

Identification of *Neisseria* spp., *Haemophilus* spp., and Other Fastidious Gram-Negative Bacteria with the MicroScan *Haemophilus-Neisseria* Identification Panel

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The *Haemophilus-Neisseria* identification (HNID) panel (American MicroScan, Sacramento, Calif.) is a 4-h microdilution format system for identification of *Haemophilus* and *Neisseria* spp., *Branhamella* (*Moraxella*) *catarrhalis*, and *Gardnerella vaginalis*. The HNID panel was evaluated by using 423 clinical isolates and stock strains of these organisms, and HNID identifications were compared with those obtained by conventional methods. In addition, 32 isolates representing six genera not included in the HNID data base were tested to determine whether these organisms would produce unique biotype numbers for possible inclusion in the data base. The HNID panel correctly identified 95.3% of 86 *Neisseria gonorrhoeae* strains, 96% of 25 *G. vaginalis* strains, and 100% of 28 *Neisseria lactamica* strains and 48 *B. catarrhalis* strains. Only 64.7% of 68 *Neisseria meningitidis* isolates were identified correctly owing to false-negative or equivocal carbohydrate and/or aminopeptidase reactions. Among the *Haemophilus* spp., 98.8% of 83 *H. influenzae* strains, 97.1% of 34 *H. parainfluenzae* strains, and 80% of 15 *H. aphrophilus* and *H. paraphrophilus* strains were correctly identified. Eight strains of *Neisseria cinerea*, a species not included in the data base, produced profiles identical with those for *B. catarrhalis* and *N. gonorrhoeae*. Isolates of other species not included in the data base, including *Eikenella corrodens*, *Kingella* spp., and *Cardiobacterium hominis*, produced unique biochemical reaction patterns on the panel. Modification of interpretative criteria for certain tests, expansion of the data base to include other species, and suggestions for additional confirmatory tests will increase the accuracy and utility of the HNID panel.

Methods for identifying fastidious microorganisms in the clinical laboratory include time-consuming conventional procedures and rapid techniques employing modified conventional biochemical tests or novel chromogenic substrates. Several commercial multitest identification systems have been marketed in recent years for the identification of *Neisseria* spp., *Haemophilus* spp., other fastidious gram-negative organisms, and anaerobes (1, 3, 4, 6, 8, 11-15, 22, 24-27). These kit systems allow the rapid determination of a variety of biochemical characteristics and enable laboratories to reliably identify not only the commonly isolated species belonging to these genera but also some of the less frequently encountered clinically significant microorganisms, such as *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Gardnerella vaginalis* (13). Biochemical reactions in these systems are used to generate numerical profiles that are compared with the computerized data bases of the systems to identify the organism.

The *Haemophilus-Neisseria* identification (HNID) panel (American MicroScan, Sacramento, Calif.) is a microdilution system for identifying *Neisseria* spp., *Branhamella* (*Moraxella*) *catarrhalis*, *Haemophilus* spp., and *G. vaginalis*. In this study, the HNID panel was evaluated for its ability to identify clinical isolates and stock strains of these organisms. Identifications obtained with the HNID panel were compared with those provided by conventional methods. In addition, other fastidious gram-negative bacteria currently not included in the HNID panel data base were

also tested to determine whether the biochemical reactions on the panel would provide unique biotype patterns, thereby allowing expansion of the data base and increasing the utility of the HNID panel for use in the clinical microbiology laboratory.

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MATERIALS AND METHODS

HNID panel. The HNID panel is a microdilution tray system that uses chromogenic enzyme substrates and modified conventional tests for the 4-h identification of *Neisseria* spp., *Haemophilus* spp., and *G. vaginalis*. Tests included on the panel are as follows: hydrolysis of indoxyl phosphate (IDX); reduction of nitrate (NO₃) and nitrite (NO₂); production of acid from glucose (GLU), sucrose (SUC), maltose (MAL), fructose (FRU), and lactose (LAC); hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (GAL); production of urease (URE), ornithine decarboxylase (ORN), and indole (IND); hydrolysis of L-prolyl-β-naphthylamide (PRO), γ-glutamyl-α-naphthylamide (NGL), benzoyl-DL-arginine-β-naphthylamide (ZAR), and *p*-nitrophenyl-α-D-glucoside (AGL); acid production from starch (STA); and an acidometric test for β-lactamase production (BL).

