Evaluation of the Oxi/Ferm Tube System with Selected Gram-Negative Bacteria

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The Oxi/Ferm test system was evaluated for accuracy and reliability for identification of nonfermentative and oxidase-positive fermentative bacteria by using 375 bacterial strains obtained from stock culture and clinical specimens. The Oxi/Ferm system is a compartmentalized tube containing eight media to provide nine biochemical test results. When combined with the oxidase test, the results corresponding to the positive reactions are totaled and the composite number is located in the coding manual to identify the organisms. The 375 isolates studied were evaluated for accuracy of identification, using both the original and revised code manuals. In comparison with the conventional media used, there was 100% correlation in tests for hydrogen sulfide and indole production, over 96% for nitrogen gas, arginine, and urease, over 92% for xylose and dextrose oxidation, and less than 90% for citrate utilization and dextrose fermentation. There was an overall accuracy in identification of 89.3% using the original manual, with accuracy revised slightly upward to 90.7% using the revised manual. There was 100% accuracy in identification with 44.0% of the strains tested (11 species) using the original manual and with 66.1% (16 species) using the revised manual. Thirteen of the 40 original misidentifications and 14 of 35 revised misidentifications resulted from failure to code and were unidentifiable by Oxi/Ferm. The remainder were incorrectly identified or could not be differentiated from closely related strains. Eleven strains of *Alcaligenes odorans* were correctly identified using the original code, whereas no code was provided in the revised manual. The Oxi/Ferm system is both simple and rapid and is satisfactory for identification of the more common isolates.

Commercial test systems for rapid identification of *Enterobacteriaceae* have been available for a number of years. Each test system, or kit, has enjoyed variable success in the ease of operation, rapidity of test performance, and accuracy of identification when compared with conventional testing systems. Specifically, the Enterotube test system received unflattering reviews in early reports (5, 9) because of difficulty in interpretation and inaccuracies of various test components. A later modification of the Enterotube provided a convenient system with much improved capacities for proper genus and species identification (2, 12, 15). However, methods used for differentiation and identification of nonfermentative bacteria (NFB) have been dependent upon lengthy biochemical batteries, incubation of tests for 7 to 10 days for proper identification, and complex formulations that are difficult to maintain in most laboratories. In addition,

choices of schema are diverse and sometimes disparate in selection of media and methods (7, 10, 13, 14). Recently, a multiple-test system has been marketed for identification of oxidase-positive and oxidase-negative NFB as well as oxidase-positive fermentative bacteria. The Oxi/Ferm (OxF) tube system (Roche Diagnostic Division, Hoffman-LaRoche, Inc., Nutley, N.J.), similar to the Enterotube, contains eight compartments and combines nine biochemical tests. The OxF tube is purported to be the first attempt to provide a rapid, concise, and convenient approach to identification of these organisms. This report presents the results of an evaluation of the OxF tube system for both the test reactions and accuracy of identification based on methods used in this laboratory.

MATERIALS AND METHODS

Organisms. The 375 cultures used in this evaluation were stock organisms retrieved from the collection of the Special Bacteriology Sub-Section, as well as

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isolates recently recovered from primary cultures. The stock organisms had been held in motility stabs at room temperature and in the lyophilized state. Methods used for identification of the NFB have been described (10). Fermentative bacteria were identified by using 27 tests according to the methods of Edwards and Ewing (4). Clinical specimens were streaked to 5% sheep blood, MacConkey, and salmonella-shigella agars for isolation, and isolates were identified by using the protocol for NFB (10) or fermentative bacteria (4). Stock cultures tested were not run concurrently by conventional and OxF procedures, whereas primary cultures were. The stock cultures were retested by conventional means only when discrepancies were found between the conventional and OxF systems.

