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Evaluation of the 4-Hour RapID NF Plus Method for Identification of 345 Gram-Negative Nonfermentative Rods

TASNA T. KITCH,[1] MICHAEL R. JACOBS,[2] AND PETER C. APPELBAUM[1]*

Department of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106[2]

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The ability of the RapID NF Plus system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) to identify 345 nonfermentative gram-negative rods was evaluated. Kits were inoculated with no. 1 McFarland suspensions, and reactions were interpreted after a 4-h incubation at 35°C. Overall, the method correctly identified 311 strains (90.1%) without additional tests and 21 strains (6.1%) with additional tests, and 13 strains (3.8%) were misidentified. Five of 13 misidentified strains were *Alcaligenes faecalis-Alcaligenes odorans* misidentified as *Alcaligenes xylosoxidans*; however, all strains were xylose negative but nitrate positive and could have been *A. faecalis* group I-*Alcaligenes piechaudii*. The system does not differentiate between *Pseudomonas fluorescens* and *Pseudomonas putida*, and all *Acinetobacter* species are identified as *Acetinobacter calcoaceticus*. Additionally, no subspecies differentiation is made between *A. xylosoxidans* subsp. *xylosoxidans* and *A. xylosoxidans* subsp. *denitrificans*. All strains of the former *Flavobacterium* group IIb are identified as *Flavobacterium indologenes-Flavobacterium gleum*, and no species identification of the genus *Methylobacterium* is attempted. The system is easy to set up and interpret and provides an accurate commercial nonautomated method for same-day identification of gram-negative nonfermenters.

The need for microbiology laboratories to identify nonfermenters without the need for extended conventional testing has led to the development of several commercial kits; some of these are specific for nonfermenters, while others are meant primarily for identification of members of the family *Enterobacteriaceae* but also identify commonly encountered nonfermenters (1, 2, 6–10, 12–17). Recently, Innovative Diagnostic Systems, Inc. (Atlanta, Ga.) applied the same principles utilized in their anaerobe and streptococcal identification methods (i.e., demonstration of preformed enzymes) to the 4-h identification of clinically significant gram-negative nonfermenters as well as some oxidase-positive glucose fermenters. This study evaluates the ability of the RapID NF Plus system to identify a spectrum of clinically isolated gram-negative nonfermentative rods, with and without the aid of supplementary conventional tests.

MATERIALS AND METHODS

Bacteria. A total of 345 clinically isolated bacteria were tested (see Table 1). These organisms were isolated at Hershey Medical Center or University Hospitals of Cleveland or were kindly provided by G. Gilardi (formerly of North General Hospital, New York, N.Y.), M. J. Pickett (University of California, Los Angeles, Calif.), G. Hall (Cleveland Clinic, Cleveland, Ohio), or A. Philippon (Hôpital St. Louis, Paris, France). Cultures were all identified in the same way, by conventional methods (4, 5), and stored in litmus milk or sterile defibrinated sheep blood at −30°C until use. Approximately 20% of the strains were fresh clinical isolates which were not frozen before testing. The identity of the latter group, as well as all strains which yielded discrepant results with the NF Plus system (see Results), was confirmed by performing the conventional tests mentioned above (4, 5) simultaneously with the NF Plus tests. Cultures were transferred and subcultured twice on sheep blood agar (BBL) before the inoculation of strips. Oxidase testing was carried out with 1.0% tetramethyl-*p*-phenylenediamine dihydrochloride (Remel, Lenexa, Kans.). Incubation took place at 35°C. Cultures were checked for purity throughout the study by Gram stain and colonial morphology.

