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Rapid Identification of Pathogenic *Neisseria* Species and *Branhamella catarrhalis*

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Two systems, the Identicult-Neisseria (IDN; Scott Laboratories, Inc., Fiskeville, R.I.) strip and the Neisseria/Haemophilus Identification Test Kit (NHI; Vitek Systems, Inc., Hazelwood, Mo.) card, were compared with the 4-h Minitek system (BBL Microbiology Systems, Cockeysville, Md.) for their ability to rapidly identify 157 pathogenic *Neisseria* and *Branhamella catarrhalis* isolates. IDN, limited in its identification to four species, when incubated at 35°C for 10 min identified 99% of the isolates. However, when IDN was incubated at 22°C for 20 min, it identified only 92% of the isolates. The NHI card, a rapid semiautomated system with the ability to identify 25 organisms to the species level, correctly identified all of the isolates. A test for β-lactamase production included in the NHI card identified the 12 *Neisseria gonorrhoeae* and 10 *B. catarrhalis* β-lactamase-positive isolates included in the study. The IDN strip (35°C) and the NHI card compared favorably with the Minitek system.

Neisseria gonorrhoeae is one of the most commonly occurring sexually transmitted pathogens that can cause a variety of sequellae (1, 3). Therefore, it is important for the laboratory to accurately and rapidly identify this pathogen. Carbohydrate-degradation tests are commonly used for *Neisseria* species identification in the diagnostic laboratory. Because they are dependent on growth of the organism, these tests require lengthy incubation and are not always reliable owing to the fastidious nature of the organism. Chromogenic enzyme substrate tests, based on preformed enzymes, have proven to be reliable for the identification of *Neisseria* species (4, 5). In this study, we compared two rapid tests, the Identicult-Neisseria (IDN; Scott Laboratories, Inc., Fiskeville, R.I.) strip and the Neisseria/Haemophilus Identification Test Kit (NHI; Vitek Systems, Inc., Hazelwood, Mo.) card, with the 4-h Minitek system (BBL Microbiology Systems, Cockeysville, Md.), all of which detect preformed enzymes.

A total of 157 isolates, which included 118 strains of *N. gonorrhoeae*, 18 strains of *Neisseria meningitidis*, 7 strains of *Neisseria lactamica*, and 14 strains of *Branhamella catarrhalis*, were used. All the isolates were obtained from clinical specimens submitted to the Medical Microbiology Laboratory at the University of California Irvine Medical Center and the Special Disease Clinic of the Orange County Health Department, Santa Ana, Calif. Specimens were inoculated onto Thayer-Martin medium (CalScott, Carson, Calif.), incubated at 35°C in 5% CO_2, and examined after 24 and 48 h for the presence of growth. Colonies growing on Thayer-Martin medium that were oxidase-positive, gram-negative diplococci were subcultured onto chocolate agar, incubated overnight, and identified by the Minitek system according to manufacturer instructions. All the *B. catarrhalis* isolates were also tested with nitrate and DNase (3). Subcultures from primary plates were either tested directly or frozen at −70°C in fetal bovine serum with 10% glycerol until they were thawed and subcultured twice onto Thayer-Martin medium, followed by testing with all the systems, including repeat testing with Minitek. All the tests were performed from 48-h subcultures. No discrepancies were found between the initial Minitek result from an 18-h isolate grown on chocolate agar and results from a 48-h Thayer-Martin medium culture. Quality control strains *N. gonorrhoeae* ATCC 19424, *N. meningitidis* ATCC 13077, *N. lactamica* ATCC 23970, and *B. catarrhalis* ATCC 25238 were tested simultaneously with the study isolates.

The IDN strip consists of three chromogenic substrates impregnated on filter paper which detect preformed enzymes β-galactosidase, γ-glutamylaminopeptidase, and prolylaminopeptidase associated with *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*. Four drops of the supplied buffer were added to each of the three test areas on the strip. Six colonies were removed from subcultures with a cotton swab, and the swab was rolled across the entire surface of each of the three test areas. The strips were incubated under two different conditions, at 35°C for 10 min or at 22°C for 20 min. If test area A (substrate for β-galactosidase) turned green, the isolate was identified as *N. lactamica* and no further testing was necessary. If test area A was colorless, two drops of color developer were added to test areas B (substrate for γ-glutamylaminopeptidase) and C (substrate for prolylaminopeptidase). Development of purple in test area B identified the isolate as *N. meningitidis* regardless of what occurred in test area C. Red color development only in test area C identified the isolate as *N. gonorrhoeae*. If test areas A, B, and C were negative, the isolate was presumptively identified as *B. catarrhalis*. The appearance of any other colors or combinations was considered uninterpretable.

