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Four Methods for Identification of Gram-Negative Nonfermenting Rods: Organisms More Commonly Encountered in Clinical Specimens

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Four commercial kits, Oxi/Ferm (OF), API 20E (AP), Minitek (MT; BBL Microbiology Systems), and Flow N/F (NF), were evaluated, without additional tests, for identification of 258 gram-negative nonfermentative rods. OF and MT were read after 48 h of incubation, and AP and NF were read after both 24 and 48 h of incubation, respectively. Overall, OF correctly identified 51% of strains, with 46% as part (but not first) of a spectrum of identifications (SI), and 3% incorrect species identification. MT yielded 85% correct identification, with 15% SI. Of 126 glucose-positive strains, or those with ≥3 positive AP reactions after 24 h, 60% were correctly identified, with 40% SI; incubation for an additional 24 h raised the rate of correct identification to 99%, with 1% SI. A total of 132 strains yielded <3 positive AP reactions after 24 h and were identified after 48 h only; of these, 82% were correctly identified, with 17% SI and 1% incorrect species identification. NF correctly identified 79% of cultures after 24 h, with 21% SI; corresponding figures after an additional 24 h of incubation were 80% and 20%, respectively. All four commercial methods show promise; OF is easiest to inoculate, but requires extra tests for optimal identification. AP reliably identifies the majority of clinically important nonfermenters, with fairly good species identification of saccharolytic strains after 24 h. MT yields reliable identification of most nonfermenters and has the advantage of flexibility. NF is easy to inoculate, yields satisfactory identification rates, and may be read after 24 h of incubation.

Gram-negative nonfermenting aerobic rods are becoming increasingly implicated in human disease, especially of nosocomial origin (11, 17, 20). The complex physicochemical properties of these organisms necessitate a battery of conventional tests for their precise identification. In addition, much confusion still exists concerning the taxonomic status of many of these organisms. Lack of personnel or other resources prevents many clinical microbiology laboratories from identifying most of these organisms by conventional means. Therefore, identification has often been neglected, especially when antimicrobial sensitivity results are available. Although such data may be adequate for patient treatment, it does not yield satisfactory epidemiological information. As interest in these organisms has increased, a number of computerized rapid identification systems have been developed and marketed (1, 2, 6, 7, 9, 10, 12-15, 18, 19, 21).

The smaller microbiology laboratory is in need of a rapid, reliable method of nonfermenter identification. The purpose of this study was to compare the ability of four commercial kits—Oxi/Ferm (OF; Roche Diagnostics, Div. Hoffmann-La Roche, Inc., Nutley, N.J.), API 20E (AP; Analytab Products, Inc., Plainview, N.Y.), Minitek (MT; BBL Microbiology Systems, Cockeysville, Md.), and Flow N/F system (NF; Flow Laboratories, Roslyn, N.Y.)—to identify commonly encountered, clinically significant gram-negative nonfermenting rods, without the aid of supplementary conventional tests.

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MATERIALS AND METHODS

Bacteria. A total of 258 commonly encountered, clinically significant nonfermenters were tested; 84% of these were kindly provided by Gerald Gilardi (Hospital for Joint Diseases and Medical Center, New York, N.Y.), and the remaining 16% were provided by commercial companies (Analytab Products, Flow Laboratories, Roche Diagnostics). Strains tested are listed in Tables 1 to 5. Cultures from G. L. Gilardi and Flow Laboratories were identified by methods previously described (5). Cultures from Roche and Analytab Products were originally obtained from G. L. Gilardi, the Center for Disease Control, and the firms' respec-

tive reference laboratories. Stock cultures stored in litmus milk at −30°C were transferred and subcultured twice onto 5% sheep blood agar plates before inoculation of kits. Oxidase testing was with 1.0% tetramethyl-*p*-phenylenediamine dihydrochloride (Marion Scientific Corp., Kansas City, Mo.). Incubation was at 35°C, unless otherwise stated. Cultures used to inoculate kits were checked for purity and viability by inoculation of Trypticase soy agar plates (BBL).