NF Plus system. The RapID NF Plus system consists of a kit with 10 wells and comprises 17 reactions (10 before and 7 after the addition of reagents). Kits were inoculated with suspensions prepared from plates in RapID inoculation fluid (Innovative Diagnostic Systems, Inc.) adjusted to the turbidity of a no. 1 McFarland standard and incubated for 4 h at 35°C. The first 10 reactions (read before reagent addition) were as follows: arginine dihydrolase; thiosulfate utilization; fatty acid esterase production; hydrolysis of *p*-nitrophenyl-phosphoester; hydrolysis of *p*-nitrophenyl-*N*-acetyl-β,D-glucosaminide; hydrolysis of *p*-nitrophenyl-α,D-glucoside; hydrolysis of *p*-nitrophenyl-β,D-glucoside; hydrolysis of *o*-nitrophenyl-β,D-galactoside (ONPG); urease production; and glucose fermentation. Two drops of RapID NF Plus reagent were then added to wells 4 to 8, two drops of Innova spot indole reagent were added to well 9, and two drops of Innova nitrate A reagent were added to well 10, and reactions were read within 3 min. Reactions detected after the addition of reagents were as follows: hydrolysis of proline-β-naphthylamide; hydrolysis of pyrrolidine-β-naphthylamide; hydrolysis of γ-glutamyl-β-naphthylamide; hydrolysis of tryptophane-β-naphthylamide; hydrolysis of *N*-benzyl-arginine-β-naphthylamide; indole production; and nitrate production. Colors were interpreted according to the manufacturer's instructions, and a six-digit computer code was constructed. All codes were referred to the firm's data base for interpretation (a code book is now available from the manufacturer). Identifications were classified as follows. (i) Correct without additional tests, corresponding to excellent, very good, good, or implicit identifications, as listed in the data base. (ii) Probability overlap with low discrimination, necessitating additional testing to delineate the correct identification from ≥2 possibilities, as listed in the data base; in such cases, all

* Corresponding author.

supplemental tests required by the data base were performed. However, in cases of probability overlap where additional tests required were already available on culture plates (pigmentation, colonial morphology), these identifications were taken as correct and placed in category i. (iii) Misidentification. For the purposes of this study, all NF Plus identifications of acinetobacters as *Acinetobacter calcoaceticus* were taken as correct. For purposes of convenience, *Acinetobacter* species were classified by conventional tests as *Acinetobacter anitratus* (glucose positive, nonhemolytic) and *Acinetobacter lwoffii* (glucose negative, nonhemolytic). All identifications of *Alcaligenes xylosoxidans* subsp. *xylosoxidans* and *denitrificans* as *A. xylosoxidans* and identifications of *Pseudomonas fluorescens* and *Pseudomonas putida* as *P. fluorescens-P. putida* were taken as correct. All identifications of organisms formerly classified as *Flavobacterium* group IIb as *Flavobacterium indologenes-Flavobacterium gleum* were taken as correct, as were the two *Methylobacterium* spp. identified to genus only.

RESULTS

Results of identifications with NF Plus are presented in Table 1. As can be seen, 311 of 345 strains (90.1%) were correctly identified, with 21 strains (6.1%) requiring additional tests for accurate identification and 13 strains (3.8%) misidentified. With the few exceptions listed in Materials and Methods, the system provides accurate identification to species level of all groups of organisms tested, even of biochemically inactive species such as those in the genus *Moraxella*.

Additional tests required for the correct identification of strains yielding codes with probability overlap are listed in Table 2. As can be seen, additional tests were most commonly required to identify *Pseudomonas cepacia*, *Alcaligenes* species, and *Moraxella* species.

A total of 13 strains were misidentified: 5 strains of *Alcaligenes faecalis-Alcaligenes odorans* (misidentified as *A. xylosoxidans*), 2 of *Moraxella nonliquefaciens* (misidentified as *Moraxella osloensis*), 3 of *Pseudomonas alcaligenes* (2 misidentified as *A. xylosoxidans* and 1 misidentified as *A. faecalis-A. odorans*), 1 of *Pseudomonas thomasii* (code yielded no identification), 1 of *Comamonas acidovorans* (misidentified as *Comamonas testosteroni*), and 1 of *Weeksella zoohelcum* (misidentified as *Weeksella virosa*). The identities of all strains yielding discrepant identifications with NF Plus were reverified by conventional methodology (4, 5).

