Accuracy and Reproducibility of the Oxi/Ferm System in Identifying a Select Group of Unusual Gram-Negative Bacilli

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The Oxi/Ferm (O/F) identification system was compared in a double-blind study to a conventional test battery for the characterization of 96 reference and clinical strains consisting of 83 nonfermentative and 13 oxidase-producing, fermentative gram-negative bacilli. The O/F tube and supplemental tests correctly identified 84% of the nonfermentative and 77% of the oxidase-producing, fermentative bacilli. However, when the supplemental tests were excluded and the biochemical profiles generated by all nine O/F tube reactions were examined, the profile accuracy reached 95% (79 of 83) for the nonfermentative and 93% (12 of 13) for oxidase-producing, fermentative bacilli. Seven of the nine O/F substrate reactions demonstrated $\geq 89\%$ agreement with conventional reactions, whereas the urea and arginine reactions provided 82 and 85% agreement, respectively. Replicate O/F tests with six selected organisms demonstrated 97% identification reproducibility and 84% overall substrate reproducibility. The mean O/F identification time was 2.6 days as compared to 3.3 days for the conventional system. Although this study suggests that the O/F system is a convenient, rapid, and accurate alternative to conventional identification methods, several modifications are recommended.

Nonfermentative and oxidase (OXI)-producing, fermentative gram-negative rods have been isolated from patients and the hospital environment with increasing frequency, and their clinical significance has been established (3, 5, 6, 12, 16, 19, 20). Despite the low degree of virulence and limited invasiveness of these organisms, they have been isolated from genitourinary and respiratory tracts, wounds, blood, and other body fluids of hospitalized patients and implicated as causative agents of sepsis, extensive suppurative lesions, or septic shock (1, 17). In addition, these organisms may demonstrate multiple drug resistance necessitating prompt identification and susceptibility testing for adequate therapy.

Simple, convenient, rapid, and standardized identification systems for these organisms have been developed recently. One of these, the Oxi/Ferm (O/F) system (Roche Diagnostics, Nutley, N.J.), proposes to provide identification of this bacterial group in 48 to 72 h by the use of miniaturized substrate quantities.

Earlier studies have evaluated certain aspects of this system (4, 7, 8, 14, 15, 18). However, our evaluation is unique in that it incorporates a double-blind methodology and challenges the system with a broad spectrum of 37 species of

atypical, nonfermentative and OXI-positive fermentative bacilli, some rarely encountered and some commonly encountered. This study investigates identification and substrate accuracy, as well as reproducibility and time and supplemental test requirements of the O/F system.

MATERIALS AND METHODS

Bacteria. The 96 organisms selected for this study reflect a comprehensive distribution of 83 nonfermentative bacilli, some commonly encountered and some less frequently encountered, and 13 OXI-positive, fermentative rods (Table 1). Forty organisms (42%) were stock cultures obtained from proficiency testing agencies (College of American Pathologists, American Society of Clinical Pathologists, and Massachusetts Department of Public Health), from an American Society for Microbiology workshop (conducted by Rudolph Hugh), and from the Center for Disease Control (Robert Weaver). The remaining 56 organisms (58%) were clinical isolates from urine, sputum, ear, eye, and wound cultures. Since *Acinetobacter* sp. and pyocyanogenic *Pseudomonas aeruginosa* may be readily identified by colonial and microscopic morphology or by a limited biochemical profile, few of these strains were included. Atypical strains included two nonfluorescent apyocyanogenic and six apyocyanogenic *P. aeruginosa* as well as one nonfluorescent *P. fluorescens* strain. All bacterial strains were maintained at -80°C on glass beads (13).

A double-blind evaluation was conducted with the cooperation of the quality control laboratory of the Lahey Clinic Foundation, which assigned randomly to each organism both an O/F system and a conventional method code number.

Unknown organisms were placed into tryptic soy broth (TSB; Grand Island Biological Co. [GIBCO], Lawrence, Mass.) and incubated for 24 h at 35°C (O/F) and at 30°C (conventional). Broths were sequentially subcultured onto tryptic soy agar plates with 5% sheep blood (sTSA; GIBCO) and to TSA (GIBCO) without blood. After an incubation period of 24 h, the organisms on TSA were used to inoculate O/F and conventional systems.