Identification of organisms. Identification was classified as correct, part (but not first) of a spectrum of identifications (SI), or incorrect. Where only genus identification was possible with a specific system, this was regarded as correct.

OF system. Procedures for inoculation and incubation were as described previously (2, 6, 7, 10, 12–14, 18). No supplemental tests were done. Where definitive code book identification was not possible without supplemental tests, identification was as SI; in such cases, no statistical information is available in the OF code book at this time.

AP system. Strips, OF, and motility media were inoculated and incubated according to standard practice (2, 5, 9, 12, 13, 19, 20). Reagents were added after 48 h in all cases. If glucose positivity or ≥3 positive reactions (excepting oxidase) was found after 24 h, tryptophan deaminase, Voges-Proskauer, and indole reactions were extrapolated to 24 h, and organisms were identified from 24-h, as well as 48-h, results, respectively. Where organisms yielded <3 positive reactions after 24 h, results were encoded after 48 h. Indole, Voges-Proskauer, and tryptophan deaminase reactions which are positive after 48 h are uniformly positive after 24 h (Karla M. Tomfohrde, personal communication). The above method of reagent addition, although differing from that used in clinical laboratories, was used to generate as much data as possible for the AP system. Interpretation of code numbers was according to the manufacturer's instructions. Where identification was as "good likelihood but low selectivity" with a spectrum of possibilities, supplemental tests were not done, and the statistically most probable identification was used for organism identification.

MT system. A dense homogenous suspension of organisms in MT nonfermenter broth (MacFarland no. 5) was used to inoculate MacConkey plates and the following range of MT disks: two of dextrose without nitrate, and one each of maltose, sucrose, D-xylose, arginine dihydrolase, ornithine and lysine decarboxylase, urease, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), nitrate reductase, starch, phenylalanine deaminase, citrate. For indole production, the inoculum was placed in an empty well. In addition, gelatin disks (Key Scientific Products, Inc., Los Angeles, Calif.), esculin, mannitol, and malonate (not commercially available in the MT panel) were inoculated. Reagents were added, and tests were interpreted with the aid of a code book, according to the manufacturer's instructions. The firm's computer facilities were consulted for organisms whose code did not appear in the book. Where supplemental tests were required for precise identification of a range of possibilities, the organism with the highest confidence value and lowest biotype validity was selected as correct.

NF system. Two-tube screens and Uni-N/F-Tek wheels were inoculated according to the manufacturer's recommendations (1, 21). *Pseudomonas aeruginosa* strains were initially inoculated into the two-tube screen; only organisms which failed to key out with the latter were inoculated onto the wheel. Strains of *Pseudomonas fluorescens* and *Pseudomonas putida* were inoculated into both tubes and wheels irrespective of whether positive identification was possible with screen tubes only; this was to generate more data for the NF system. Oxidase-negative strains were inoculated onto the wheel only. 42P screen tubes were incubated at 42°C, and GNF screens plus wheels were incubated at 35°C. Interpretation of reactions was according to the manufacturer's recommendations, utilizing a computer code book (1, 21). Numbers which did not appear in the code book were referred to the firm's computer facilities. Where the code book revealed a spectrum of more than one organism as the possible species, identification was with the organism of greatest statistical likelihood, without performance of supplemental tests.

RESULTS

Biochemical reactions of strains in the four systems are available from the authors on request. In general, most tests gave expected reactions. OF nitrogen (N_2) gas production was often difficult to interpret, owing to accumulation of bubbles under the wax overlay. Citrate utilization tests also presented difficulty with OF, owing to equivocal greenish-blue color production, which could have been interpreted as either positive or negative. Nitrate reactions with AP were satisfactory only if the method recommended by the manufacturer was strictly adhered to: if powdered instead of granular zinc was used, or if strips were not left open for at least 10 to 15 min after zinc addition, equivocal reactions resulted. Motility was the most difficult test to interpret in AP. In many cases, only a few motile outgrowths from the primary inoculum were found, necessitating interpretation by an experienced technologist for optimal results. Positive AP indole reactions were often weak and took 1 to 2 min to develop satisfactorily. MT nitrate reductase tests gave rapid results, but inaccuracies occurred, especially false-negative tests for N_2 gas production. The sensitivity of Key gelatin disks, as presently available in the MT system, was low. NF gave rise to problems in interpretation of N_2 gas and indole production. N_2 gas interpretation difficulties arose when scanty, small bubbles were produced on the butt surface beneath the constriction; these were read as positive, whereas bubbles or cracks along the inoculation stab line were taken as negative. The Flow indole method was difficult to interpret: when a red color did develop, this