TABLE 1. Identification of nonfermenters by the RapID NF Plus system

Organism (*n*)	No. of strains identified[a]: Correctly[b]	With PO	Incorrectly or with no code
Pseudomonas aeruginosa (57)	57	0	0
Pseudomonas fluorescens (7)	7[b,c]	0	0
Pseudomonas putida (4)	4[b,c]	0	0
Xanthomonas maltophilia (26)	25	1	0
Pseudomonas cepacia (27)	24	3	0
Acinetobacter anitratus (23)	23[b,d]	0	0
Acinetobacter lwoffii (11)	10[b,d]	1	0
Alcaligenes faecalis-Alcaligenes odorans (24)	17	2	5
Alcaligenes xylosoxidans subsp. *xylosoxidans* (29)	28[b]	1	0
Alcaligenes xylosoxidans subsp. *denitrificans* (12)	9[b]	3	0
Moraxella osloensis (10)	8	2	0
Moraxella nonliquefaciens (2)	0	0	2
Oligella urethralis (4)	4	0	0
Oligella ureolytica (1)	1	0	0
Ochrobactrum anthropi (1)	1	0	0
Pseudomonas stutzeri (8)	8	0	0
Pseudomonas putrefaciens (4)	4	0	0
Pseudomonas diminuta (10)	10	0	0
Pseudomonas paucimobilis (4)	3	1	0
Pseudomonas alcaligenes (5)	2	0	3
Pseudomonas thomasii (1)	0	0	1
Pseudomonas pickettii (1)	1	0	0
Pseudomonas vesicularis (4)	2	2	0
Pseudomonas mendocina (3)	3[e]	0	0
Pseudomonas group 2 (2)	1	1	0
Flavimonas oryzihabitans (6)	6[f]	0	0
Chromobacterium violaceum (2)	2	0	0
Flavobacterium odoratum (15)	15	0	0
Flavobacterium meningosepticum (13)	12	1	0
"*Flavobacterium* group IIb" (8)	8[b,g]	0	0
Methylobacterium spp. (2)	2[b]	0	0
Comamonas acidovorans (4)	2	1	1
Weeksella virosa (2)	2	0	0
Weeksella zoohelcum (1)	0	0	1
Sphingobacterium multivorum (7)	7	0	0
Agrobacterium tumefaciens (1)	1	0	0
Bordetella bronchiseptica (2)	2	0	0
CDC group IV C-2 (1)	0	1	0
CDC group M6 (1)	0	1	0

[a] The total percentages were 90.1% correct, 6.1% PO (probability overlap), and 3.8% incorrect or no identification.
[b] Correct to level of species, genus, or group of subspecies (see Materials and Methods). Where not signified, all identifications were to species level.
[c] Identified as *P. fluorescens-P. putida*.
[d] See Discussion for current taxonomic thinking.
[e] Two of three strains differentiated from *P. aeruginosa* in a probability overlap identification by brownish pigment.
[f] Includes one strain with a probability overlap differentiated from *X. maltophilia* by colonial morphology and pigmentation.
[g] Identified as *F. indologenes-F. gleum*.

DISCUSSION

Previous reports of the capability of commercial kits to accurately identify nonfermenters with and without conventional tests have yielded various results, and all show some deficiencies in the identification of weakly oxidizing and nonreactive strains (1, 2, 6–10, 12–17). In the current study, the RapID NF Plus method yielded excellent results in the identification of clinically significant gram-negative nonfermenters. The method was easy to set up, and, with a little experience, easy to interpret and comprises, together with the Biolog system (Biolog, Inc., Hayward, Calif.) (9), the only commercial nonautomated system of which we are aware that yields nonfermenter identification in 4 h. It is, however, important to note that the 4-h identification time comes only after the initial 24 to 72 h required to achieve adequate growth of a pure culture. All groups of organisms were accurately identified to species level, with the exception of *P. fluorescens-P. putida*, *Acinetobacter* species, *A. xylosoxidans* subspecies, *F. indologenes-F. gleum*, *P. alcaligenes*, and methylobacteria.

The taxonomy of the genus *Acinetobacter* is currently in a state of flux (3, 11). Recent DNA hybridization studies have identified 17 hybridization groups (genospecies) within this genus: *Acinetobacter baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. lwoffii*, and 11 unnamed

TABLE 2. Extra tests recommended by the RapID NF Plus system data base for correct identification