Conventional media. The conventional tests against which the OxF reactions were compared included: triple sugar-iron agar (TSI) for dextrose fermentation and H₂S production; rapid arginine broth (11) for dihydrolase activity; potassium nitrate broth with inverted tube for nitrogen gas production; Casitone broth with xylene extraction and peptone broth, respectively, for indole production by NFB and fermentative bacteria; OF special medium (phenol red indicator) with 1% dextrose and xylose for acidification tests of NFB and Andrade base with 1% sugars for fermenters; Christensen agar for urease; and Simmons agar for citrate utilization. Results of tests were based on 48-h incubation to coincide with 48-h results in the OxF system. Susceptibility tests using the standardized single-disk method (1) were performed routinely.

OxF system. Except for three gratuitous boxes of 16 tubes each, the OxF tubes were commercially purchased. The OxF tube is a compartmentalized container consisting of agar media for testing, consecutively, acid from dextrose (anaerobic), arginine dihydrolase, denitrification, hydrogen sulfide and indole production, acid from dextrose, acid from xylose, and urease and citrate utilization. The compartments used to test for dextrose fermentation, arginine dihydrolase, and gas formation are sealed in paraffin to provide an anaerobic environment. The remaining compartments are perforated to provide oxygenation.

OxF test procedure. The conduct of the OxF tests followed the procedure described in the manufacturer's directions, with a few innovations. Stock cultures were streaked for isolation on the plated media and incubated for 18 to 24 h at 35°C. Plates containing the stock isolates were examined for morphological characteristics, with pertinent observations recorded. A Gram stain was prepared and an oxidase test (Kovacs reagent) was performed from isolated colonies, and the results were also recorded. OxF tubes and Trypticase soy agar (TSA) plates were warmed to room temperature before use. A large inoculum of the culture was gathered on the OxF inoculating needle and drawn through the tube with a single twisting motion. The needle was then used to streak the TSA plate, reinserted through the anaerobic dextrose, arginine, and gas compartments, and broken off at the designated point. The caps were replaced, and the adhesive strip covering the perforations was removed and placed across the top of the tube for future use.

Concurrently, a TSI slant and a tube of semisolid motility medium were inoculated using colonies from the same plate as for OxF, followed by incubation of all tests at 35°C. The TSA plates and TSI slants were removed after overnight incubation and allowed to remain at room temperature to enhance pigment production. The motility stabs were also removed at this time and observed for motility, with nonmotile cultures further tested by the slide method. Plates containing the recent isolates were handled in a similar manner, except that the oxidase, TSI, and motility tests were integrated into the routine battery of tests. The OxF tubes were examined at 48 h, the observations were recorded, and reagents were added as appropriate. It was necessary to read the tube reactions before adding reagents, since this caused leakage between compartments. The adhesive strip was removed from the top of the tube and used to cover the needle holes on the underside.

Test reactions. Fermentation or oxidation of carbohydrates changed the indicator from green to various shades of yellow, as compared with an uninoculated control. A positive arginine dihydrolase test was denoted by an obvious change of the medium from colorless to purple. Nitrogen gas production was evidenced by a separation of the paraffin seal from the agar. A test for nitrite production was performed in the same compartment as gas production, by penetrating the paraffin seal with a separate needle and syringe containing each of conventional reagents and implanting a few drops of each reagent under the seal. A bright red color resulted in a positive test. This test was not included in the scheme of the manufacturer. Hydrogen sulfide was demonstrated by a blackening along the inoculum wire. The indole test was performed by penetrating the plastic film with a needle and syringe and allowing a few drops of reagent (Kovacs) to run down the plastic film. A positive urease test was shown by a change in indicator from yellow to pink or red. Utilization of citrate was noted by a change in indicator from green to blue.

Positive reactions in OxF were checked on the pad provided; the checked positive numbers were totaled, and the composite numbers were then located in the code manual. Suggested supplemental tests to separate two or more organisms of a given code were also given in the code manual, and these were performed when necessary. Specific tests which showed discrepancies between the two systems were repeated with the conventional media, using as inoculum isolates from the TSA plate. The OxF test was not repeated.