was often weak and difficult to see.

Only the NF system offered species identification of most strains. With the exception of *Moraxella phenylpyruvica*, *Moraxella* strains were identifiable to genus only by OF and MT; AP offers only genus identification of moraxellas. OF and AP identify *Alcaligenes* strains to genus only, whereas MT identifies these organisms as *Alcaligenes/Pseudomonas* species. *Acinetobacter calcoaceticus* biotypes *haemolyticus* and *alcaligenes* were identified as biotypes *anitratus* and *lwoffi*, respectively, by all four systems. The differentiation of *A. calcoaceticus* into four biotypes on the grounds of sugar oxidation, hemolysis, and proteolysis (4) is not recognized by all taxonomists (G. L. Gilardi, personal communication). Differentiation between *Moraxella urethralis* and *Moraxella osloensis* strains may be difficult (8); therefore, when the Flow system (the only method which offered species identification of moraxellas) included *M. osloensis* in the SI for *M. urethralis* strains, this was taken as correct group identification. MT and NF systems did not differentiate between *P. fluorescens* and *P. putida* strains. *Flavobacterium meningosepticum* strains were identified to genus only with OF, and to genus (two strains) or species (seven strains) with AP.

Identification of nonfermenter strains with the various systems is listed in Tables 1 to 5. OF correctly identified 51% of strains (mainly *P. aeruginosa*,, *Pseudomonas maltophilia*, *A. calcoaceticus* biotype *alcaligenes*, *Alcaligenes odorans*, *F. meningosepticum*) without supplemental tests; 46% of remaining organisms required additional tests, not included in OF, for precise identification, and 3% of strains were incorrectly identified. Of 126 strains with positive AP glucose reactions or ≥3 positive reactions after 24 h, 60% were correctly identified (mainly *P. maltophilia*, *A. calcoaceticus* biotypes *anitratus* and *haemolyticus*, *Pseudomonas cepacia*), with 40% requiring additional tests. Incubation for an additional 24 h raised the rate of correct identification to 99%, with 1% SI. All *P. aeruginosa* strains keyed as SI (mainly *P. fluorescens* group) after 24 h and required OF, motility, nitrate reduction, and MacConkey tests for precise identification. A total of 132 strains yielded <3 positive AP reactions after 24 h and were identified after 48 h only; of these, 82% were correctly identified, with 17% SI (mainly *P. fluorescens*, *Alcaligenes* species) and 1% incorrect identification. Of 77 organisms identified as the first likelihood of a "good likelihood but low selectivity" range, 61% yielded the correct result, with 39% SI. MT correctly identified 85% of strains, with 15% SI; the latter

TABLE 1. *Identification of nonfermenters with OF*

Organism	Correct identification[a]	One of a spectrum of identifications[b]	Incorrect identification
P. aeruginosa (52)[c]	51[c]	1	—[d]
P. fluorescens (11)	—	11	—
P. putida (7)	—	7	—
P. maltophilia (34)	28	1	5
A. calcoaceticus biotype *anitratus* (26)	2	24	—
A. calcoaceticus biotype *lwoffi* (16)	7	9	—
A. calcoaceticus biotype *haemolyticus*[e] (15)	5	10	—
A. calcoaceticus biotype *alcaligenes*[f] (14)	14	—	—
A. faecalis[g] (12)	—	12	—
A. odorans[g] (14)	13	1	—
A. denitrificans[g] (12)	3	9	—
M. osloensis[g] (12)	—	7	—
M. phenylpyruvica (2)	—	1	1
M. urethralis[g] (4)	2	2	—
M. nonliquefaciens[g] (11)	—	11	—
F. meningosepticum[g] (9)	7	1	1
P. cepacia (12)	—	11	1

[a] Includes free tests (reactions on Trypticase soy agar, hemolysis).
[b] Extra tests necessary for correct identification.
[c] Number of strains.
[d] —, None.
[e] Identified as biotype *anitratus*.
[f] Identified as biotype *lwoffi*.
[g] Organisms identifiable to genus only with this system.