To inoculate the panel, a suspension of the organism equivalent to a McFarland no. 3 turbidity standard is prepared in the inoculum broth provided with the panel. This suspension is prepared from a pure culture grown on a suitable medium, such as blood or chocolate agar. Each of the 18 test wells on the panel is inoculated with 50 μl of the

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organism suspension, the urease well is overlaid with sterile mineral oil, and the panel is incubated for 4 h at 35°C in a non-CO₂ incubator. Color reactions are read either immediately or after the addition of reagents. Those tests requiring reagent addition include the NO₃ and NO₂ reduction tests (nitrate reagents A and B), the IND test (Kovac's indole reagent), and the PRO, NGL, and ZAR aminopeptidase tests (cinnamaldehyde reagent). Positive and negative results for each of the HNID tests are interpreted and scored as recommended by the manufacturer, and a six-digit biotype number is generated. Identifications are obtained by consulting the HNID biotype code book.

During this evaluation, individual tests were interpreted as positive, negative, or equivocal. Biotypes were generated that included combinations of interpretations, and all biotype numbers were looked up in the code book. Identifications having 90% or greater likelihood were considered correct. Identifications of isolates for which one or several HNID tests were difficult to interpret were scored as equivocal identifications. Isolates that produced code numbers providing low-probability (less than 90%) identifications or that resulted in unlisted code numbers were considered to be unidentified by the HNID panel. Isolates having biotype numbers that resulted in incorrect identifications were considered misidentified by the HNID panel.

Organisms. The 455 organisms tested in this study were clinical isolates (417 organisms) or stock strains (38 organisms) derived from clinical sources. Most were obtained from specimens submitted to the microbiology laboratory of the University of Illinois Hospital. Many gonococcal and meningococcal isolates were from specimens obtained from patients attending the Howard Brown Memorial Clinic, a private sexually transmitted diseases clinic in Chicago. Organisms were recovered from a variety of specimen types, including endocervical, urethral, and rectal cultures, blood and cerebrospinal fluid, respiratory tract cultures, wound and abscess cultures, vaginal cultures, conjunctival cultures, and cultures of other sterile body fluids (e.g., peritoneal fluid and prostatic fluid). Stock strains were maintained frozen at -70°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.)-decomplemented horse serum (Remel, Lenexa, Kans.) (1:1) and were subcultured at least three times before testing. Inocula for the HNID panel and for conventional tests were prepared from 18- to 24-h blood or chocolate agar (GIBCO Diagnostics, Madison, Wis.) subcultures, except for *G. vaginalis* strains, which were subcultured on human blood-Tween 80 bilayer medium (GIBCO Diagnostics) (28). Testing by conventional procedures and by using the HNID panel was performed in a blinded fashion. Identifications obtained with each procedure were subsequently compared, and isolates with discrepant identifications were retested by both methods.

Conventional methods. Conventional identification tests for *Neisseria* spp. and *B. catarrhalis* included the Gram stain; oxidase and catalase tests; growth on modified Thayer-Martin medium (GIBCO Diagnostics); production of acid from glucose, maltose, sucrose, fructose, and lactose; reduction of nitrate; DNase production; susceptibility to colistin (10-μg disk); and β-lactamase production in the chromogenic cephalosporin spot test (BBL Microbiology Systems) (5, 17, 20, 21-23). *G. vaginalis* isolates were identified on the basis of the Gram stain, oxidase and catalase tests, growth and hemolysis on human blood-Tween 80 bilayer medium, production of acid from glucose, maltose, mannitol, and starch, and hydrolysis of hippurate (9, 28). *Haemophilus* spp. and other fastidious gram-nega-

TABLE 1. Identification of *Neisseria* spp., *B. catarrhalis*, and *G. vaginalis* isolates with the MicroScan HNID panel

Organism(s)	No. tested	No. (%) of isolates:			
		Identified ^a	Equivocal ^b	Not identified ^c	Misidentified ^d
<i>N. gonorrhoeae</i>	86	82 (95.3)	3 (3.5)	1 (1.2)	0
<i>N. meningitidis</i>	68	44 (64.7)	16 (23.5)	5 (7.4)	3 (4.4)
<i>N. lactamica</i>	28	28 (100)	0	0	0
<i>Neisseria</i> spp. ^e	36	28 (77.8)	0	7 (19.4)	1 (2.8)
<i>B. catarrhalis</i>	48	48 (100)	0	0	0
<i>G. vaginalis</i>	25	24 (96.0)	0	1 (4.0)	0

^a Organism correctly identified with greater than 90% probability.

^b Multiple equivocal reactions resulting in low-probability identification prevent reliable species designation.