RESULTS

The 375 organisms used in this evaluation were identified by conventional methods (10) and are listed in Table 1. Comparisons of biochemical tests in the OxF system and conventional media are shown in Table 2. Confirmed conventional test reactions were considered correct, and the results indicate the final correlation. With the exception of the anaerobic dextrose test, there were more positive tests in conventional media than in OxF. Tests for hy-

TABLE 1. Summary of isolates used in evaluation

Organism	No. tested
<i>Pseudomonas aeruginosa</i>	46
<i>P. acidovorans</i>	8
<i>P. alcaligenes</i>	2
<i>P. cepacia</i>	6
<i>P. diminuta</i>	7
<i>P. fluorescens</i>	14
<i>P. maltophilia</i>	23
<i>P. mendocina</i>	3
<i>P. pseudoalcaligenes</i>	15
<i>P. putida</i>	25
<i>P. putrefaciens</i>	4
<i>P. stutzeri</i>	15
<i>P. stutzeri</i> -like	6
<i>P. testosteroni</i>	9
<i>Archromobacter xylosoxidans</i>	16
<i>Achromobacter</i> sp.	6
<i>Flavobacterium meningosepticum</i>	6
<i>Flavobacterium</i> IIb	15
<i>Flavobacterium</i> IIc	1
CDC IIk-1	3
CDC IIk-2	4
CDC VE-1	3
CDC VE-2	9
<i>Alcaligenes faecalis</i>	7
<i>A. odorans</i>	11
<i>Bordetella bronchiseptica</i>	6
CDC IVc (IVe)	3
<i>Acinetobacter anitratus</i>	35
<i>A. lwoffii</i>	31
<i>Moraxella osloensis</i>	7
<i>Moraxella</i> M-4f	1
<i>Aeromonas hydrophila</i>	17
<i>Pasteurella multocida</i>	11
Total	375

drogen sulfide, indole, nitrogen gas production, arginase activity, and urea hydrolysis showed over 96% agreement. Agreement was better than 92% for nitrite production, acid from xylose, and acid from dextrose. Utilization of citrate and acid from anaerobic dextrose agreed less than 90% for the two methods. Overall agreement was 95.0% between the two systems.

Hydrogen sulfide and indole production. The hydrogen sulfide and indole reactions in the OxF tubes were identical to those in the conventional media (100%). The hydrogen sulfide reactions of the four strains of *Pseudomonas putrefaciens* were easily interpreted. An occasional latent pigment-producing strain of *P. aeruginosa* caused a darkening of the medium, although not the definite blackening expected along the line of inoculation. Indole reactions were clear and precise. It was essential that the reagent be applied to the plastic film, since reactions were not evident when reagent was added to the agar. All strains of *P. multocida*, 16 strains of 17 *Aeromonas hydrophila*, and 15 of the 22 strains of flavobacteria produced indole in both tests.

Nitrogen gas production. Gas formation in OxF correlated well with that in nitrate broth (98.4%). The production of gas was sufficient to be evidenced at the ceiling of the compartment in addition to lifting the paraffin seal. Four strains of *P. aeruginosa* produced gas in broth but not in OxF, although each was identifiable. One strain each of *P. stutzeri* produced gas in broth but not in OxF and conversely, with the OxF-negative culture not identifiable.

Arginine dihydrolase. The arginine tests agreed extremely well in the two systems (97.1%). The test in OxF was easily interpreted, and positive reactions, with two exceptions (one

TABLE 2. Comparison of results of conventional tests and the Oxi/Ferm system using 375 organisms

Test	No. positive		No. negative		No. positive in agreement	No. negative in agreement	Total agreement	
	C ^a	OxF ^b	C	OxF			No.	%
Hydrogen sulfide	4	4	371	371	4	371	375	100.0
Indole	42	42	333	333	42	333	375	100.0
Nitrogen gas	77	72	298	303	72	297	369	98.4
Arginine	113	106	262	269	104	260	364	97.1
Urease	76	69	299	306	66	296	362	96.5
Nitrite production	110	100	265	275	100	258	358	95.5
Xylose oxidation	178	168	197	207	162	192	354	94.4
Dextrose oxidation	216	190	159	185	188	158	346	92.3
Citrate utilization	259	234	116	141	225	111	336	89.6
Anaerobic dextrose	28	53	347	322	16	309	325	86.7
								Avg 95.0

^a C, Conventional tests.