TABLE 2. *AP results of nonfermenters with ≥3 positive reactions after 24 h*[a]

Organism	Correct identification		One of a spectrum of identifications
	Time (h)	No.	
P. aeruginosa (48)	24	—[b]	48
	48	48	—
P. fluorescens (1)	24	—	1
	48	—	1
P. putida (1)	24	—	1
	48	1	—
P. maltophilia (23)	24	23	—
	48	23	—
A. calcoaceticus biotype *anitratus* (25)	24	25	—
	48	25	—
A. calcoaceticus biotype *haemolyticus*[c] (15)	24	15	—
	48	15	—
F. meningosepticum (2)	24	2[d]	—
	48	2[d]	—
P. cepacia (11)	24	10	1
	48	11	—

[a] There were no incorrect identifications.
[b] —, None.
[c] Identified as biotype *anitratus*.
[d] One organism identified to species, the other to genus only.

TABLE 3. *AP results at 48 h of nonfermenters with <3 positive reactions after 24 h*

Organism	Correct identification	One of a spectrum of identifications	Incorrect identification
P. aeruginosa (4)	4[a]	—[b]	—
P. fluorescens (10)	1	9	—
P. putida (6)	6	—	—
P. maltophilia (11)	11[c]	—	—
A. calcoaceticus biotype *anitratus* (1)	1[a]	—	—
A. calcoaceticus biotype *lwoffi* (16)	16	—	—
A. calcoaceticus biotype *alcaligenes*[d] (14)	14	—	—
A. faecalis[e] (12)	8	4	—
A. odorans[e] (14)	7	7	—
A. denitrificans[e] (12)	10	1	1
M. osloensis[e] (7)	7	—	—
M. phenylpyruvica[e] (2)	1	1	—
M urethralis[e] (4)	3	1	—
M. nonliquefaciens[e] (11)	11	—	—
F. meningosepticum (7)	7[f]	—	—
P. cepacia (1)	1	—	—

[a] All keyed out as SI after 24 h.
[b] —, None.
[c] All keyed out as *P. maltophilia* after 24 h.
[d] Identifiable as biotype *lwoffi*.
[e] Identifiable to genus only with this system.
[f] Six correct species identifications, one identification to genus only.

TABLE 4. *Identification of nonfermenters with MT*[a]

Organism	Correct identification	One of a spectrum of identifications
P. aeruginosa (52)	35	17
P. fluorescens (11)	10[b]	1
P. putida (7)	7[b]	—[c]
P. maltophilia (34)	34	—
A. calcoaceticus biotype *anitratus* (26)	18	8[d]
A. calcoaceticus biotype *lwoffi* (16)	10	6
A. calcoaceticus biotype *haemolyticus*[e] (15)	15	—
A. calcoaceticus biotype *alcaligenes*[f] (14)	14	—
A. faecalis[g] (12)	11	1
A. odorans[g] (14)	14	—
A. denitrificans[g] (12)	12	—
M. osloensis[h] (7)	7	—
M. phenylpyruvica (2)	1[h]	1
M. urethralis[h] (4)	3	1
M. nonliquefaciens[h] (11)	11	—
F. meningosepticum (9)	5	4[d]
P. cepacia (12)	12	—