O/F identification system. The nine biochemical substrates of the O/F tube (lot numbers 0046 and 0051) were inoculated by using an isolated colony according to the manufacturer's instructions. Simultaneously, a 16- to 18-h TSB motility (MOT) test, a Kirby-Bauer (2) susceptibility battery (penicillin, ampicillin, carbenicillin, kanamycin, colistin, novobiocin, and nalidixic acid; Difco Laboratories, Detroit, Mich.), and a Kovacs OXI test (Marion Scientific, Kansas City, Mo.) were conducted. All O/F tubes and other subcultures were incubated at 35°C while cultures for flagella staining remained at room temperature. All media and corresponding reagents used in this evaluation were prepared commercially (GIBCO).

TSA cultures were observed for purity and colonial characteristics. As the manufacturer instructed, the O/F tube reactions were interpreted after 48 h of incubation. The second edition of the O/F computer manual was used for organism identification or selection of supplemental tests for differentiation of similar organisms.

Standard biochemical battery (conventional). Isolated colonies from 24-h-old TSA cultures were subcultured with an inoculating wire to a conventional biochemical battery consisting of: O/F—dextrose, lactose, maltose, and xylose (open and closed); Moeller's decarboxylase—lysine, ornithine, and control; arginine dihydrolase; triple sugar iron; Simmon's citrate (CIT); Christensen's urea (URE); nitrate broth with Durham's tube; TSB (xylene extraction for indole); and a semisolid MOT agar plate (M. Bennett, H. Nadler, and H. George, Am. J. Med. Technol., in press). A Kovacs OXI test (tetramethyl reagent) and a Kirby-Bauer antibiogram (2) were also performed. All screw caps of tubes were loosened, except tubes with oil overlay, and were incubated with plates at 30°C. MOT agars, brain heart infusion cultures for flagella staining, and other subcultures held for pigment production were incubated at room temperature. All conventional media and corresponding reagents were obtained from a commercial source (GIBCO). Of all media utilized in the evaluation 5% were tested for sterility at 30°C for 48 h, and samples from each lot of medium were tested for quality of performance by using reference strains of *Pseudomonas* species, *A. calcoaceticus* var. *lwoffi*, and *A. anitratum*.

All inoculated biochemicals were incubated and examined for 4 consecutive days. Organisms were identified according to standard criteria (9, 21; *Identification of Glucose Non-Fermenting Gram-Negative Rods*, American Society for Microbiology, 1975). If

adequate criteria for identification were not provided by data from the initial biochemical battery, supplemental tests were performed. These tests were selected from the following: flagella stain (Chroma-Gesellschaft Schmid and Co., West Germany) (*Identification of Glucose Non-Fermenting Gram-Negative Rods*, 1975); Gram stain (Hucker method); catalase; MacConkey; eosin-methylene blue; *Salmonella shigella*; sTSA; Sella's differential agar; cetrimide agar; deoxyribonuclease agar; o-nitrophenyl- β -D-galactopyranoside test; Mueller-Hinton agar (amylase test); phenylalanine deaminase agar; nutrient gelatin; nutrient broth; TSB (42°C growth, 6.5% NaCl tolerance); and phenol red broth with carbohydrate disks (Difco).

If inconclusive biochemical profiles were obtained, OXI, indole (IND), or nitrate tests were repeated, using different substrates (1% aqueous tetramethyl paraphenylenediamine dihydrochloride [Sigma Chemical Co., St. Louis, Mo.] and IND-nitrate broth).

Substrate reproducibility. To determine substrate variability of the O/F system, 11 coded replicate cultures of each of 6 reference strains (*P. aeruginosa* pyocyanogenic, *Aeromonas hydrophila*, *Flavobacterium meningosepticum*, *Bordetella bronchiseptica*, *P. putrefaciens*, and *Alcaligenes denitrificans*) were inserted randomly into the study.