^b OxF, Oxi/Ferm system.

isolate each of *P. fluorescens* and *P. putida*), were quite intense. On a few occasions there was a slight discoloration of the media in the negative tests, which was ignored. Six strains of *P. stutzeri*-like (CDC Vb-3), two *Achromobacter* sp., and one *P. fluorescens* were arginine positive in the rapid test, but not in OxF. Two strains of *Achromobacter xylosoxidans* were arginine positive in OxF and negative in the rapid test.

Urea hydrolysis. Agreement in the urease tests was 96.5%. The reactions were, for the most part, strong in intensity. One strain of *Acinetobacter lwoffii* and two strains of *P. aeruginosa* gave weak but unequivocally positive tests in OxF and repeated negative tests in conventional media. Three strains of *P. cepacia* and CDC VE-2, two of *P. aeruginosa*, and one each of *P. maltophilia* and CDC IVc failed to give a urease reaction in OxF, although a positive test was obtained in conventional medium.

Nitrite production. The manufacturer makes no claim to the ability of the OxF system to detect nitrites, nor are codes provided in the manuals. Since the nitrogen gas compartment apparently contains nitrate, it seemed advantageous to perform the test for nitrite. Such tests proved quite satisfactory (95.5%), considering that 12 of the 17 discrepancies were found with gas-producing cultures of *P. aeruginosa*, *P. stutzeri*, and *A. xylosoxidans*. For the non-gas-producing cultures, there was 99% agreement between the two systems.

Xylose and dextrose oxidation. Difficulty in interpreting acid production in the xylose compartment of OxF resulted in a 94.4% agreement with the conventional test. When compared with uninoculated OxF tubes, the slight yellowing reactions observed in the compartments could not be discounted and were considered to be positive tests. Nevertheless, false negative xylose oxidation reactions in OxF accounted for 17 of the 21 discrepancies, with 11 due to the *P. stutzeri* group (three strains), *Flavobacterium* IIB (three strains), and *P. multocida* (five strains). Repeat xylose tests in conventional media confirmed a false negative result. Extended incubation for xylose oxidation could not be accomplished after addition of the indole reagent because of leakage between compartments. The dextrose compartment remained unaffected by the reagent. The OxF dextrose test (92.3% agreement) was not as difficult to read as the xylose test, although there were 27 false negative and two false positive reactions after the 2-day incubation period. Again, 11 *P. multocida*, 6 *P. stutzeri* group, and 3 *Flavobacterium* IIB strains accounted for most of the discrepancies. One strain each of *P. stutzeri* required 3 and 4 days, respectively, for a

positive reaction to appear in the compartment. Conversely, two strains of *Flavobacterium* IIB were frankly dextrose positive in OxF, whereas 5 days were necessary for the conventional reactions.

Citrate utilization. There was only an 89.6% correlation between the two tests with negative OxF reactions, accounting for most of the discrepancies. Two strains of *P. acidovorans* and one of *A. xylosoxidans* were repeatedly positive in 24 h in Simmons medium, yet each required 3 days in OxF. Thirty-six other organisms clearly showed discrepant results; of these, 2 *Alcaligenes faecalis*, 2 *Acinetobacter anitratus*, 2 *P. aeruginosa*, 14 *P. maltophilia*, and 5 *Flavobacterium* IIB were positive in Simmons agar and negative in OxF. It should be noted that *P. maltophilia* is not coded for a positive citrate test. One strain each of *Achromobacter* sp. and *Acinetobacter lwoffii* and three strains of CDC Ilk-2, were positive in OxF and repeatedly negative in Simmons medium. This latter organism is considered to have a negative characteristic for citrate utilization, although it is coded as a positive test in OxF.