[a] There were no incorrect identifications.
[b] Identified as *P. fluorescens/P. putida* with this system.
[c] —, None.
[d] Eight strains of biotype *anitratus* and four of *F. meningosepticum* required mannitol/esculin (not included in regular panel) for correct identification.
[e] Identified as biotype *anitratus*.
[f] Identified as biotype *lwoffi*.
[g] Identifiable as *Alcaligenes/Pseudomonas* species in this system.
[h] Identifiable to genus only with this system.

included *P. aeruginosa*, *A. calcoaceticus* biotypes *anitratus* and *lwoffi*, *F. meningosepticum*. False-negative tests for N_2 gas production accounted for problems with *P. aeruginosa*; all 12 *A. calcoaceticus* biotype *anitratus* and *F. meningosepticum* strains identified as SI keyed out correctly when results of mannitol-esculin tests were used. Positive tests for N_2 gas-urease were responsible for SI of *A. calcoaceticus* biotype *lwoffi* strains. The Flow two-tube screening method identified 98% of *P. aeruginosa*, 18% of *P. fluorescens*, and 45% of *P. putida* strains after 24 h; all remaining organisms in these three groups were correctly identified on addition of the wheel. The Flow method identified 79% of cultures after 24 h, with 21% SI; corresponding figures after an additional 24-h incubation were 80% and 20%, respectively. Organisms identified as SI were mainly *P. maltophilia*, *Alcaligenes faecalis*, and *Moraxella* strains.

Extra tests most commonly needed for correct identification of SI strains are listed in Table 6. As can be seen, some members of all organism groups required a variety of additional tests to pinpoint a correct identification from an SI spectrum. Incorrect identifications are presented in Table 7. Eight strains were misidentified by OF; false-positive citrate tests were responsible for identifying three strains of *P. maltophilia* as *A. calcoaceticus* biotype *lwoffi* and two strains as *P. cepacia*. A false-positive citrate test was also responsible for one *F. meningosepticum* strain not being coded. One *M. phenylpyruvica* strain was misidentified as group 4E *Alcaligenes*-like, due to a false-positive alkaline dextrose and rapid urease tests. One strain of *P. cepacia* was misidentified as *Pseudomonas* species/*A. faecalis*/M-4 *Moraxella*-like, due to a false-negative aerobic dextrose reaction. The one incorrect identification by AP of an *Alcaligenes denitrificans* strain as *Pseudomonas* species other/*Moraxella*/*Pasteurella* was due to a false-negative reaction in API motility medium.

DISCUSSION

This study attempted to evaluate the relative ability of each of four commercially available systems to identify a group of commonly encountered, clinically significant gram-negative nonfermenters. The potential of these organisms

TABLE 5. *Identification of nonfermenters with NF*[a]

Organism	Correct identification		One of a spectrum of identifications
	Time (h)	No.	
P. aeruginosa (52)	24	52	—[b]
	48	52	—
P. fluorescens[c] (11)	24	11	—
	48	11	—
P. putida[c] (7)	24	7	—
	48	7	—
P. maltophilia (34)	24	23	11
	48	21	13
A. calcoaceticus biotype *anitratus* (26)	24	26	—
	48	26	—
A. calcoaceticus biotype *lwoffi* (16)	24	16	—
	48	15	1
A. calcoaceticus biotype *haemolyticus*[d] (15)	24	15	—
	48	15	—
A. calcoaceticus biotype *alcaligenes*[e] (14)	24	14	—
	48	14	—
A. faecalis (12)	24	—	12
	48	—	12
A. odorans (14)	24	6	8
	48	8	6
A. denitrificans (12)	24	6	6
	48	10	2
M. osloensis (7)	24	—	7
	48	—	7
M. phenylpyruvica (2)	24	—	2
	48	—	2
M. urethralis[f] (4)	24	—	4
	48	—	4
M. nonliquefaciens (11)	24	6	5
	48	6	5
M. meningosepticum (9)	24	9	—
	48	9	—
P. cepacia (12)	24	12	—
	48	12	—

[a] There were no incorrect identifications.

[b] —, None.

[c] Identified as *P. fluorescens/P. putida* with this system.

[d] Identified as biotype *anitratus*.

[e] Identified as biotype *lwoffi*.