RESULTS

Identification accuracy. Accurate identifications were obtained with 69 of 82 (84%) nonfermentative bacilli and 10 of 13 (77%) OXI-positive, fermentative bacilli for an overall accuracy of 83%. One misidentified strain of *Eikenella corrodens* was excluded from calculation of the O/F system's identification accuracy since the computer coding manual did not incorporate this organism into its data base. Similar identification accuracies were obtained with clinical isolates (88% accuracy) and reference strains (75% accuracy).

All strains of *P. cepacia*, *P. putrefaciens*, *Acinetobacter*, *Flavobacterium*, *Achromobacter xylosoxidans*, *Vibrio*, and *Chromobacterium violaceum* were correctly identified.

The 16 misidentified bacteria and the corresponding discrepant test reactions responsible for their misidentifications are listed in Table 1. Of the 16 misidentifications by the O/F system, only 5 resulted from inaccurate (false-negative) reactions within the O/F tube. The remaining 11 resulted from inaccurate MOT tests, flagella stain misinterpretations, coding system inadequacies, or from discrepancies in other test procedures that were supplemental to the O/F tube.

Substrate agreement. The overall agreement of all O/F tube substrates plus the supplemental MOT tests with conventional substrates was 92% for nonfermentative and 93% for OXI-producing, fermentative gram-negative bacilli. The percent agreement of individual O/F substrates and the composite O/F system with con-

TABLE 1. O/F system identifications

Organism	No. of strains misidentified/total	Misidentification	Discrepant reaction(s)
<i>P. aeruginosa</i> (pigmented)	0/2		
<i>P. aeruginosa</i> (nonpigmented)	1/8	<i>Achromobacter</i> spp.	No code for ADH (–) strains
<i>P. fluorescens</i> , <i>P. putida</i>	2/11	<i>P. putida</i> , <i>P. fluorescens</i>	Gelatin liquefaction
<i>P. maltophilia</i>	1/10	<i>Alcaligenes</i> spp.	OXI, weak (+)
<i>P. cepacia</i>	0/3		
<i>P. stutzeri</i> (mendocina variety)	2/6	<i>Achromobacter</i> spp. <i>P. vesicularis</i>	XYL (–), inadequate code ADH and N ₂ (–)
<i>P. putrefaciens</i>	0/1		
Nonsaccharolytic <i>Pseudomonas</i> spp.	1/5	<i>Alcaligenes</i> spp.	Flagella stain
<i>A. anitratum</i>	0/5		
<i>A. lwoffii</i>	0/2		
<i>Flavobacterium</i> spp.	0/7		
<i>Moraxella</i> spp.	1/7	<i>Alcaligenes</i> spp.	MOT (+)
<i>Alcaligenes</i> spp.	2/5	Nonsaccharolytic <i>Pseudomonas</i> spp. <i>Moraxella</i> -like, CDC 4f	Flagella stain
<i>B. bronchiseptica</i>	2/4	<i>Alcaligenes</i> spp. CDC IVe	MOT (–) URE (–) No growth (nutrient broth)
<i>A. xylosoxidans</i>	0/3		
CDC Ve, CDC Va	0/2		
CDC IIj	1/1	CDC IVe	IND (–), inadequate code
<i>E. corrodens</i>	1/1	<i>P. haemolytica</i>	No code
<i>P. multocida</i>	0/3		
<i>P. haemolytica</i>	1/1	<i>P. ureae</i>	No growth (MacConkey)
<i>P. ureae</i>	1/1	<i>P. haemolytica</i>	URE and OXI (–), inadequate code
<i>A. hydrophilia</i>	0/4		
<i>Plesiomonas shigelloides</i>	1/1	<i>V. parahaemolyticus</i>	Lysine (–)
<i>Vibrio cholerae</i> , <i>C. violaceum</i> , <i>V. parahaemolyticus</i>	0/3		

ventional biochemical reactions for nonfermentative and OXI-positive, fermentative bacilli is illustrated in Table 2. Six of the 10 tests—anaerobic glucose (AN-GLU), nitrogen gas (N₂), hydrogen sulfide (H₂S), IND, aerobic xylose (XYL), and aerobic glucose (GLU)—showed ≥95% agreement between O/F and conventional biochemical reactions.

