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Comparative Evaluation of Five Commercial Systems for the Rapid Identification of Pathogenic *Neisseria* Species

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Prompt diagnosis and effective treatment of urogenital gonococcal infections require rapid isolation and identification of *Neisseria gonorrhoeae* from urogenital specimens. We evaluated a new, rapid (30-min) test called Gonochek II (E-Y Laboratories, San Mateo, Calif.) which utilizes chromogenic substrates for the identification of pathogenic *Neisseria* species. It was compared with the API NeIdent (Analytab Products, Inc., Plainview, N.Y.), Minitek (BBL Microbiology Systems, Cockeysville, Md.), and RapID NH (Innovative Diagnostics, Atlanta, Ga.), systems and the Phadebact GC (Pharmacia Diagnostics, Piscataway, N.J.) test for its performance in identifying known strains of *N. gonorrhoeae* (39 strains), *Neisseria meningitidis* (22 strains), *Neisseria lactamica* (12 strains), and *Branhamella catarrhalis* (17 strains). The Gonochek II system correctly identified 100% of *N. gonorrhoeae*, *N. lactamica*, and *B. catarrhalis* strains and 95.4% of *N. meningitidis* strains. The percent agreement for correct identification of all strains tested was 98.8%. In contrast, the Minitek, RapID NH, and API NeIdent systems correctly identified 86.6, 80.0, and 73.3% of the strains, respectively. The Phadebact GC test identified 94.9% of the *N. gonorrhoeae* isolates but also cross-reacted with 41.6% of the *N. lactamica* strains. The Gonochek II system is rapid, simple to perform, and easy to interpret, requires 1 to 2 min to set up, and more accurately identifies pathogenic *Neisseria* species when compared with other systems used in this study.

Identification of *Neisseria gonorrhoeae* from urogenital specimens requires the isolation of an oxidase-positive, gram-negative diplococcus which is subject to a confirmatory test. Currently, a variety of methods are available to confirm the identification of pathogenic *Neisseria* species, including carbohydrate utilization, direct fluorescent-antibody test, chromogenic substrates, radiometry, and coagglutination (6, 10).

Recently, Chu et al. (A. E. Chu, P. K. Chun, and D. M. Yajko, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C257, p. 279) described a new test system called Gonochek II (E-Y Laboratories, San Mateo, Calif.) for the rapid identification of *N. gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* and presumptive identification of *Branhamella catarrhalis*. The Gonochek II system is currently marketed exclusively in the United States by the Du Pont Co., Wilmington, Del.

The Gonochek II is a single-tube test system consisting of three chromogenic substrates, namely, 5-bromo-4-chloro-3-indoyl-β-D-galactoside, gamma-glutamyl *para*-nitroanilide, and prolyl-β-naphthylamide, each of which is specific for a single pathogenic *Neisseria* species. The hydrolysis of these substrates by the appropriate enzyme results in the formation of a color which is visible directly or after the addition of a coupling diazo salt.

For comparison purposes, we evaluated the Gonochek II system with four other commercially available *Neisseria* identification systems. These were the Minitek (BBL Microbiology Systems, Cockeysville, Md.), API NeIdent (Analytab Products, Inc., Plainview, N.Y.), and RapID NH (Innovative Diagnostics, Atlanta, Ga.) systems and the Phadebact GC test (Pharmacia Diagnostics, Piscataway, N.J.). In the minitek system, paper disks containing glucose, maltose, sucrose, or *o*-nitrophenyl-β-D-galactopyranoside are used for determining carbohydrate utilization by pathogenic *Neisseria* species. In the API NeIdent and RapID NH systems, both conventional biochemical and chromogenic substrates are used for the identification of *Neisseria* spp. and *Neisseria*-like organisms of the genera *Branhamella*, *Kingella*, and *Moraxella* and Centers for Disease Control M groups. In both systems, the utilization of carbohydrates is determined by a pH indicator, and the hydrolysis of chromogenic substrates is demonstrated by color development after the addition of coupling reagents. In the Phadebact GC test, the sensitized Cowan 1 strain of *Staphylococcus aureus* is used to demonstrate coagglutination when reacted with a suspension of *N. gonorrhoeae* cells.