[f] Does not key out to species with this system (see text).

for causing nosocomial infection, as well as their specific antibiotic susceptibility spectrum (11, 17, 20), makes accurate identification mandatory.

The OF system, without supplemental tests, correctly identified (to genus or species) 98% of *P. aeruginosa*, 82% of *P. maltophilia*, 100% of *A. calcoaceticus* biotype *alcaligenes*, 93% of *A. odorans*, and 78% of *F. meningosepticum* strains. Other isolates were identified with varying degrees of accuracy (Table 1), and most required additional tests for more precise identification (Table 7). Previous workers (2, 6, 7, 10, 12-14, 18) have reported the ability of OF to identify most commonly encountered nonfermenters, if recommended supplemental tests are performed. If these tests are not carried out, accuracy of OF drops (2, 6, 13, 18). Difficulties in interpretation of N_2 gas reactions have been described before (10). Conflicting reports on the accuracy of the OF citrate test have appeared in the literature (2, 6, 7, 10, 12-14). In our hands, citrate utilization reactions were difficult to interpret and responsible for mis-identification of five *P. maltophilia* strains, as well as non-identification of one strain of *F. meningosepticum* (code number 0251). Advantages of OF include ease of inoculation and the infrequent need to consult the manufacturer's computer facilities with numbers not present in the code book. In our opinion, the value of OF, especially in the smaller laboratory without facilities for many recommended conventional tests, would be improved by inclusion of more reactions (e.g., mannitol, lactose, maltose, deoxyribonuclease, esculin, ONPG) in the tube, with correspondingly longer code numbers and a more comprehensive code book. When a spectrum of identifications is encountered, statistical data on the likelihood of each would also be valuable. Improvement of existing N_2 gas and citrate tests would further enhance the value of the OF system.

Identification by AP of saccharolytic nonfermenters after 24 h was satisfactory for the majority of strains, with the exception of *P. aeruginosa* (Table 2). All 48 strains of the latter organism were identified as SI (mainly *P. fluorescens* group) after 24 h, with precise species identification only after additional expanded AP tests. Gelatin negativity of 10 out of 11 *P. fluorescens* strains in AP tests precluded accurate identification of this organism group. Problems in interpretation of the AP motility test were responsible for SI of *Alcaligenes* strains and for the one incorrect AP identification (Table 7); improvement in sensitivity of the latter test would enhance the capability of the AP system to identify nonfermenters. Reports in the literature on the accuracy of AP nonfermenter identification have varied; most workers agree that oxidase-negative bacteria, such as *P. maltophilia* and *A. calcoaceticus* biotypes, are reliably identified by this system (2, 6, 9, 12, 13, 19, 21), but problems have been encountered in the species identification of *P. aeruginosa* (6, 9, 13, 21). In the light of these findings as well as our own, it seems that the expanded 48-h AP system may be necessary for differentiation of *P. aeruginosa* from other saccharolytic oxidase-positive nonfermenters. Problems in AP identification of *P. cepacia* reported by others (2, 6, 9, 21) were not encountered in our study. A practical difficulty

TABLE 6. *Extra tests most commonly needed for correct identification (SI)*[a]

Organism	OF	AP	MT	NF
P. aeruginosa	—[b]	—	42°C; CET; ACE (*P. fluorescens* group)[c]	—
P. fluorescens/P. putida	42°C; GEL; PB; AMP (*P. fluorescens* group)	Conventional GEL (*P. fluorescens* group)	—	—
A. calcoaceticus biotype *anitratus*	MOT (*P. cepacia*)	—	MANN; ESC (CDC VE-1, 2)	—
A. calcoaceticus biotype *lwoffi*	MOT (*P. maltophilia*)	—	MOT (*B. parapertussis; Alcaligenes/ Pseudomonas*)	—
Alcaligenes species	MOT; NAL; PEN; MAC; ANG; FLA; BB ( )[d]	CET; ACE; SS; FLA; 6.5% NaCl; N_2 from nitrite ( )[d]	MOT ( )[d]	MOT; NIT; PAD; SS; FLA ( )[d]
Moraxella species	PEN; MOT; MAC; ANG; FLA ( )[d]	SS; PEN; TSI ( )[d]	MOT ( )[d]	NIT; MOT; PAD; FLA ( )[d]
P. maltophilia	—	—	—	MOT (*P. paucimobilis*)
F. meningosepticum	MOT; ONPG; SUC; LYS (*Vibrio-Aeromonas*)	—	MANN; ESC; CAT (*Flavobacterium* IIB)	—
P. cepacia	PB; CAR; KAN; AMP; MOT (*P. fluorescens; P. stutzeri*; CDC VE-2; *P. pickettii*; *Achromobacter*)	10% LAC; PB (CDC VE-1; *P. maltophilia*; *P. paucimobilis*)	—	—

