## New Technique (the NOW Test) for Rapid Detection of *Streptococcus* pneumoniae in the Nasopharynx

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Although the NOW test was originally introduced as a rapid pneumococcal antigen detection test for use with urine samples, it was successfully adapted to nasopharyngeal samples in the present study. The sensitivity, specificity, positive predictive value, and negative predictive value of the test were 92.2, 97.7, 95.9, and 95.5%, respectively. These results demonstrate that nasopharyngeal colonization with *Streptococcus pneumoniae* can be documented within 15 min of sample collection.

*Streptococcus pneumoniae* is the leading bacterial cause of respiratory infections in children and of acute otitis media (AOM) in particular (8). Simultaneous cultures from nasopharynx and middle ear samples from patients with AOM demonstrate the same pathogen in a high proportion of cases (1–5, 9). This study was designed to determine the reliability of a rapid pneumococcal antigen test in detecting the presence or absence of *S. pneumoniae* in the nasopharynxes of children with or without AOM.

Children below the age of 15 years were enrolled at three office sites after we obtained informed consent. The enrollees had to be either healthy or ill with AOM. The children were enrolled without regard to sex or race. Children were excluded from the study if they had been treated with antibiotics within the past month. Nasopharyngeal samples were obtained with Mini-tip Culturettes (Becton Dickinson, Sparks, Md.). The specimens were cultured within 12 h of collection. The specimens were cultured on sheep blood agar and chocolate agar. The plates were incubated at 36°C in 5%  $CO_2$  for 18 to 24 h. S. pneumoniae was identified by colonial morphology, Gram stain characteristics, optochin sensitivity, and bile solubility. Nontypeable Haemophilus influenzae was identified by growth on chocolate agar, colonial morphology, Gram strain characteristics, a growth requirement for X and V factors, and failure to agglutinate with typing antisera. Moraxella catarrhalis was identified by colonial morphology, Gram stain characteristics, and the biochemical reaction of butyrate esterase.

The NOW test was obtained from Binex, Inc., Portland, Maine. It is an in vitro rapid immunochromatographic assay for the detection of *S. pneumoniae* cell wall polysaccharide in urine specimens from patients with symptoms of pneumonia. The test kit incorporates rabbit anti-*S. pneumoniae* antibody adsorbed onto a nitrocellulose membrane. If pneumococcal cell wall polysaccharide is in the specimen, an easily discernible pink-to-purple line appears within 15 min on the membrane. A control is included to ensure the validity of the test. We adapted the test to detect pneumococcal antigen in the nasopharynxes of children colonized with *S. pneumoniae*. The same swab used to collect the sample for culture was used for the NOW test. The procedure was conducted according to the directions in the kit. The sensitivity, specificity, positive predictive value, and negative predictive value of the NOW test were calculated for the total population and for the healthy and AOM subpopulations as well. Differences between the groups were assessed by chi square analysis.

One hundred thirty-eight children were enrolled from three offices. The children ranged in age from 4 to 168 months, with a median of 22.5 months. Seventy-two subjects were male, and 66 were female. Fifty-three children were classified as healthy, and 85 were classified as having AOM.

Nasopharyngeal cultures were collected from every subject. S. pneumoniae was recovered from 37% of the children. Healthy children were colonized less often with pathogens than were children with AOM (45.3 versus 87.1%, P < 0.001). S. pneumoniae was recovered from 20.8% of healthy children and 47.1% of children with AOM (P < 0.01). The NOW test result was positive for 35.5% of the samples and negative for 64.5%. The NOW test had an overall sensitivity, specificity, positive predictive value, and negative predictive value of 92.2, 97.7, 95.9, and 95.5%, respectively. The results for the two subpopulations were similar.

The NOW test was successfully adapted for use with respiratory specimens. It reliably detected the presence and absence of S. pneumoniae in the nasopharynxes of children. The test was equally reliable for healthy and ill children. The success of the NOW test in the present report suggests that the NOW test could complement the culture for S. pneumoniae in the airway when a rapid result is desired. Although other rapid tests for pneumococcal antigen detection have been marketed, the NOW test has several distinct advantages. Unlike other tests such as countercurrent immunoelectrophoresis or latex agglutination, which utilize multiple capsular polysaccharide antigens, the NOW test uses a single cell wall polysaccharide that is present on all clinical strains of S. pneumoniae (6, 7). This enables the NOW test to detect all isolates of S. pneumoniae rather than the more common types tested for by the countercurrent immunoelectrophoresis and latex agglutination kits. The NOW test also has advantages over PCR assays because it

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is less expensive, it is technically simple, and it does not require specially trained technicians or sophisticated equipment.

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## REFERENCES

- Dickinson, D. P., B. G. Loos, D. M. Dryja, and J. M. Bernstei. 1988. Restriction fragment mapping of *Branhamella catarrhalis*: a new tool for studying the epidemiology of this middle ear pathogen. J. Infect. Dis. 158: 205–208.
- Faden, H., J. Stanievich, L. Brodsky, J. Bernstein, and P. L. Ogra. 1990. Change in nasopharyngeal flora during otitits media of childhood. Pediatr. Infect. Dis. J. 9:623–626.
- Gehanno, P., G. Lenoir, B. Barry, J. Bons, I. Boucot, and P. Berche. 1996. Evaluation of nasopharyngeal cultures for bacteriologic assessment of acute otitis media in children. Pediatr. Infect. Dis. J. 15:329–332.

- Howie, V. M., and J. H. Ploussard. 1971. Simultaneous nasopharyngeal and middle ear exudate cultures in otitis media. Pediatr. Digest 13:31–35.
- Loos, B. G., J. M. Bernstein, D. M. Dryja, T. F. Murphy, and D. P. Dickinson. 1989. Determination of the epidemiology and transmission of nontypeable *Haemophilus influenzae* in children with otitis media by comparison of total genomic DNA restriction fingerprints. Infect. Immun. 57:2751–2757.
- Miller, M., P. Koltai, and S. Hetherington. 1990. Bacterial antigens and neutrophil granule proteins in middle ear effusions. Arch. Otolaryngol. Head Neck Surg. 116:335–336.
- Palva, T., and T. Lehtinen. 1987. Pneumococcal antigens and endotoxin in effusions from patients with secretory otitis media. Int. J. Pediatr. Otorhinolaryngol. 14:123–128.
- Peter, G., and J. O. Klein. 1997. Streptococcus pneumoniae, p. 828–835. *In* S. S. Long, L. K. Pickering, and C. G. Prober (ed.), Principles and practice of pediatric infectious diseases. Churchill Livingstone, New York, N.Y.
- Schwartz, R., W. J. Rodriguez, R. Mann, W. Khan, and S. Ross. 1979. The nasopharyngeal culture in acute otitis media. JAMA 241:2170–2173.