The purpose of this study was to evaluate the performance of the Gonochek II system in comparison with the other commercially available test systems in identifying pathogenic *Neisseria* species and to assess its cost effectiveness and utility in the clinical laboratory.

(This work was presented previously [A. Philip and G. C. Garton, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 115, 1984].)

MATERIALS AND METHODS

Organisms. A total of 90 strains of pathogenic *Neisseria* species, consisting of *N. gonorrhoeae* (39 strains), *N. meningitidis* (22 strains), and *N. lactamica* (12 strains), and *B. catarrhalis* (17 strains) were used in this study. Of these, 38 *N. gonorrhoeae* strains, 13 *N. meningitidis* strains, 1 *N. lactamica* strain, and 16 *B. catarrhalis* strains were recovered from clinical specimens submitted to the microbiology laboratory of the Wilmington Medical Center, Wilmington, Del. The remainder of the isolates were stock strains obtained from the Centers for Disease Control, Atlanta, Ga., and Mt. Sinai Hospital, New York, N.Y. Species identification of each isolate was verified by a rapid sugar fermentation method (1). All isolates were stored at −70°C until used.

The Gonochek II system. The Gonochek II system was

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TABLE 1. Comparison of five test systems for the identification of pathogenic *Neisseria* species

Organism (no. of isolates)	No. (%) correctly indentified by[a]:				
	Minitek	RapID NH	API NeIdent	Gonochek II	Phadebact GC
N. gonorrhoeae (39)	29 (74.3)[b] 4 (10.2)[c]	39 (100)	32 (82.1)	39 (100)	32 (82.1) 5 (12.8)[d]
N. meningitidis (22)	18 (81.8)	20 (90.9)	12 (54.5)	21 (95.4)	0
N. lactamica (12)	12 (100)	11 (91.6)	6 (50.0)	12 (100)	5 (41.6)
B. catarrhalis (17)	15 (88.2)	2 (11.8) 13 (76.5)[c]	16 (94.1)	17 (100)	0
% Correct identification	86.6	80	73.3	98.8	94.9

[a] Species identification was established by the modified rapid fermentation test of Kellog and Turner as modified by Brown (1).
[b] 4 h.
[c] 22 h.
[d] Repeat tested.
[c] Multiple identification.

used according to the instructions of the manufacturer. Briefly, 3 drops of sterile H_2O was added to Gonochek II tubes to dissolve the substrates. Five to ten colonies of each organism to be identified were scraped from an 18-h-old culture (chocolate agar) and inoculated into Gonochek II tubes. The caps of the Gonochek II tubes were tightly sealed, and tubes were incubated at 37°C for 30 min. After incubation, the tubes were examined for the presence of either a blue or a yellow color. Prussian blue was indicative of the activity of β-galactosidase specific for *N. lactamica*. On the other hand, yellow was indicative of the activity of gamma-glutamyl aminopeptidase (GGA) specific for *N. meningitidis*. If neither of these colors was present, 2 drops of the E-Y 20 reagent (reconstituted by the addition of 1.0 ml of sterile H_2O) was added to the tube. Formation of a pink color immediately after the addition of the diazonium salt indicated the activity of prolyl iminopeptidase specific for the identification of *N. gonorrhoeae*. Lack of a pink color after the addition of the E-Y 20 reagent constituted a presumptive identification of *B. catarrhalis*.

Comparative test systems. The API NeIdent, Minitek, and RapID NH systems and the Phadebact GC test were used as previously described, according to instructions of the respective manufacturers (2, 5, 7, 8). The test kits were stored at 4°C until used. All test systems were used to identify 18- to 24-h-old cultures grown on chocolate agar plates at 37°C.