[a] 42°C, Growth at 42°C; MOT, motility; CET, growth on cetrimide; ACE, growth on acetamide; MAC, growth on MacConkey agar; SS, growth on salmonella-shigella agar; 6.5% NaCl, growth in 6.5% NaCl; NIT, nitrate reduction; PAD, phenylalanine deamination; GEL, gelatin liquefaction; FLA, flagellar staining; ANG, anaerobic growth; BB, barred appearance on Gram stain from broth; TSI, growth on triple sugar iron agar; ONPG, ONPG breakdown; SUC, sucrose oxidation; 10% LAC, utilization of 10% lactose; MANN, mannitol oxidation; ESC, esculin hydrolysis; CAT, catalase production; LYS, lysine decarboxylase production; PEN, penicillin G sensitivity; AMP, ampicillin sensitivity; PB, polymixin B sensitivity; CAR, carbenicillin sensitivity; KAN, kanamycin sensitivity; NAL, nalidixic acid sensitivity.

[b] —, None.

[c] Organisms in parentheses represent other SI possibilities.

[d] Other asaccharolytic/weakly saccharolytic pseudomonads; *Pasteurella* spp.

with the AP system was the large number (approximately 25%) of profile numbers which did not appear in the code book, necessitating lengthy delays in identification because of the need for computer assistance. Enlargement of the existing code book so as to include more nonfermenter profile numbers would help obviate this problem. The relatively high (61%) rate of correct identifications when the first likelihood of a "good likelihood but low selectivity" SI was used as the organism's identification reflects a possible use for this system in smaller laboratories with fewer facilities.

Few published reports on the use of MT for nonfermenter identification could be found. Slifkin and Pouchet (M. Slifkin and G. R. Pouchet, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C88, p. 50), in a preliminary study, found the system to be satisfactory when supplemented with motility testing, in the identification of a wide variety of nonfermenters. In our hands the MT system yielded satisfactory results for most organism groups. Recourse to manufacturer's computer facilities was low (approximately 5%). Problems with SI of *P. aeruginosa* strains may be obviated by modifications in the nitrate reductase test; also, addition of mannitol and esculin disks to the existing panel would improve identification of *A. calcoaceticus* biotype *anitratus* and *F. meningosepticum*, as well as CDC VE-1, CDC VE-2, and *Flavobacterium* group IIB (P. C. A., unpublished data). If inclusion of the gelatin test in the routine MT panel is envisaged, increased sensitivity is required. Differentiation of organisms presently classified as *Alcaligenes/Pseudomonas* could be effected by inclusion of acetamide, deoxyribonuclease, mannitol disks, as well as a test for N_2 gas production from nitrate.

