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Use of the API NeIdent System for Identification of Pathogenic *Neisseria* spp. and *Branhamella catarrhalis*

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The API NeIdent system (Analytab Products, Plainview, N.Y.) was evaluated for identifying *Neisseria* spp. and *Branhamella catarrhalis* commonly isolated from clinical specimens. The system identified 90% of 303 *Neisseria gonorrhoeae* isolates, 71% of 113 *Neissera meningitidis* isolates, and 63% of 16 *Neisseria lactamica* isolates but failed to identify any of 22 *B. catarrhalis* isolates. Testing of gonococcal strains of various auxotypes revealed no relationship between nutritional requirements and NeIdent profile numbers. With the *Neisseria* species, interpretation of the cinnamaldehyde-coupled β-naphthylamine reactions was difficult and resulted in profile numbers not listed in the Profile Register. Positive resazurin-glucose reactions resulted in unlisted numbers for all *B. catarrhalis* strains. Inconsistent results were also obtained when 62 *N. gonorrhoeae* isolates were tested more than once on the strip. In all cases, profile variability and failure to identify these organisms were related to the β-naphthylamide substrate tests. Expansion of the data base and modification of the substrate formulations or their interpretive criteria may increase the reliability of the NeIdent system for identifying *Neisseria* spp. and *B. catarrhalis*.

Most conventional and rapid methods for identifying pathogenic *Neisseria* spp. depend on degradation of appropriate carbohydrates by the organisms (8). These procedures require a pure culture for reliable results, and some strains may behave aberrantly, e.g., glucose-negative gonococci (2, 8) and maltose- and glucose-negative meningococci (4, 8). Consequently, alternative rapid methods for identifying *Neisseria* spp., including coagglutination (1, 8) and the use of chromogenic enzyme substrates such as β-naphthylamine derivatives of amino acids (12), have been developed. Using chromogenic substrates to detect bacterial esterases and aminopeptidases, D'Amato et al. identified stock strains of gonococci and meningococci grown on both selective and nonselective media in 4 h (3).

The API NeIdent system (Analytab Products, Plainview, N.Y.) uses both conventional biochemical and chromogenic enzyme substrate reactions to identify *Neisseria* spp. and neisseria-like organisms of the genera *Branhamella*, *Kingella*, *Moraxella*, and the Centers for Disease Control M groups. We evaluated the API NeIdent system with stock strains and fresh clinical isolates of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, and *Branhamella catarrhalis* to assess its accuracy for identifying these organisms and to determine whether NeIdent profiles were related to gonococcal auxotypes.

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MATERIALS AND METHODS

Stock organisms. The stock strains had been isolated from a variety of clinical material and were maintained frozen at −86°C. These included 102 *N. gonorrhoeae*, 51 *N. meningitidis*, 14 *N. lactamica*, and 22 *B. catarrhalis* strains. All *N. gonorrhoeae* strains had been auxotyped on a modified Catlin medium (9). For testing, the stock strains were subcultured three times on chocolate agar (BBL Microbiology Systems, Cockeysville, Md.) before inoculation into the NeIdent strip.

Clinical isolates. Fresh isolates of *N. gonorrhoeae* (201 isolates), *N. meningitidis* (62 isolates), and *N. lactamica* (2 isolates) were obtained from patients at the University of Chicago Medical Center (UCMC) and from homosexual men at the Howard Brown Memorial Clinic (HBMC) in Chicago. The UCMC specimens were received on JEMBEC plates containing modified Thayer-Martin (MTM) medium (Remel Laboratories, Lenexa, Kans.), or the specimens were plated in the laboratory on chocolate agar and MTM medium (BBL). HBMC specimens were plated on MTM medium (GIBCO Diagnostics, Madison, Wis.). After incubation at 35°C in a CO_2 atmosphere, colonies of oxidase-positive, gram-negative diplococci were subcultured on chocolate agar. All fresh isolates were identified by the rapid carbohydrate fermentation technique (7), and growth from the first or second subculture was used to inoculate the NeIdent test strips. In one part of this study, 62 urethral gonococcal isolates from HBMC patients were tested on the NeIdent strip directly from the primary MTM culture plate. On the following day conventional identification tests and repeat NeIdent tests were performed from pure subcultures grown on chocolate agar. All other isolates were tested only once on the strip.