Less than 90% correlation was observed with URE (82%), arginine dihydrolase (ADH; 85%), and MOT tests (86%) (Table 3). Fifteen of 17 URE test disagreements were due to false-negative URE reactions in the O/F tube, especially with *Pseudomonas* spp. The majority of ADH test discrepancies were attributable to false positives in the conventional system. Ten of 13 MOT test disagreements resulted from inaccurate MOT tests using the manufacturer's broth procedure. In addition, agreement of the alkalinity reaction in O/F and conventional GLU or XYL substrates occurred in only 38 of 70 (54%) instances.

Substrate and identification reproducibility. O/F substrate reproducibility was eval-

TABLE 2. Overall agreement between O/F and conventional substrates

O/F substrate	Agreement ^a
AN-GLU	81/83 (98)
ADH	79/93 (85)
N ₂	89/94 (95)
H ₂ S	93/93 (100)
IND	87/90 (97)
XYL ^b	87/92 (95)
GLU ^b	88/93 (95)
URE	75/92 (82)
CIT	84/94 (89)
MOT	80/93 (86)

^a Values indicate number in agreement per total tested; those in parentheses indicate percent. Average agreement was 92%.

^b Observation of alkalization in carbohydrate compartments was 38/70 (54%).

uated by replicate testing of six reference strains (Table 4). Fewer replicate tests were performed with *P. putrefaciens* because of overgrowth of several tests by another organism. Overall reproducibility for all positive O/F test responses and

TABLE 3. *Organisms with less than 90% agreement between O/F and conventional substrates*

Substrate	Organism	Agreement ^a
URE	<i>P. aeruginosa</i>	6/9
	<i>P. fluorescens</i> , <i>P. putida</i>	5/10
	<i>P. cepacia</i>	0/3
	<i>P. stutzeri</i>	5/6
	<i>Moraxella</i> spp.	6/7
	<i>Bordetella</i>	3/4
	CDC Ve, Va, IIj	2/3
	<i>Pasteurella</i> spp.	4/5
	<i>P. fluorescens</i> , <i>P. putida</i>	7/10
	<i>P. stutzeri</i>	5/6
ADH	Nonsaccharolytic	2/4
	<i>Pseudomonas</i> spp.	5/6
	<i>Acinetobacter</i> spp.	5/6
	<i>Flavobacterium</i> spp.	3/7
	<i>Bordetella</i>	3/4
	<i>Vibrio</i> spp.	1/2
MOT (flagellated organisms)	<i>P. stutzeri</i>	2/6
	Nonsaccharolytic	4/5
	<i>Pseudomonas</i> spp.	4/5
	<i>Alcaligenes</i> spp.	4/5
MOT (nonflagellated organisms)	<i>Acinetobacter</i> spp.	5/6
	<i>Flavobacterium</i> spp.	5/7
	<i>Moraxella</i> spp.	5/7
	<i>Pasteurella</i> spp.	4/5

^a Values indicate number of strains in agreement per total number tested.

MOT tests was 84%, and overall reproducibility for all negative test reactions was 99.6%.

Substrate reproducibility for positive test reactions ranged from 86 to 100% for ADH, H₂S, IND, XYL, CIT, and MOT tests. Substrate reproducibilities of less than 86% were detected with N₂, URE, AN-GLU, GLU, and ALK (alkalinization of carbohydrates) reactions. Alkalization of GLU and XYL compartments in the O/F tube was a variable observation with only 65% overall reproducibility for all organisms tested. The reproducibility for a negative test reaction was ≥90% for all O/F substrates and MOT tests.