Quality control. *N. gonorrhoeae* ATCC 9828, *N. meningitidis* ATCC 13090, *N. lactamica* ATCC 23970, *B. catarrhalis* ATCC 25240, and a clinical isolate of *Neisseria sicca* were used with each test run to control the performance of each test system.

Cost analysis. A comparative analysis was performed to determine the cost per test with each system. All media, reagents, and ancillary supplies used in these tests were obtained commercially. For cost comparison purposes, we used only the list prices of these supplies. The direct cost of labor for performing a single test was calculated on the basis of the actual time required to perform an identification by each test system.

RESULTS

Table 1 shows the percentages of correct identification of pathogenic *Neisseria* species by each test system. The Gonochek II system correctly identified 100% of *N. gonorrhoeae* strains, 100% of *N. lactamica* strains, 100% of *B. catarrhalis* strains, and 95.4% of *N. meningitidis* strains. One strain of *N. meningitidis* was misidentified as *N. gonorrhoeae* because of a negative reaction for GGA activity.

The Minitek system identified 74.3% of *N. gonorrhoeae* strains in 4 h. Four strains (10.2%) required an additional 18 h of incubation for a positive identification. Of the six strains of *N. gonorrhoeae* misidentified, two were identified as *B. catarrhalis* because of a false-negative glucose test and four were identified as *N. meningitidis* because of a false-positive maltose test. The Minitek system correctly identified 100% of *N. lactamica* strains, 88.2% of *B. catarrhalis* strains, and 81.8% of *N. meningitidis* strains. Four (18.2%) *N. meningitidis* strains were misidentified, of which two were identified as *N. gonorrhoeae* or *B. catarrhalis* because of failure to utilize maltose or both glucose and maltose. The remaining two strains were identified as *N. lactamica*. The Minitek system correctly identified 86.6% of all the pathogenic *Neisseria* species tested in this study.

The RapID NH system identified 100% of *N. gonorrhoeae* strains, 90.9% of *N. meningitidis* strains, 91.6% of *N. lactamica* strains, and 11.8% of *B. catarrhalis* strains. Of the two strains (9.1%) of *N. meningitidis* misidentified, one failed to give a valid profile number and the other was identified as *N. gonorrhoeae* because of a false-negative gamma-glutamyl transpeptidase test. One strain (8.3%) of *N. lactamica* was misidentified as *N. gonorrhoeae* because of a negative *o*-nitrophenyl-β-D-galactopyranoside test. Thirteen *B. catarrhalis* strains (76.5%) gave profile numbers for *Moraxella* spp. and *B. catarrhalis*, and two strains were identified as either *Actinobacillus actinomycetemcomitans* or *N. gonorrhoeae*.

The API NeIdent system correctly identified 82.1% of *N. gonorrhoeae* strains, 54.5% of *N. meningitidis* strains, 50% of *N. lactamica* strains, and 94.1% of *B. catarrhalis* strains. Seven *N. gonorrhoeae* strains (17.9%) were misidentified as either *Neisseria subflava* or *Neisseria* species other than gonococcus because of false-positive glycyl phenylalanine aminopeptidase and glycyl proline test results. Of the 10 *N. meningitidis* strains (45.4%) misidentified, 5 were identified as *N. subflava*, 1 as *N. gonorrhoeae*, and 4 as other *Neisseria* species. Six *N. lactamica* strains (50%) were misidentified as *N. subflava*. One *B. catarrhalis* strain (5.8%) was identified as *Neisseria flavescens* because of false-positive resazurin reduction and gamma-glutamyl transferase reactions. The *N. gonorrhoeae* and *N. lactamica* strains misidentified as *N. subflava* gave profile numbers not listed in the API code compendium. Their identification was established in a call to the API computer center.