^c Biotype code numbers not included in the data base of the system, preventing organism identification.

^d Incorrect species identification provided by the data base.

^e Includes 30 *N. subflava*, 4 *N. mucosa*, and 2 *Neisseria sicca* strains.

tive bacilli were identified on the basis of the following tests: Gram stain; oxidase and catalase tests; growth and hemolysis on 5% sheep blood agar and on 5% horse blood agar (GIBCO Diagnostics); requirements for X and V factors; the aminolevulinic acid-porphyrin test; requirement of CO₂ for growth; production of β-lactamase; reduction of nitrate; production of urease, ornithine decarboxylase, and indole; and production of acid from glucose, maltose, sucrose, lactose, mannitol, mannose, xylose, and ribose (1, 10, 16, 18-20).

RESULTS

Table 1 shows the results obtained by using the HNID panel for the identification of *Neisseria* spp., *B. catarrhalis*, and *G. vaginalis*. Of 86 gonococcal strains, 3 (3.5%) produced weak, equivocal PRO reactions; regardless of the interpretation of this single test, however, these strains were still identified as *N. gonorrhoeae* on the basis of positive GLU reactions. One strain (1.2%) was not identified (*N. gonorrhoeae*, 86.3%/*B. catarrhalis*, 13.9%). If the strains producing equivocal PRO reactions are included among the correctly identified isolates, then 98.8% of the gonococci were correctly identified with the HNID panel. Among the meningococci, only 44 (64.7%) of 68 isolates produced reactions on the HNID panel that allowed an unequivocal identification of *Neisseria meningitidis*. For 16 strains (23.5%), combinations of the results of several key biochemical tests, including GLU, MAL, PRO, and NGL, were negative on the basis of the interpretive criteria for the panel. Five (7.4%) meningococcal strains were not identified; two were identified as *N. meningitidis*, 53.6%/*Neisseria* spp., 46.3%; one was identified as *Neisseria gonorrhoeae*, 86.6%/*B. catarrhalis*, 13.9%; and two produced unlisted biotype numbers. Three strains were misidentified as *N. gonorrhoeae* on the basis of positive GLU and PRO reactions and negative MAL and NGL reactions.

Among the other *Neisseria* spp. tested, 7 (19.4%) of 36 saprophytic isolates were not adequately distinguished from pathogenic species; 5 *N. subflava* isolates were identified as *N. gonorrhoeae*, 86.6%/*B. catarrhalis*, 13.9%; 1 was identified as *N. meningitidis*, 53.6%/*Neisseria* spp., 46.3%; and 1 produced an unlisted biotype number. One *N. subflava* isolate was misidentified as *N. gonorrhoeae*. All *Neisseria lactamica* and *B. catarrhalis* isolates were correctly identified. Of the 25 *G. vaginalis* isolates tested, only 1 produced an unlisted biotype number.

TABLE 2. Identification of *Haemophilus* spp. with the MicroScan HNID panel

Organism	No. tested	No. (%) of isolates ^a :		
		Identified	Not identified	Misidentified
<i>H. influenzae</i>	83	82 (98.8)	0	1 (1.2)
Biotype I	26	26 (100)	0	0
Biotype II	32	32 (100)	0	0
Biotype III	14	14 (100)	0	0
Biotype IV	8	7 (87.5)	0	1 (12.5)
Biotype V	2	2 (100)	0	0
Biotype VI	1	1 (100)	0	0
<i>H. parainfluenzae</i>	34	33 (97.1)	1 (2.9)	0
Biotype I	11	11 (100)	0	0
Biotype II	16	16 (100)	0	0
Biotype III	6	6 (100)	0	0
Biotype IV	1	0	1 (100)	0
<i>H. aphrophilus</i>	8	6 (75.0)	0	2 (25.0)
<i>H. paraphrophilus</i>	7	6 (85.7)	0	1 (14.3)

^a See Materials and Methods and the footnotes to Table 1 for an explanation of the subheadings. No *Haemophilus* isolate identifications were equivocal on the basis of the HNID panel.

Of the 48 *B. catarrhalis* strains, 39 were β -lactamase positive on the basis of the chromogenic cephalosporin test; 3 of the 86 *N. gonorrhoeae* strains were β -lactamase positive on the basis of the chromogenic cephalosporin test. The acidometric BL test on the HNID panel detected all of these strains.