NeIdent system. The API NeIdent panel consists of 11 miniaturized conventional and chromogenic enzyme substrate tests in 10 microcupules on a plastic strip. Organisms to be identified are suspended in 2 ml of balanced salts; bovine serum albumin buffer to a turbidity equivalent to a no. 3 McFarland standard and 2 or 3 drops of the bacterial suspension are used to inoculate each microcupule. After inoculation, the strips are incubated at 35°C for 4 h in a non-CO_2 atmosphere. After incubation, the reactions are interpreted as follows: β-galactosidase, phosphatase, and proline aminopeptidase activities are detected by the presence of the yellow end products nitrophenol, *p*-nitrophenol, or *p*-nitroaniline released from nitrophenyl or nitroanilide derivatives of β-D-galactoside, phosphate, and proline, respective-

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ly; fructose utilization results in a pH change, turning the cresol red indicator from red to yellow or orange; resazurin reduction in the presence of sodium pyruvate or glucose (RGL) is indicated by a color change from deep blue or purple to lavender, pink, or clear; bacterial aminopeptidase and transferase enzymes are detected by the release of β-naphthylamine from the substrates hydroxyproline β-naphthylamide (HOP), γ-glutamyl β-naphthylamide (GGT), glycylphenylalanine β-naphthylamide (GPA), and glycylproline β-naphthylamide (GPR). The last four reactions are read within 30 s after the addition of a cinnamaldehyde reagent which complexes with any free β-naphthylamine and results in a pink to magenta-red color. The catalase test is performed by adding 3% hydrogen peroxide to the β-galactosidase cupule. From the results of these 11 reactions, a four-digit profile number is generated that is used with the NeIdent Profile Register to identify the organism.

RESULTS

Table 1 summarizes the data for the identification of gonococci, meningococci, *N. lactamica*, and *B. catarrhalis* by the NeIdent strip. The system identified 90% of 303 goncoccal isolates, 71% of 113 meningococcal isolates, and 63% of 16 *N. lactamica* isolates but failed to identify any of 22 *B. catarrhalis* isolates.

Table 2 shows the auxotypes and NeIdent profile numbers of stock strains of *N. gonorrhoeae*. Three of the four possible profile numbers (4412, 4452, 4012, 4052) were obtained. Of all strains tested, only one isolate (Arg-Orn$^+$ auxotype) had a NeIdent profile number that was not listed in the Profile Register (4413). A majority of both prototrophic gonococci (zero auxotype, requires none of the compounds tested) and multiply auxotrophic gonococci (AHU auxotype, requires arginine, hypoxanthine, and uracil) had the same profile number (4412).

For eight of the stock strains, the GPA and RGL color reactions were ambiguous and not clearly positive or negative; however, the correct organism identification was obtained regardless of the interpretation of these tests. Overall, 99% of the stock gonococcal strains were correctly identified.

TABLE 1. Identification of pathogenic *Neisseria* spp. and *B. catarrhalis* by the NeIdent system

Organism	No. tested	No. identified (%)[a]
N. gonorrhoeae		
Stock strains	102	101 (99)
Clinical isolates	201	173 (86)
N. meningitidis		
Stock strains	51	36 (71)
Clinical isolates	62	44 (71)
N. lactamica		
Stock strains	14	10 (71)
Clinical isolates	2	0 (0)
B. catarrhalis (stock strains)	22	0 (0)

[a] Of 303 *N. gonorrhoeae* strains and isolates, 274 (90%) were identified; of 113 *N. meningitidis* strains and isolates, 80 (71%) were identified; of 16 *N. lactimica* strains and isolates, 10 (63%) were identified; of 22 *B. catarrhalis* strains and isolates, none (0%) were identified. Of 454 strains and isolates tested, 364 (80%) were identified.

TABLE 2. Auxotypes and NeIdent profile numbers for stock strains of *N. gonorrhoeae*

Auxotype[a]	No. tested	No. with following NeIdent profile number:			% Identified[b]
		4412	4452	4012	
Zero	33	27	4	2	100
Pro	15	10	2	3	100
Arg-Orn$^+$	13	6	3	3	92
Other[c]	12	6	5	1	100
AHU	29	26	0	3	100

[a] Isolates were tested for their requirements for arginine (Arg), proline (Pro) methionine, histidine, leucine, lysine, valine, hypoxanthine (Hypx), uracil, and thiamine pp$_i$. Orn$^+$ denotes that ornithine could replace the requirement for arginine. AHU strains required arginine, hypoxanthine, and uracil for growth. Zero indicates the lack of a requirement for any of the compounds tested.