The NF system offered the most accurate

TABLE 7. *Major identification differences between OF, AP, MT, and NF methods*

Organism[a]	OF	AP	MT	NF
P. maltophilia (two strains)	*P. cepacia*	*P. maltophilia*	*P. maltophilia*	*P. paucimobilis; P. maltophilia*[b]
P. maltophilia (three strains)	*A. calcoaceticus* biotype *lwoffi*	*P. maltophilia*	*P. maltophilia*	*P. maltophilia*
A. denitrificans	*A. faecalis; Achromobacter* biotype 1[c]	*Pseudomonas* other; *Moraxella; Pasteurella*[b]	*Alcaligenes/ Pseudomonas*	*A. denitrificans*
M. phenylpyruvica	4E *Alcaligenes*-like	*Moraxella* spp.	CDC IV-E; *M. phenylpyruvica*	*M. nonliquefaciens; M. osloensis; P. testosteroni; A. faecalis; P. alcaligenes; A. denitrificans; M. phenylpyruvica; P. acidovorans*[b]
F. meningosepticum	Not coded (0251)	*F. meningosepticum*	*F. meningosepticum*	*F. meningosepticum*
P. cepacia	*Pseudomonas; A. faecalis;* M-4 *Moraxella*-like[c]	*P. cepacia*	*P. cepacia*	*P. cepacia*

[a] Previously identified by conventional methods (see text).
[b] Where a spectrum of possibilities was given, identifications are listed, in descending order of probability, for all systems except OF.
[c] The spectrum of possibilities with OF is not listed in order of probability (see text).

identification (to species or biotype) of all four systems. Other advantages of this system include identification of most *P. aeruginosa* strains by the two-tube screen, good identification of most strains after 24 h (1, 21), and very little need to consult manufacturer's computer facilities (<1%). Prolongation of the incubation period to 48 h increased the correct identification of *A. odorans* and *A. denitrificans* strains, but led to a slight decrease in the identification rate of *P. maltophilia*. In contrast to findings by Warwood et al. (21), all *P. cepacia* strains tested were correctly identified by NF. Problems in interpretation of the NF N_2 gas reaction and lack of precise identification of asaccharolytic nonfermenters have been described before (1, 21). In our opinion, additional tests for phenylalanine deaminase (possibly in the center of the wheel) and motility/nitrate reductase (possibly as a third combination screen tube) would enhance the ability of the NF system to identify the *Alcaligenes/Moraxella* group. More precise package instructions on interpretation of N_2 gas results and increased sensitivity of the indole test would also improve this system.

Utilization of stock organisms supplied by kit manufacturers could have introduced bias into results reported in this study; however, we feel that the small number of strains from such sources (16%) as compared to cultures from G. L. Gilardi's laboratory (84%) argues against this possibility. Additionally, identification of organisms provided by firms did not differ significantly, when results of the supplier's kit were compared with those of competitors.

The clinical microbiology laboratory at Hershey Medical Center currently uses the API 20E system for routine nonfermenter identification: in the absence of facilities for conventional tests, it was felt that utilization of well-characterized stock cultures of clinical isolates would yield optimal information, under the circumstances. Edberg et al. (3) have suggested that direct comparison of two commercial kit systems without conventional tests merely reflects percentage agreement figures which represent the sum of innate errors of both; therefore, comparison of commercial kits would best be made on the basis of organism identification, rather than test-by-test comparison. In our study, no attempt was made to compare results of individual tests in each of the four respective systems; rather, emphasis was placed on accuracy of identification and shortcomings of individual tests in the same system.

Other methods for nonfermenter identification which were not evaluated in this study include the Micro-media system (Micro-media, Inc., Palo Alto, Calif.) and Microscan (Microscan, Inc., Ramsey, N.J.). A method which is not commercially available, but which seems to offer rapid and accurate identification of most nonfer-

menters from clinical specimens, is the oxidative attack (OA) system developed by Otto and coworkers (15, 16). If the OA method is marketed, it may prove to be one of the best commercial methods for identification of this organism group.

In summary, all four systems evaluated in this study show promise for identification of common, clinically significant nonfermenters: OF is the easiest to inoculate, whereas AP has the advantage of identifying *Enterobacteriaceae*. The MT system has the advantage of flexibility. The NF method is the only system to identify most organisms reliably after 24 h and has the added advantage of identifying most *P. aeruginosa* strains by the two-tube screen only. All four systems may be improved with a view to use without additional tests, by addition of new tests or modification of existing ones. In this way, accurate nonfermenter identification could be placed within the reach of smaller laboratories which do not possess facilities for extended conventional testing.

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