The review of individual organism performance with the variable O/F substrates is shown in Table 5. Production of nitrogen gas was detectable in only 4 of 11 (36%) tests performed with *A. denitrificans*. Hydrolysis of the O/F URE was a variable demonstration with the weak urease-producing organisms; only 5 of 11 (45%) tests with *A. denitrificans* and 1 of 11 (9%) tests with *P. aeruginosa* were positive. The O/F AN-GLU and GLU reactions were variable with *F. meningosepticum*, a positive AN-GLU

TABLE 4. *Overall O/F reproducibility*

O/F substrate	Agreement ^a
Positive reaction	
AN-GLU	14/21 (67)
ADH	22/22 (100)
N ₂	15/22 (68)
H ₂ S	6/7 (86)
IND	21/21 (100)
XYL ^b	11/11 (100)
GLU ^b	26/32 (81)
URE	16/33 (48)
CIT	42/44 (95)
MOT	48/51 (94)
Negative reaction	
AN-GLU, ADH, N ₂ , H ₂ S, IND, XYL, GLU, URE, and CIT	336/336 (100)
MOT	9/10 (90)

^a Values indicate number in agreement per total tested, and those in parentheses indicate percent. Average reproducibility was 84 and 99.6% for positive and negative reactions, respectively.

^b Observation of alkalization in carbohydrate compartments was 51/79 (65%).

TABLE 5. *Problems of O/F substrate reproducibility^a*

O/F substrate	Organism	Agreement ^b
URE	<i>P. aeruginosa</i>	1/11
	<i>A. denitrificans</i>	5/11
	<i>B. bronchiseptica</i>	10/11
N ₂	<i>A. denitrificans</i>	4/11
AN-GLU	<i>F. meningosepticum</i>	3/10
GLU	<i>F. meningosepticum</i>	4/10

^a Substrates with <86% agreement in replicate tests.

^b Values indicate number of replicates in agreement per total tested.

reaction was demonstrated in only 3 of 10 tests (30%), and a positive GLU reaction was observed in only 4 of 10 tests (40%).

Variability in the O/F URE test of one *B. bronchiseptica* and the O/F H₂S test of one *P. putrefaciens* was responsible for their misidentification as *Alcaligenes* species. All other organisms were identified correctly in the 63 replicate inoculations.

Time required for O/F system identifications. A comparison of the time required for O/F and conventional system identifications revealed that 47% of the organisms were more rapidly identified by the O/F system with a mean identification time of 2.6 days, in contrast to 3.3 days for the conventional system. Forty-five other organisms required the same identification time using both methods, and 42% of the organisms required an average of 1.7 supplemental tests to provide a final O/F identification.

Ease in interpretation of substrates. The O/F system was convenient to use, and the

reaction end points were easily discernible by using manufacturer's color charts in 89% (136 of 153) of all O/F tubes inoculated for either organism identification or reproducibility testing. Ambiguous end points in several compartments (GLU, XYL, and CIT) and URE color changes not described by the manufacturer did not influence final identifications.

DISCUSSION

As of this report, we are aware of six earlier evaluations of the O/F system. Isenberg et al. (7), using the first edition of the computer manual, compared O/F and conventional substrate reactions and identification accuracy for 21 species of commonly encountered, nonfermentative rods and *Aeromonas*, and reported an identification accuracy of 97%. Nord et al. (14), also using the first edition of the computer manual, conducted a similar evaluation with coded organisms of three bacterial genera (*Pseudomonas*, *Aeromonas*, and *Alcaligenes*) and reported an identification accuracy of 92%. Oberhofer et al. (15), employing the second edition of the computer manual, evaluated the system in its identification of 33 species of common and occasionally encountered, nonfermentative, and OXI-producing fermentative rods and found an overall identification accuracy of 89%. Unlike previously described studies, that of Dowda (4) compared the identification accuracy of the O/F system with the API system, rather than with conventional methods. This comparison used 17 species of common nonfermentative and OXI-positive fermentative rods and yielded a 94% identification accuracy. The most recent investigators, Shayegani et al. (18), evaluated 48 species of common and of less frequently encountered nonfermentative bacilli with the O/F and conventional systems and indicated that 27.2% were misidentified and 16.7% were unidentified.

Unlike these earlier reports, this composite study was designed to combine a number of criteria which previously had been evaluated individually. We compared O/F and conventional systems by using a double-blind methodology, identified a comprehensive and selective distribution of reference and clinical strains, and determined O/F system reproducibility, identification time, and supplemental test requirements, using the second edition of the computer manual.