TABLE 2. Cost per identification

Expense	Cost ($) per identification by following system:				
	Gonocheck II	Minitek[a]	API NeIdent	RapID NH	Phadebact GC
Purity chocolate agar plate		0.35	0.35	0.35	
Strip or panel[b]			1.66	2.00	
Phadebact GC test[c]					1.26
Gonocheck II	1.85				
Ancillary					
Sterile swab		0.01	0.01	0.01	
Disposable inoculating loop	0.03				0.03
Pipette			0.04	0.04	0.04
Pipette tips		0.80			
Carbohydrate disks		0.48			
Minitek plates		0.01			
Neisseria broth		0.62			
Spot indole				0.01	
Nitrate reagents				0.02	
RapID NH inoculating fluid				0.30	
Glass tubes					0.14
Labor[d]	0.23	0.90	0.54	0.90	0.61
Total cost per test	2.11	3.17	2.60	3.63	2.08

[a] For the Minitek system, the use of a humidor ($7.50), dispenser ($62.62), and pipetter ($88.27) was not included in the direct cost.
[b] API NeIdent $24.75/15 tests, RapID NH, $40/20 tests.
[c] Phadebact GC test, $63/50 tests.
[d] The times required to perform an identification were 1.3 min (Gonocheck II), 5 min (Minitek), 3 min (API NeIdent), 5 min (RapID NH), and 3.4 min (Phadebact GC).

The Phadebact GC test correctly identified 94.9% of *N. gonorrhoeae* strains. Five strains (12.8%) required repeat testing because of equivocal reaction or agglutination in the negative control. Two other strains remained negative upon subsequent testing. Although none of the *N. meningitidis* or *B. catarrhalis* strains cross-reacted with the Phadebact reagent, five *N. lactamica* strains (41.6%) gave strong cross-reactivity with the Phadebact test.

The overall costs were calculated on the basis of direct costs of kits, ancillary supplies, and labor required to run a single test (Table 2). As indicated above, all media, reagents, and supplies were obtained commercially. The cost per test was estimated to be $3.17 (Minitek), $2.60 (API NeIdent), $3.63 (RapID NH system), $2.08 (Phadebact GC), and $2.11 (Gonochek II).

DISCUSSION

Our results indicate that the Gonochek II system is more sensitive than the Minitek, RapID NH, and API NeIdent systems and the Phadebact GC test for the identification of pathogenic *Neisseria* species. Of the 90 strains of pathogenic *Neisseria* species tested, the Gonochek II system correctly identified 89 (98.8%) strains. The sensitivity and specificity of Gonochek II in our hands was similar to that reported by Welborn et al., who found it to be 100% sensitive and specific for the identification of pathogenic *Neisseria* species (9). The Gonochek II misidentified one *N. meningitidis* strain as *N. gonorrhoeae* because of a negative GGA reaction. Recently, Hoke and Vedros demonstrated that the presence of GGA is specific for *N. meningitidis* and differentiates this organism from *N. gonorrhoeae* (3). They also showed that an *N. gonorrhoeae* strain which utilized glucose, maltose, and sucrose was negative for GGA activity (4). In a study of 26 fresh clinical isolates of *N. meningitidis* in which lectins and chromogenic substrates were used, Yajko et al. found a 100% positive result for GGA activity (10). It appears, therefore, that GGA is a specific marker for *N. meningitidis* and is more reliable than maltose utilization for species identification.

We found that the single-tube configuration of the Gonochek II system makes it easier to handle in the clinical laboratory, requires less than 2 min of personnel time to set up, and allows its use on colonies isolated on the primary plate. The colors generated in the Gonochek II system are distinct, easy to read, and stable. All of these features, in our opinion, make it a very attractive system for rapid identification of *N. gonorrhoeae* from urogenital cultures. The manufacturer recommends that the Gonochek II system be inoculated with 5 to 10 colonies. We observed that with some strains of *N. gonorrhoeae* and *N. lactamica*, as few as three colonies produced clear-cut reactions. The size of the inoculum is strain dependent, and we therefore recommend that the Gonochek II system be used according to the instructions of the manufacturer. The Gonochek II system is not designed to identify all *Neisseria* species. It is formulated to aid in the identification of pathogenic *Neisseria* species recovered on a selective medium. Because many nongonococcal *Neisseria* species produce prolyl iminopeptidase, it is essential that the test organism be evaluated for its ability to grow on a selective medium or that it be recovered on such a medium to avoid misidentification of *Neisseria* species.