Anaerobic dextrose. There was an 86.7% correlation in fermentation reactions by OxF and conventional methods, with 38 of the 50 discrepancies due to positive OxF reactions. Six *F. meningosepticum*, 13 *Flavobacterium* IIB, 4 *Acinetobacter anitratus*, 2 *P. cepacia*, and 1 *P. putida* failed to ferment glucose in TSI but did so in OxF. The flavobacteria and flavobacterium-like organisms are questionable fermenters since they can acidify a TSI slant. A few of the organisms did, indeed, give delayed acid slants, but were considered negative for the 2-day comparison. Fermentation of dextrose was coded for *A. anitratus* cultures, giving an accurate identification. The seal in the compartment used for *P. putida* was separated from the agar and apparently accounts for the true false positive test. In contrast, all of the 11 strains of *P. multocida* fermented dextrose in TSI but not in OxF. This negative characteristic was coded for an accurate identification.

Accuracy of identification. Table 3 shows the accuracy of the OxF system to identify the various species of NFB and oxidase-positive, fermentative bacteria. Of the 375 cultures tested, the OxF method correctly identified 335 (89.3%) based on information contained in the original code manual. Recently a revised manual became available, and each culture was re-evaluated by the new criteria. In the revision, some organisms lost their species designation. This is reflected in Table 3 as equivocal results. Under the new code, accuracy was revised slightly upwards to 90.7%. Eleven species (165 strains) were

TABLE 3. Accuracy of the Oxi/Ferm system in comparison with conventional methods

Organism	No. tested	Original code ^a		Revised code ^a		
		No. correct	% Correct	No. correct	No. equivocal ^b	% Correct
<i>Pseudomonas diminuta</i>	7	7	100.0		7	100.0
<i>P. fluorescens</i>	14	14	100.0	14		100.0
<i>P. maltophilia</i>	23	23	100.0	23		100.0
<i>P. pseudoalcaligenes</i>	15	15	100.0		15	100.0
<i>P. putrefaciens</i>	4	4	100.0	4		100.0
<i>Acinetobacter anitratus</i>	35	35	100.0	35		100.0
<i>A. lwoffii</i>	31	31	100.0	31		100.0
<i>Alcaligenes odorans</i>	11	11	100.0	0		0.0
<i>Moraxella</i> sp.	8	8	100.0	8		100.0
<i>P. multocida</i>	11	11	100.0	11		100.0
<i>Achromobacter</i> sp.	6	6	100.0	6		100.0
<i>A. xylosoxidans</i>	16	15	93.8	13		81.3
<i>P. aeruginosa</i>	46	43	93.5	46		100.0
<i>Bordetella</i> sp. ^c	9	8	88.9	8		88.9
CDC VE ^d	12	10	88.3	9		75.0
<i>Aeromonas hydrophila</i>	17	15	88.2	16		94.1
<i>P. putida</i>	25	22	88.0	25		100.0
<i>P. stutzeri</i> group ^e	24	21	87.5	16		66.7
<i>Flavobacteria</i> ^f	22	19	86.4	19		86.4
<i>Alcaligenes faecalis</i>	7	6	85.7	7		100.0
CDC IIk ^g	7	5	71.4	5		71.4
<i>P. cepacia</i>	6	3	50.0	6		100.0
<i>P. testosteronei</i>	9	2	22.2		8	88.9
<i>P. acidovorans</i>	8	1	12.5		8	100.0
<i>P. alcaligenes</i>	2	0	0.0		2	100.0
Total	375	335	89.3	300	40	90.7

^a Results were judged for accuracy using the original code book. Cultures were re-evaluated for accuracy of identification using the revised code book.

^b Number of strains equivocal, identified as *Pseudomonas* sp. only.

^c *B. bronchiseptica*, six strains; CDC IVc (IVe), three strains.

^d CDC VE-1, three strains; CDC VE-2, nine strains.

^e *P. mendocina*, 3 strains; *P. stutzeri*, 15 strains; *P. stutzeri*-like, 6 strains.

^f *F. meningosepticum*, 6 strains; *Flavobacterium* IIb, 15 strains; CDC IIc, 1 strain.