In this evaluation, the O/F system, including supplemental tests, identified accurately 83% of all bacteria tested. This finding falls within the range of accuracy (70 to 82%) estimated by Lapage et al. for conventional and computer-assisted identification methods for nonfermenta-

tive bacilli (10). If the supplemental test reactions recommended by the computer manual had provided appropriate test responses, the O/F system's identification accuracy would have increased to 95% (90 of 95).

O/F substrate agreements with conventional biochemicals of $\geq 95\%$ for AN-GLU, N_2 , H_2S , IND, XYL, GLU, and 89% for CIT are consistent with other studies (8, 14, 15). Our findings of an 18% disagreement for URE and 15% disagreement for O/F MOT suggest that the manufacturer should consider improving these specific tests.

The less reproducible reactions noted in the O/F tube were N_2 , URE, AN-GLU, and GLU. Our observation of marked variability of the rapid N_2 test with *A. denitrificans* is in agreement with the work of Isenberg et al. (7), and substitution of the N_2 test by a more reliable procedure may be advisable. Our finding that urease production by *Alcaligenes* demonstrated only 48% reproducibility is in agreement with other reports (7, 8, 14) of variability and inaccuracy of the URE reaction. In contrast to earlier reports (7, 8), our evaluation did not detect problems with the CIT reaction. We did experience problems with the AN-GLU and GLU reactions of *Flavobacterium* spp., as did other workers (15).

An increase in overall substrate reproducibility from 84 to 91% would be achieved by improving the URE and N_2 substrates in the O/F test battery. The utility of the ALK test in organism identification should be reevaluated, since we observed an agreement of only 54% between O/F and conventional GLU and XYL alkalization and an average reproducibility of only 65% for the O/F ALK test.

As a result of the problems experienced while performing this study, we recommend the following changes to improve overall accuracy of O/F system identification. Improvement of the supplemental test battery would be accomplished by providing detailed instructions for each supplemental test, e.g., inoculation of inhibitory media or determination of growth at 42°C, to ensure standardized results. Semisolid agar plates may provide a more reliable MOT test than the manufacturer's recommended broth procedure. Replacement of gelatin liquefaction by a lecithinase test could provide a less variable and more rapid result. Increased use of the *o*-nitrophenyl- β -D-galactopyranoside and the 6.5% NaCl tolerance tests could improve the system's overall performance. Incorporation of amylase (11), deoxyribonuclease and fluorescein (Flo and Tech agars; BBL) tests into the supplemental test battery could provide additional

identification guidelines. Uniqueness of flagella morphology could be used to differentiate non-saccharolytic pseudomonads, e.g., *P. diminuta* and *P. acidovorans*.

To facilitate more rapid identification, we recommend the simultaneous inoculation of the O/F tube and semisolid MOT plate and performance of a Kirby-Bauer antibiogram incorporating the special drugs indicated in the O/F coding manual.

Suggested revisions of the computer manual include addition of organisms and corresponding differentiation criteria to six coding manual numbers: 0000 *Pasteurella* spp. (OXI negative); 0001 *E. corrodens* and *P. putrefaciens* (H₂S negative); 0021 CDC IIj (IND negative); 1071 *P. stutzeri*; 1171 *P. aeruginosa* (ADH negative); and 4271 *V. parahaemolyticus*. The computer manual should also print each test result of the biochemical profile next to the code number to minimize misidentifications arising from clerical errors in computing the four-digit code.

The code numbers of three organisms were not incorporated into the O/F coding manual: 1041 *P. stutzeri*, 0200 *P. multocida*, and 4271 *V. parahaemolyticus*.

Overall evaluation and conclusion. This double-blind evaluation suggests that the O/F system is a convenient, rapid, and acceptable alternative to conventional methods for identification of both commonly and rarely encountered nonfermentative and OXI-positive fermentative, gram-negative bacilli in only 48 to 72 h. Until revisions are made, we suggest each O/F numerical identification be evaluated against the manufacturer's bacterial identification chart based on expected biochemical reactions.