The NHI card consists of 16 test wells containing conventional and single-substrate chromogenic tests to differentiate *Neisseria* and *Haemophilus* species. The catalase test was performed before the test card was inoculated. The NHI cards were filled with a 1.8-ml suspension of organisms in saline equivalent to a McFarland no. 4 to 6 standard. The sealed cards were then incubated aerobically for 5 h at 35°C. Manufacturer instructions suggest a 4-h incubation, but we found color development at 5 h to be easier to interpret. The test reaction was interpreted by using a viewer box and a color guide. The reaction was then manually entered into the Vitek computer, and the identity of the isolate with the level

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TABLE 1. Comparison of identifications with the IDN strip and the NHI card

Organism	No. (%) correctly identified by:		
	IDN		NHI
	22°C	35°C	
N. gonorrhoeae	106 (90)	118 (100)	118 (100)
N. meningitidis	17 (94)	17 (94)	18 (100)
N. lactamica	7 (100)	7 (100)	7 (100)
B. catarrhalis	14 (100)	14 (100)	14 (100)
Total	144 (92)	156 (99)	157 (100)

of confidence and contradicting results, if any, was displayed.

All *N. gonorrhoeae* and *B. catarrhalis* isolates were tested for β-lactamase production using Cefinase disks (BBL) and Betatest strips (Microdiagnostics, Cleveland, Ohio).

The overall results of the IDN (22 and 35°C) and NHI systems are shown in Table 1. Of the 157 isolates tested, the IDN system when incubated at 22°C correctly identified 106 of the 118 (90%) *N. gonorrhoeae*, 17 of the 18 (94%) *N. meningitidis*, and all of the 7 *N. lactamica* and 14 *B. catarrhalis* isolates tested. The misidentified strain of *N. meningitidis*, which failed to produce visual purple in the appropriate test well, was identified as *N. gonorrhoeae*. The 12 *N. gonorrhoeae* isolates that failed to produce color in any of the test wells were presumptively identified as *B. catarrhalis*. However, when incubated at 35°C, the IDN system correctly identified 100% of the *N. gonorrhoeae*, *N. lactamica*, and *B. catarrhalis* isolates. One *N. meningitidis* isolate did not produce color in the appropriate test well and was misidentified as *N. gonorrhoeae*.

The NHI system correctly identified all of the isolates tested. In addition, of the 118 *N. gonorrhoeae* isolates and 14 *B. catarrhalis* isolates tested, 12 (10%) and 10 (71%), respectively, were β-lactamase positive by the NHI card and the acidometric and chromogenic cephalosporin methods.

The estimated technical time required for test performance was 4.5 min for the IDN strip and 8.0 min for the NHI card. The costs of supplies were similar: $2.64 and $2.68, respectively, for the IDN strip and the NHI card. Although the costs of supplies were similar, the overall cost of the NHI card ($4.01) was higher than that of the IDN strip ($3.41) because of the additional technical time required for test performance.

The results of our study indicate that the NHI card compares favorably with the Minitek system for the identification of pathogenic *Neisseria* species from clinical isolates. Although it agreed with the Minitek system for 99% of the organisms, the IDN card (35°C) misidentified one *N. meningitidis* isolate as *N. gonorrhoeae*, a major error. With the exception of this isolate, our results at 35°C correlate with those of Hosmer et al (2). We cannot recommend use of this system at 22°C, because of its poor performance. Also, it must be emphasized that colonies to be tested by the IDN system must be from a selective medium. Three *Neisseria* species, *N. flavescens*, *N. sicca*, and *N. subflava*, are γ-glutamylaminopeptidase and prolylaminopeptidase producers and consequently would be misidentified as *N. meningitidis* if tested from nonselective media. Also, it is important to note that the IDN strip presumptively identifies *B. catarrhalis* and that further testing should be performed to confirm identification.

In summary, the advantages of the IDN strip (35°C) were the small inoculum required, rapid turnaround time, and minimal technical time (4.5 min). The advantages of the NHI card were its 100% correlation with conventional methods, accurate β-lactamase detection, ability to identify a broad range of organisms, computer-assisted identification, and semiautomation.

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