^g CDC IIk-1, three strains; CDC IIk-2, four strains.

100% correct in identification and accounted for 44.0% of the strains tested. With the new code, 248 strains (66.1%) were identifiable 100% of the time. All 11 of the *Alcaligenes odorans* strains were misidentified as *A. faecalis* using the new code. *P. aeruginosa* and *Achromobacter xylosoxidans* (16.5% of the strains tested) were correctly identified more than 93% of the time. A change in the code allowed only 81.3% of the *A. xylosoxidans* strains to be identified, although all of the *P. aeruginosa* were correct. Results with *Bordetella* sp., CDC VE, *Aeromonas hydrophila*, *P. putida*, *P. stutzeri* group, flavobacteria, and *Alcaligenes faecalis* (30.9% of strains) were over 85% correct. The remainder of the organisms (8.5%) were identifiable in less than 75% of instances using the original coding system, whereas use of the revised manual increased accuracy of the OxF system to 100% with *P. cepacia* and to equivocal with *P. acidovorans* and *P. alcaligenes*. The revised code

sacrificed species identification of the accurately identified *P. diminuta* and *P. pseudoalcaligenes*.

Table 4 shows the source of identification errors with selected organisms. Of the 32 errors listed, 14 were the result of the failure of the system to code for various reactions. *Flavobacterium* IIb and CDC IIk-2 were in total agreement in both systems, but neither manual properly coded for the reactions obtained. Five cultures of *P. stutzeri* and 11 *A. odorans* were properly coded in the original manual, but the codes were either eliminated or misrepresented in the revised manual. The system clearly proved to be inadequate to identify to species the remaining organisms.

DISCUSSION

The Oxi/Ferm system is a convenient, simple, and rapid approach to the identification of NFB.

TABLE 4. Source of discrepancies between the Oxi/Ferm system and conventional tests with 32 selected organisms

Organism	No.	OxF identification	Discrepancy ^a
<i>Pseudomonas stutzeri</i>	2	No code (O,R) ^b	Variable reactions
<i>P. stutzeri</i>	5	No code (R)	D-negative in OxF
<i>Achromobacter xylosoxidans</i>	1	No code (O,R)	C-negative in OxF
<i>A. xylosoxidans</i>	2	No code (R)	A-positive in OxF
<i>Flavobacterium</i> IIb	1	No code (O,R)	X-positive both systems
<i>Aeromonas hydrophila</i>	1	No code (O,R)	AD-negative in OxF
CDC IIk-2	1	No code (O,R)	C-negative both systems
CDC VE-1	1	No code (R)	AD-negative in OxF
<i>P. stutzeri</i>	1	<i>P. pseudoalcaligenes</i> (O)	D-negative in OxF
CDC IVc (IVe)	1	<i>Alcaligenes faecalis</i> (O,R)	U-negative in OxF
<i>Flavobacterium</i> IIb	1	CDC IIk-1 (O,R)	Atypical strain
<i>Flavobacterium</i> IIb	1	<i>Vibrio cholerae</i> (O,R)	X-positive in OxF
CDC IIk-1	1	Flavobacteria (O,R)	D-positive in OxF
CDC VE-1	2	CDC VE-2 (O,R)	AD-positive in OxF
<i>Alcaligenes odorans</i>	11	<i>A. faecalis</i> (R)	Positive for odor

^a D, Dextrose; C, citrate; A, arginine; X, xylose; AD, anaerobic dextrose; U, urease.

^b O, Original manual; R, revised manual.

Eight media representing nine, and possibly ten, biochemical tests are simultaneously tested in a matter of minutes. The inoculation and labeling procedure, to include streaking of a TSA plate, takes considerably less time than does inoculating the equivalent conventional battery of tests. In addition, colonies can be picked directly from primary isolation plates and processed immediately. Notwithstanding the temptation to take advantage of such conveniences, parallel inoculations of a TSI slant and tube of semisolid motility medium should also be performed. The TSI tube determines whether the organism is fermentative or nonfermentative. The motility stab is a convenience; nonmotile cultures must be retested by the slide method. Use of the TSA plate should not be casually overlooked, since the additional information provided is indispensable to proper identification. Principally, pigment production is favored at room temperature and is more evident than on MacConkey or blood agar medium. The plate should also be used to discern odor and wrinkling of colonies as they occur.