Organism[a] (n)	Identification by RapID NF Plus	Extra tests required[b]
X. maltophilia (1)	*P. aeruginosa-P. cepacia-X. maltophilia*	LAC, MAL, LDC, FLA, PIG
P. cepacia (2)	*P. cepacia-A. calcoaceticus*	PIG, MAN, MOT
P. cepacia (1)	*P. cepacia-X. maltophilia*	MAN, DNA
A. lwoffii (1)	*P. cepacia-A. calcoaceticus*	MAN, MOT
A. faecalis-A. odorans (1)	*A. faecalis-A. odorans-Kingella kingae*	CAT, HEM, GLU, MOT
A. faecalis-A. odorans (1)	*A. faecalis-A. odorans-B. bronchiseptica-Pasteurella multocida-A. xylosoxidans*	MAC, URE, XYL
A. xylosoxidans subsp. *xylosoxidans* (1)	*A. xylosoxidans-P. aeruginosa*	PIG, FLA
A. xylosoxidans subsp. *denitrificans* (3)	*A. faecalis-A. odorans-A. xylosoxidans-B. bronchiseptica*	XYL, URE
M. osloensis (1)	*M. osloensis-P. cepacia*	PIG, GLU
M. osloensis (1)	*M. nonliquefaciens-A. faecalis-A. odorans-M. osloensis*	SS, MOT, NO_3
P. paucimobilis (1)	*P. paucimobilis-F. indologenes-F. gleum*	PIG, MAN
P. vesicularis (1)	*P. vesicularis-P. diminuta*	PIG, ESC
P. vesicularis (1)	*P. vesicularis-A. faecalis-A. odorans*	MAC, SS, FLA
Pseudomonas group 2 (1)	*Pseudomonas* group 2-*P. pickettii*	FLA
F. meningosepticum (1)	*Flavobacterium* spp. (*F. indologenes-F. gleum-F. meningosepticum*)	PIG, DNA
C. acidovorans (1)	*C. acidovorans-C. testosteroni*	MAN, ACE
CDC group IV C-2 (1)	*B. bronchiseptica*-IV C-2-*A. faecalis-A. odorans*	SS, NO_3, URE
CDC group M-6 (1)	CDC M group-*P. fluorescens-P. putida*	CAT, MAC, SS, GLU

[a] Identified by conventional methods.

[b] FLA, flagella stain; PIG, pigment; MOT, motility; CAT, catalase; HEM, β-hemolysis; MAC, growth on MacConkey agar; SS, growth on salmonella-shigella agar; LDC, lysine decarboxylase; DNA, DNase; URE, conventional urease test; NO_3, reaction in nitrate tests after addition of zinc; ESC, esculin hydrolysis; ACE, acetamide hydrolysis; GLU, glucose oxidation; LAC, lactose oxidation; MAN, mannitol oxidation; MAL, maltose oxidation; XYL, xylose oxidation.

genospecies (11). Because the clinical relevance of this expanded classification remains to be established, no definitive data are available on how far commercial kits should go in the genospecies identification of these organisms.

Breakdown to subspecies level of *A. xylosoxidans* may not be essential, with the exception of cases of nosocomial infections. Differentiation between *F. indologenes* and *F. gleum* is difficult, and most of these strains were submitted to us as "*Flavobacterium* group IIb" (11). Although *F. indologenes* has been proposed as the binomial for some strains of the IIB group (18), current evidence suggests that both this and other binomials such as *F. gleum* and *Flavobacterium balustinum* represent taxa within and not synonyms for group IIb (11). For these reasons, identifications as *F. indologenes-F. gleum* were taken as correct in our study. The five strains of *A. faecalis-A. odorans* misidentified as *A. xylosoxidans* were all nitrate positive but xylose negative and may be classified as *A. faecalis* group I-*Alcaligenes piechaudii* (11).

NF Plus yielded accurate identification to species level of even relatively nonreactive species such as *Moraxella* spp., *Pseudomonas diminuta*, and *Pseudomonas vesicularis*. Two *Moraxella* strains, one *Weeksella* strain, and one *Comamonas* strain were correctly identified to genus level but not to species level; correct identification to genus level within these groups would usually be deemed satisfactory for clinical purposes. Because the method does not rely on growth, the identification of slow-growing, biochemically inactive nonfermenters such as *Pseudomonas aeruginosa* and *P. cepacia* from patients with cystic fibrosis is facilitated. In the current study, 85% of *P. cepacia* strains were isolated from cystic fibrosis patients, and identification was satisfactory in all cases. Our experience with other commercial methods in the identification of isolates from these patients has been uniformly poor.

Evaluations of the kind described in the current study are hampered by a lack of availability of less commonly isolated nonfermenters. Adequate challenge of NF Plus (or any other commercial identification method) must include as many of the more recondite species as possible, in numbers weighted according to their clinical significance. Because of the rarity of these strains, a central clinical microbiology reference laboratory in which a library of nonfermenters (commonly encountered as well as rare) could be stored and, when required, easily provided for others is important.

The decision of how far to go in the identification of nonfermenters from patients is an individual one predicated upon the size and capability of the clinical microbiology laboratory and the source and clinical significance of the isolate. Results of this study show that the NF Plus method is an important new method for same-day, accurate identification of all groups of clinically significant nonfermenters.

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