[b] Ninety-nine percent of the strains were identified.

[c] Includes seven Arg-Pro strains, four Pro-Hypx strains, and one Arg-Hypx strain.

Table 3 lists the NeIdent profiles of 201 fresh clinical isolates of gonococci from a variety of body sites. All 32 extragenital isolates were identified correctly. Among 22 cervical and 147 male urethral isolates, 86 and 83%, respectively, were identified by NeIdent. Of these fresh clinical isolates, 86% had one of the four numbers currently listed in the Profile Register. Among the 28 isolates that were not identified, 24 (86%) had a positive GPR reaction within 30 s after cinnamaldehyde reagent was added, and 4 (14%) had a negative HOP reaction at 30 s. According to the NeIdent package insert, only 1% of gonococci are GPR positive, and 1% are HOP negative.

To determine the effect of timing on the interpretation of the cinnamaldehyde-coupled tests, 85 fresh clinical isolates of *N. gonorrhoeae* from chocolate agar subcultures were tested on the NeIdent strip. Reactions in the HOP, GGT, GPA, and GPR microcupules were recorded at 15 and 30 s after the addition of cinnamaldehyde reagent. Of the isolates, 57% produced a valid profile number at both 15 and 30 s after the reagent addition, and 22% were correctly identified only at 30 s (Table 4). Therefore, 79% of the strains were identified as gonococci when the directions of the manufacturer were followed. On the other hand, 9% of the strains produced a valid profile number at 15 but not at 30 s. In these cases, a positive GPR reaction developed at between 15 and 30 s, resulting in a nonvalid profile number. Ten strains (12%) were not identified at either reading time: six developed a positive GPR reaction at between 0 and 15 s, and four had a negative HOP reaction at 30 s. Similar results were obtained when 62 of these 85 strains were tested directly from MTM primary cultures; 64% had valid profile numbers after 15 and 30 s and 13% had valid profile numbers at 30 but not at 15 s after the reagent addition (data not shown).

Sixty-two urethral isolates of *N. gonorrhoeae* were tested on NeIdent from primary cultures with sufficient growth and from chocolate agar subcultures to assess profile reproducibility. Of these, 57 were identified. Of these 57, 24 produced the same profile in both tests, and 14 produced different profiles which still resulted in correct identifications. Ten were identified when tested from the primary culture but not the subculture, and for nine isolates only growth from the subculture provided a correct identification. Five strains produced noninterpretable profile numbers when tested from both the primary growth and the subculture. Profile differences and failure to identify the organisms were always related to the cinnamaldehyde-coupled tests.

TABLE 3. Fresh clinical isolates of *N. gonorrhoeae* identified with the NeIdent system

Site of isolation	No. of isolates	No. of isolates with following gonococcal profile number:				No. identified (%)[a]	No. of isolates with following unlisted profile number:		
		4412	4452	4012	4052		4413[b]	4453[b]	4442[c]
Oropharynx	8	5	2	0	1	8 (100)	0	0	0
Rectum	19	14	5	0	0	19 (100)	0	0	0
Other[d]	5	5	0	0	0	5 (100)	0	0	0
Cervix	22	8	5	4	2	19 (86)	2	1	0
Urethra	147	73	44	1	4	122 (83)	8	13	4

[a] Of 201 isolates, 173 (86%) were identified.
[b] GPR reaction positive at 30 s.
[c] HOP reaction negative at 30 s.
[d] Includes two isolates from blood, one isolate from joint fluid, one isolate from amniotic fluid, and one isolate from an intrauterine device.

For both stock and clinical isolates of *N. meningitidis*, problems were also encountered with the β-naphthylamine reactions. The NeIdent system correctly identified 80 of 113 strains (Table 1), all of which appropriately had a positive GGT reaction only. Thirty-two strains were not identified because one, two, or three β-naphthylamine reactions were positive in addition to the GGT reaction. Only one unidentified isolate was GGT negative. Among 16 *N. lactamica* stock strains, 10 were identified correctly. Again, the β-naphthylamine reactions were responsible for unlisted profile numbers.

None of the 22 *B. catarrhalis* strains was identified correctly. All were RGL positive, rather than the expected RGL negative, resulting in profile numbers not listed in the Profile Register. In contrast to the results with *Neisseria* spp., the β-naphthylamine reactions produced by *B. catarrhalis* were easily interpreted and corresponded with the expected results.