The OxF test is rapid in the sense of performance. Although time studies were not in the scope of this investigation, many reactions were apparently complete in 24 h (*Acinetobacter anitratus*) and proceeded no further after an additional 24-h incubation. However, gas formation by most organisms generally required 48 h, as did manifestation of a strong arginine and citrate reaction. These observations are supported by a recent study wherein only 52% of the expected reactions were elicited after 24 h of incubation (8). Therefore, experience with the system is necessary to attempt readings earlier than 48 h, the time recommended by the manufacturer.

Overall, the results of this evaluation showed a remarkably good correlation between OxF and conventional counterparts. Many of the discrepancies found among the individual tests were undoubtedly due to differences in materials used in the two systems. For example, reactions in the anaerobic dextrose compartment of OxF were quite consistent for the yellow-pigmented flavobacteria and CDC groups IIk and VE. These organisms are considered to be questionable fermenters (14), although this effect is often delayed or absent in the TSI agar. Experience in this laboratory has been that, occasionally, acidification of the slant is apparent only after 3 or 4 days of incubation. Virtually all of the disagreements in anaerobic dextrose were with the yellow-pigmented organisms. In addition, many of the disagreements in xylose were also due to these organisms as well as to *P. multocida*. The disparity in results with these substrates between this and a previous evaluation (8) is reflected in the selection of organisms used for testing. *Alcaligenes odorans* failed to produce nitrogen gas in the OxF system. The nitrogen gas compartment apparently contains nitrate, and since *A. odorans* fails to reduce nitrate to nitrite and, hence, gas (14), a valid comparison with gas formation using a medium such as Sellers could not be made. Thus, a discrepancy was not recorded for this test. Nineteen of the 30 citrate tests positive in Simmons medium and negative in OxF were the *P. maltophilia* (14 strains) and *Flavobacterium* IIb (5 strains). Despite these discrepancies, most of the organisms were coded for a proper identification. The remaining citrate tests were clearly false negative in OxF. It is noteworthy that three strains of group IIk-2 were citrate positive in the OxF tubes and coded as such, but are listed

as citrate negative using conventional tests (10, 14, 16).

The test for nitrites was shown to be a useful adjunct to the battery of tests incorporated in OxF. Twelve of the 17 discrepant results were with gas-producing organisms, which was not surprising since reduction of nitrate to nitrite and gas is a continuum, and results are not always exactly duplicated. The greatest value of the nitrite test lay in the differentiation of nonoxidative bacteria, as well as *P. multocida*, from similarly coded organisms. The results of the nitrite test could either be included in the code manual or used as a supplemental test.

Two additional considerations would increase the reliability of the system. First, inclusion of lysine in the H₂S-indole medium would measurably increase the chances of a proper identification of both *P. maltophilia* and *P. cepacia*. The test for lysine would reduce the chance of error in identifying the occasional oxidase-positive or nonmotile strain of *P. maltophilia* (10). Likewise, lysine would have assisted in identifying the three strains of *P. cepacia* misidentified using the original manual. Lysine is compatible with a ferric compound as well as with tryptophan (3). Lysine decarboxylation and hydrogen sulfide and indole formation do not occur simultaneously among the oxidase-positive organisms, except for the geographically rare *Vibrio* species, with the resultant reactions free from a potential inhibitory or masking effect. Second, effective and prudent utilization of tests already at hand would reduce reliance on some supplemental tests. The manufacturer's manual suggests such tests, yet does not fully exploit this potential. For example, the supplemental test for motility is used in an inconsistent manner. Whereas motility is listed as a differential characteristic between *P. maltophilia* and *Acinetobacter lwoffii*, it is omitted between *Moraxella* sp. and *Alcaligenes* sp. and related organisms. Similarly, "barred-from-broth" is a useful characteristic for identification of *Achromobacter xylosoxidans* and has been all but eliminated from the revised manual. Furthermore, use of susceptibility tests for differentiation could be expanded. Tests for susceptibility to tetracycline and streptomycin of *P. stutzeri* (susceptible) and *A. xylosoxidans* (resistant) would enhance the assurance of differentiation of these biochemically similar organisms. Use of the inverse susceptibility of the fluorescent pseudomonads to carbenicillin and kanamycin also would be helpful (6, 10). Susceptibility to carbenicillin (*P. aeruginosa*) and kanamycin (*P. putida-fluorescens*) would be more advantageous than discerning the variable characteris-