Table 2 shows the HNID results for 132 strains of *Haemophilus* spp. A single biotype IV *Haemophilus influenzae* strain was misidentified as a biotype III strain because of a false-negative ORN reaction on the panel. A *Haemophilus parainfluenzae* biotype IV strain produced an unlisted code number, although all three biotyping reactions (i.e., URE, ORN, and IND) were the same by conventional methods. Two *Haemophilus aphrophilus* strains and one *Haemophilus paraphrophilus* strain were misidentified as *H. parainfluenzae* biotype IV because of false-negative LAC test results on the panel.

Six biotype I, eight biotype II, and one biotype V *H. influenzae* strains and one biotype I *H. parainfluenzae* strain were β -lactamase positive. All of these strains were detected by the BL test on the HNID panel.

Table 3 shows the results obtained for 32 isolates representing six genera not currently included in the HNID data base. Eight *Neisseria cinerea* strains generated biotypes that were identical to those for *B. catarrhalis* (six strains) or for low-confidence identifications between *N. gonorrhoeae* and *B. catarrhalis* (two strains). All but one of the *E. corrodens* strains tested produced unique biotype numbers; the NO₃, ORN, and PRO reactions were usually positive. The *E. corrodens* strain that was only NO₃ and PRO positive was called *B. catarrhalis*. All *Kingella* spp., *C. hominis*, and *A. actinomycetemcomitans* strains were biochemically active with the substrates on the HNID panel. One of two *Kingella denitrificans* isolates, one *Kingella kingae* isolate, and both *C. hominis* isolates produced unique, unlisted biotype numbers. The three *A. actinomycetemcomitans* strains produced reaction patterns that were identical to those of *Neisseria mucosa*.

When stock strains and clinical isolates were compared, no differences in the distribution of discrepant biochemical activities on the HNID panel were noted for any of the organism groups included in the study. As described above, discrepant reactions were associated more with individual isolates than with the source of the isolates. Those strains that were misidentified or not identified with the panel because of aberrant individual biochemical tests showed similar results on repeat testing, again indicating the sensitivity of the HNID panel to the biochemical activity of individual isolates among certain groups of organisms.

DISCUSSION

The MicroScan HNID panel is one of several commercially available systems for the identification of fastidious, clinically significant microorganisms. Most of the organisms in the current data base were identified with likelihood probabilities of greater than 98%. Many isolates of *N. meningitidis*, however, produced reactions that could not be interpreted on the basis of the test reading criteria and consequently were the most frequently misidentified and unidentified organisms encountered during the study. While most *N. meningitidis* isolates were positive in the GLU, MAL, PRO, and NGL tests, several were GLU negative, MAL negative, or both. The MAL reaction was often red-orange rather than yellow. The NGL aminopeptidase reaction, another key test for identification of *N. meningitidis*, was frequently orange rather than red or magenta, the color of a positive reaction according to the manufacturer. Modifications of the interpretive criteria for distinguishing

TABLE 3. HNID panel reactions for other fastidious bacteria not included in the HNID data base

Organism	No. tested	Positive HNID panel reactions ^a	No. with indicated positive reactions	Current data base identification (ID)
<i>N. cinerea</i>	8	NO ₂ , PRO PRO	6 2	<i>B. catarrhalis</i> 97.3%/ <i>N. gonorrhoeae</i> 2.6%
<i>E. corrodens</i>	16	NO ₃ , ORN, PRO NO ₃ , ORN ORN, PRO NO ₃ , PRO	13 1 1 1	No ID listed No ID listed No ID listed <i>B. catarrhalis</i> 99.9%
<i>K. denitrificans</i>	2	NO ₃ , NO ₂ , PRO NO ₃ , NO ₂ , GLU, PRO	1 1	<i>B. catarrhalis</i> 99.9% No ID listed
<i>K. kingae</i>	1	IDX, NO ₂ , GLU, MAL, PRO	1	No ID listed
<i>C. hominis</i>	2	NO ₂ , GLU, SUC, MAL, FRU, IND, NGL, ZAR	2	No ID listed
<i>A. actinomycetemcomitans</i>	3	NO ₃ , NO ₂ , GLU, MAL, FRU	3	<i>N. mucosa</i> 99.9%

^a See Materials and Methods for an explanation of the reaction abbreviations.

positive and negative reactions, particularly for the PRO, NGL, and ZAR aminopeptidase reactions, would probably decrease the numbers of equivocal identifications. Since the ZAR reaction was negative for all of the *Neisseria* spp. tested, this reaction could serve as a negative control for comparison with the PRO and NGL reactions of individual isolates. Use of an inoculum with a turbidity greater than a no. 3 McFarland turbidity standard may also decrease the number of equivocal test interpretations, particularly with the carbohydrate utilization tests. False-negative MAL, SUC, and FRU carbohydrate test results were largely responsible for the failure of the panel to adequately distinguish a number of the saprophytic *Neisseria* spp. from the pathogenic species.