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LITERATURE CITED

1. Abrutyn, E., and S. Plotkin. 1975. *Flavobacterium meningosepticum* and *Alcaligenes faecalis* meningitis: a review, p. 113-123. In A. von Graevenitz and T. Sall (ed.), *Pathogenic microorganisms from atypical clinical sources*. Marcel Dekker, Inc., New York.
2. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turek. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45:493-496.
3. Cabrera, H. A., and M. A. Drake. 1975. An epidemic in a coronary care unit caused by *Pseudomonas* species. *Am. J. Clin. Pathol.* 64:700-704.
4. Dowda, H. 1977. Evaluation of two rapid methods for identification of commonly encountered nonfermenting or oxidase-positive, gram-negative rods. *J. Clin. Microbiol.* 6:605-609.
5. Gilardi, G. L. 1972. Infrequently encountered *Pseudomonas* species causing infection in humans. *Ann. Intern. Med.* 77:211-215.
6. Henriksen, S. 1973. *Moraxella*, *Acinetobacter*, and the *Mimeae*. *Bacteriol. Rev.* 37:522-561.
7. Isenberg, H. D., and J. Sampson-Scherer. 1977. Clinical laboratory evaluation of a system approach to the recognition of nonfermentative or oxidase-producing gram-negative, rod-shaped bacteria. *J. Clin. Microbiol.* 5:336-340.
8. Kilbourn, J. P. 1977. Oxi-Ferm system: letter to the editor. *Am. J. Med. Technol.* 43:1167-1168.
9. King, E. O. 1972. The identification of unusual pathogenic gram negative bacteria. United States Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, Ga.
10. Lapage, S. P., S. Bascomb, W. R. Willcox, and M. Curtis. 1973. Identification of bacteria by computer: general aspects and perspective. *J. Gen. Microbiol.* 77:273-290.
11. Lee, W. S. 1976. Use of Mueller-Hinton agar as amylase testing medium. *J. Clin. Microbiol.* 4:312.
12. Louria, D. B. 1974. Superinfection: a partial overview, p. 1-18. In J. E. Prier and H. Friedman (ed.), *Opportunistic pathogens*. University Park Press, Baltimore.
13. Nagel, J. G., and L. J. Kunz. 1972. Simplified storage and retrieval of stock cultures. *Appl. Microbiol.* 23:837-838.
14. Nord, C. E., B. Wretling, and A. Dahlback. 1977. Evaluation of two test-kits—API and Oxi/Ferm tube—for identification of oxidative-fermentative gram-negative rods. *Med. Microbiol. Immunol.* 163:93-97.
15. Oberhofer, T. R., J. W. Rowen, G. F. Cunningham, and J. W. Higbee. 1977. Evaluation of the Oxi/Ferm tube system with selected gram-negative bacteria. *J. Clin. Microbiol.* 6:559-566.
16. Rosenthal, S. L. 1974. Sources of pseudomonas and acinetobacter species found in human culture materials. *Am. J. Clin. Pathol.* 62:807-811.
17. Sanford, J. P. 1973. Approach to the evaluation and prevention of aerobic gram-negative bacillary nosocomial infections, p. 43-55. In *Seminar on gram-negative infections*. Schering Corp., Kenilworth, N.J.
18. Shayegani, M., A. M. Lee, and D. M. McGlynn. 1978. Evaluation of the Oxi/Ferm tube system for identification of nonfermentative gram-negative bacilli. *J. Clin. Microbiol.* 7:533-538.
19. United States Public Health Service. 1974. Nosocomial *Pseudomonas cepacia* bacteremia caused by contaminated pressure transducers. *Morbidity Mortal. Weekly Rep.* 23:423.
20. von Graevenitz, A., and J. Weinstein. 1971. Pathogenic significance of *Pseudomonas fluorescens* and *Pseudomonas putida*. *Yale J. Biol. Med.* 44:265-273.
21. Weaver, R. 1974. Gram-negative organisms: an approach to identification. Center for Disease Control, Atlanta, Ga.