tics of pyocyanine and fluorescein production on a TSA plate.

The organisms in this evaluation were selected for the explicit purpose of challenging the OxF system to find its limitations, even though many of the organisms tested would be found infrequently. In all, 40 isolates were unidentifiable or misidentified by the OxF using the original manual. Thirteen could not be identified, since the number designating each did not appear in the key manual. Using the revised manual, 35 isolates were not identified, and 11 of these were strains of *Alcaligenes odorans*. In some instances, such as coding for urease-positive *A. lwoffii* and anaerobic dextrose-positive *A. anitratus*, the system was quite good. The codes for the various species of achromobacteria were similarly favorable. In others, such as coding for the nonoxidative NFB, it was less satisfactory. The differences between these organisms were maximized in the original code, whereas in most instances the differences are too subtle to be resolved by a few biochemical tests. This was rectified in the revised code registry by combining most of the pseudomonads. However, some of the easily recognized organisms lost species identity. *P. diminuta* and *P. pseudoalcaligenes* were accurately identified using the original manual, but were designated as *Pseudomonas* species with the revised manual. In Table 3, these were scored as equivocal. Conversely, *P. testosteroni*, *P. acidovorans*, and *P. alcaligenes* were invariably misidentified with OxF according to the original code, but are listed as equivocal because of genus identification. Of course, such designations are not entirely accurate since the new code failed to identify at the species level. The recent report by Isenberg and Sampson-Scherer (8) lists similar organisms in their evaluation. Misidentification was not noted, suggesting that the identification of species was attained. It should be pointed out that the manual used to code these organisms was not specified. Significantly, all of the 11 strains of *A. odorans* tested in the present study were misidentified using the new code. *Alcaligenes faecalis*, with a listing of a variable characteristic for odor, was substituted for *A. odorans*. Nevertheless, the coding system, to the extent that it is presented, is sound since it accounts for almost all of the members of the most frequently encountered species.

Identification of NFB is a difficult task, and with the requirements for numerous media and appropriate quality control attendant with each, many laboratories may forego the attempt to routinely identify these organisms. The OxF system, even with its limitations of nine tests and

oxidase reaction to identify the numerous organisms that possibly can be recovered from clinical specimens, seems to offer a distinct advantage over the use of conventional methods. It is evident that there are deficiencies in the system. But, since this is the first attempt to miniaturize the protocol for identification of NFB, and since it does reduce identification to a simplified coding system, the OxF system is a welcomed addition to the microbiology laboratory. The aforementioned intent of the study was to challenge the extent to which the OxF system could identify a wide range of NFB and several fermentative bacteria. Thirty-one species or groups of NFB were tested, many of which were specifically selected to challenge the OxF system. The study showed that the system is acceptable for identifying most frequently encountered organisms, namely: *P. aeruginosa*, *P. putida*, *P. fluorescens*, *P. maltophilia*, *Acinetobacter anitratus*, and *A. lwoffii*. It is also satisfactory for the flavobacteria, *Aeromonas hydrophila*, *P. multocida*, moraxella, the bordetella group, and, to a lesser extent, *Achromobacter xylosoxidans* and *P. stutzeri*. Most of the remaining NFB are infrequently recovered from clinical specimens. Those isolates that cannot be identified to species, as well as those for which no code exists or otherwise show aberrant reactions, should be sent to a reference center for definitive identification.

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