The Minitek system correctly identified 86.6% of patho-

genic *Neisseria* species evaluated in this study. The performance of the Minitek system in our hands was similar to the experience of other workers (8). One major disadvantage with the Minitek system is that isolates giving a negative reaction in 4 h must be incubated overnight before final scoring. Consequently, in the laboratory *B. catarrhalis* isolates from urogenital specimens would necessarily by identified after overnight incubation. Other disadvantages with the Minitek system include the need for a large inoculum, the use of a pure subcultured isolate which further delays the identification process, and the need for other expendables such as plastic trays, disks, dispenser, broth, and humidor. Furthermore, the Minitek reactions are not always clear-cut, vary from lot to lot, and often lead to misinterpretation.

The RapID NH system correctly identified 100% of the *N. gonorrhoeae* strains in 4 h. Of the 22 strains of *N. meningitidis* tested, only 20 were correctly identified by the RapID NH system. The *N. meningitidis* identification failure with this system was related to difficulties with the detection of proline aminopeptidase or 5-β-glutamyl aminotranspeptidase activity or both. The RapID NH system correctly identified 2 of 17 *B. catarrhalis* strains. Thirteen isolates were coded for *Moraxella* species and *B. catarrhalis*, requiring a Gram stain to differentiate between these species. In an evaluation of the RapID NH system, Robinson and Oberhofer found that only 1 of 23 *B. catarrhalis* strains was correctly identified, whereas the remaining strains were identified as *Moraxella* or *Kingella* spp. (7). A major contributing factor to the misidentification of *B. catarrhalis* was the variability in the nitrate reaction.

The API NeIdent system correctly identified 82.1% of *N. gonorrhoeae* strains and 94.1% of *B. catarrhalis* strains. It did not perform satisfactorily in identifying *N. meningitidis* and *N. lactamica* strains (Table 1). The primary reason for failure to identify *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* strains was consistently related to the difficulty in detecting hydroxyproline aminopeptidase, gamma-glutamyl β-naphthylamide transferase, glycyl phenylalanine aminopeptidase, and glycyl proline aminopeptidase activity. In most cases, these reactions were borderline positive in 30 s, which led to the misidentification of these species. In a recent evaluation of the API NeIdent system, Janda et al. found that their failure to identify *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* strains was related to the results of these chromogenic substrate reactions (5). In their study, 14 of 85 *N. gonorrhoeae* strains could not be correctly identified because of a positive glycyl aminopeptidase reaction in less than 30 s. The API NeIdent system needs considerable improvements, and the code compendium needs expansion for improved performance in identifying pathogenic *Neisseria* species.

The Phadebact GC test correctly identified 34 of 39 *N. gonorrhoeae* strains. Five of these strains were negative on the primary test but became positive on retest. In our hands, the sensitivity of the Phadebact GC test (95%) in identifying *N. gonorrhoeae* strains was similar to the experience of other investigators (2, 11). The specificity of the test, however, was compromised by its cross-reactivity with *N. lactamica*. Of the 12 strains of *N. lactamica* tested, 7 were positive by the Phadebact GC test. Recently, Young and Reid reported that 7 of 13 stock cultures and 2 of 3 clinical isolates of *N. lactamica* gave a positive result with the Phadebact GC test (11). Although the incidence of *N. lactamica* in urogenital specimens is less than 1%, its cross-reactivity with the Phadebact GC test affects the specificity of the test.

Table 2 shows the cost of supplies and labor entered into the calculation of cost per test with each test system. The cost of labor was based on the actual time required to set up and read each test system.

The Gonochek II system is an efficient test procedure for the rapid laboratory confirmation of *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* and for presumptive identification of *B. catarrhalis*. It is easy to use, does not require expandables as the other kits do, and fits in very well with the work flow in most clinical laboratories.

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