>&gt;0</td>
<td>5/36 (13.9)</td>
<td>23/36 (63.9)</td>
</tr>
</tbody>
</table>

* a No. of children with a positive urine antigen test result/no. with nasopharyngeal carriage (% with concordant results).