DISCUSSION

The ability of the API NeIdent system to identify both stock strains and clinical isolates of *Neisseria* spp. and *B. catarrhalis* was evaluated. Of the 454 stock strains and clinical isolates tested, 80% were correctly identified. The strip performed best with *N. gonorrhoeae* (90% identified) and less well with *N. meningitidis* (71%) and *N. lactamica* (63%); it did not identify any of the *B. catarrhalis* strains.

Of the 11 tests on the NeIdent strip, 7 were almost always clear-cut and easy to read. The four reactions that require the addition of cinnamaldehyde reagent, however, were often difficult to interpret or became falsely positive within the 30-s reading time. Failure to identify *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* was consistently related to the results of these chromogenic substrate reactions. For all *Neisseria* strains tested, the time required for color development varied considerably. For example, failure to identify 14 of 85 *N. gonorrhoeae* strains resulted from a positive GPR reaction in less than 30 s (Table 4). Inconsistent results were also obtained when a number of gonococcal isolates were tested more than once; that is, the profile numbers were not reproducible. The problems encountered with the β-naphthylamine amino acid derivatives may be related to differences in bacterial enzyme levels or in enzyme kinetics or to instability of these derivatives on the strip.

Another explanation may be the local prevalence of gonococci and meningococci that exhibit enzymatic abilities different from those strains included in the NeIdent data base. Most GPR-positive and all HOP-negative *N. gonorrhoeae* isolates were isolated from homosexual men at the HBMC rather than from UCMC patients (6).

In contrast to the *Neisseria* strains, *B. catarrhalis* strains were not identified because the resazurin reduction test (RGL), which is not a chromogenic substrate test, was always positive rather than the expected negative. Despite discrepant reactions, the profile numbers of the unidentified *Neisseria* and *B. catarrhalis* strains were never those of the other organisms identified by NeIdent. Thus, they were never misidentified as nonpathogenic *Neisseria* spp. or organisms belonging to closely related but different genera. The system may be improved by expansion of the NeIdent data base, as well as by modifications of substrate formulations and of interpretative criteria for the cinnamaldehyde-coupled reactions.

No relationship was observed between nutritional requirements of the gonococcal strains as determined by auxotyping and degradation of the NeIdent substrates. Both fastidious AHU strains and non-AHU auxotypes produced the same profile numbers; therefore, enzymatic differences between prototrophic and auxotrophic gonococci were not reflected in the NeIdent reactions. In their study, D'Amato et al. found no direct correlation between amino acid requirements and aminopeptidase activity (3). The NeIdent system readily identified AHU gonococci, which often produce acid from glucose slowly in conventional identification media (9).

Although the NeIdent system identified 90% of both genital and extragenital gonococci, further refinements are needed to improve this performance and to increase its reliability for identifying the other pathogenic *Neisseria* spp. and *B. catarrhalis*. In comparison to conventional methods,

TABLE 4. Gonococcal identification by the NeIdent system at 15 and 30 s after the addition of cinnamaldehyde reagent[a]

Identification at 15 s/30 s	No. of isolates (%)	Discrepant reactions
N. gonorrhoeae/*N. gonorrhoeae*	48 (57)	None
No identification/*N. gonorrhoeae*	19 (22)	None
N. gonorrhoeae/no identification	8 (9)	GPR positive at 15–30 s
No identification/no identification	6 (7)	GPR positive at 0–15 s
No identification/no identification	4 (5)	HOP negative at 30 s

[a] All non-β-naphthylamine reactions were read before the cinnamaldehyde addition.

organisms may be inoculated into the NeIdent strip directly from the primary isolation media, and the results are obtained in 4 h. Other methods, however, such as coagglutination for gonococci and rapid carbohydrate degradation tests, also provide rapid identification and do not require interpretation of equivocal colorimetric endpoints. (1, 2, 10, 11). A potential advantage of the NeIdent system is the identification of nonneisserial organisms such as *Moraxella* and *Kingella* species that may be confused with *N. gonorrhoeae* (5). This would be of particular value for isolates from nongenital sites.

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ADDENDUM IN PROOF

Since the acceptance of this manuscript for publication, Analytab Products, Inc. has expanded the data base for the NeIdent system. *N. gonorrhoeae* strains which produce a 4013 or a 4413 profile number are now listed in the Profile Register included with the kit. The API Computer Service provides some assistance in the identification of organisms with other unlisted profiles. A color interpretation chart for the tests on the strip is also included with the kit.

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