The HNID panel generally provided reliable identifications for *Haemophilus* spp. The three biotyping reactions—urease, ornithine decarboxylase, and indole—were identical to those obtained with the conventional procedure for all isolates except for a single biotype IV *H. influenzae* isolate, for which the conventional ornithine decarboxylase test was positive and the HNID reaction was negative. The biotype IV *H. parainfluenzae* isolate was not identified with the HNID panel because of the data base of the system. The data base contains the reactions of *H. parainfluenzae* biotype IV strains described by Oberhofer and Back (24), for which all three biotyping reactions are negative, instead of those described by Kilian (16), for which all three reactions are positive. Hence, the single Kilian biotype IV strain included in the present study was not identified even though all HNID reactions corresponded with the reference results. Additionally, *H. aphrophilus* (two strains) and *H. paraphrophilus* (one strain) were incorrectly identified as *H. parainfluenzae* biotype IV with the HNID panel because of the negative biotyping reactions and a negative LAC test. These isolates were GAL positive on the panel yet were positive for both GAL and lactose utilization tests in conventional tests. Most of the biotype IV strains described by Oberhofer and Back (24) were GAL negative. Although not included in Table 2, another *H. paraphrophilus* isolate, received in a College of American Pathologists proficiency sample, was also similarly misidentified as *H. parainfluenzae* biotype IV by using the HNID panel. Modification of the data base to correct for discrepant LAC and GAL test results and reconciliation of the data base with currently accepted biotype designations for *Haemophilus* spp. (19) will help to resolve these discrepancies.

N. cinerea strains produced profiles for *B. catarrhalis* or for low-likelihood identifications as *N. gonorrhoeae* (Table 3) because of negative GLU reactions and positive PRO reactions on the panel. While the glucose reaction is generally negative for *N. cinerea*, positive glucose tests have been noted in other carbohydrate utilization systems and such strains have been misidentified as glucose-negative gonococci (2, 7). *N. cinerea* is also positive for the prolyl aminopeptidase reaction in other chromogenic substrate systems (2, 14). Unlike Gonochek II and Identicult-*Neisseria*, which rely solely on the detection of prolyl aminopeptidase for identifying *N. gonorrhoeae* (3, 11, 12, 14, 15, 25), the PRO (prolyl aminopeptidase) substrate in the HNID panel is hydrolyzed not only by *N. gonorrhoeae* and *N. cinerea*, but also by *B. catarrhalis*. In the other systems, *B. catarrhalis* strains are prolyl aminopeptidase negative, and the absence of this and other enzymatic activities provides a presumptive identification of *B. catarrhalis* (3, 11, 12, 14, 15). The HNID panel relies on NO₃ and NO₂ reduction and the negative GLU reaction to identify *B. catarrhalis*, and

indeed, all isolates of this species were uniform in these characteristics (Table 1). Distinct differences in colony morphology and failure to grow on selective *Neisseria* media, coupled with negative GLU and NO₃ reactions on the panel, may help to distinguish *N. cinerea* from *B. catarrhalis* and *N. gonorrhoeae* (Table 3). These same HNID panel reactions would also help to distinguish *N. gonorrhoeae* and *B. catarrhalis* from those *N. cinerea* strains that may be recovered on selective media (17). Combinations of these characteristics may be incorporated easily into the data base of the HNID system to direct the performance of confirmatory tests such as susceptibility to colistin (17, 23).

The data presented indicate that the HNID panel may be useful for identifying other fastidious gram-negative coccobacilli (Table 3). Most *E. corrodens* isolates produced identical unique biotype numbers not listed in the data base. Those isolates that were misidentified as *B. catarrhalis* or *Neisseria* spp., such as *K. denitrificans* and *A. actinomyces-temcomitans*, could easily be identified on the basis of Gram stain morphology and oxidase and catalase tests. Such tests could be used to generate additional data base information when cell morphology and other rapid tests could help distinguish between gram-negative bacilli having identical biochemical patterns on the panel. Such an approach has been used with success in other rapid identification systems for fastidious gram-negative bacilli and anaerobic bacteria (13, 27). Expansion of the data base to include these and possibly other species and modification of the interpretive criteria for certain tests will improve the utility of the HNID panel in the clinical laboratory.

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