Evaluation of the Oxi/Ferm Tube System for Identification of Nonfermentative Gram-Negative Bacilli

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Received for publication 13 December 1977

The Oxi/Ferm tube system designed for identification of oxidative-fermentative gram-negative bacteria was evaluated for identification of nonfermentative gram-negative bacilli by comparing it with the conventional system. The nine biochemical reactions used as the initial tests in the Oxi/Ferm tube system showed a close agreement with the same conventional reactions. However, the system was only 41% accurate to species or biotype for complete identification of 239 isolates which were well distributed in 48 species and biotypes and included organisms not listed in the Oxi/Ferm tube identification list. The system correctly identified 56% of the test organisms when the degree of identification was based on the manufacturer's guidance list. However, all isolates of *Acinetobacter calcoaceticus*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, *P. diminuta*, and group III were completely identified by the system.

Nonfermentative gram-negative bacteria as opportunistic pathogens are being encountered with increasing frequency in individual and nosocomial infections. Complete identification is essential for proper handling of the infection and for epidemiological studies. Such identification presents a challenge for the clinical laboratories because of the large battery of tests to be performed and the complexity of the profiles required.

Roche Diagnostics (Nutley, N.J.), manufacturer of Enterotube, a rapid system for the identification of *Enterobacteriaceae*, has designed a similar system (Oxi/Ferm tube) for the identification of oxidative-fermentative (OF) gram-negative rods. Four recent studies of this system found it satisfactory for use with the organisms commonly encountered in clinical laboratories (2, 4-6).

In the present study, the Oxi/Ferm tube system was evaluated with a broader range of nonfermentative gram-negative bacilli for possible use in reference laboratories. The system was compared with the conventional system for the identification of 239 isolates well distributed among 48 species.

MATERIALS AND METHODS

Bacteria. The 239 organisms used for evaluation (see Table 3) were nonfermentative gram-negative rods, including some not listed in the Oxi/Ferm tube manual. The fermentative organisms included in the manual (*Aeromonas*, *Pasteurella*, *Plesiomonas*, and *Vibrio*) were not evaluated in this study. The nonfermentative organisms *Pseudomonas mallei*, *P. pseu-*

domallei, and *P. vesicularis* were also not included in this study.

One hundred and eighty-four of the 239 isolates used in this study were from the Division of Laboratories and Research culture collection. These originated from clinical specimens and were submitted to our laboratory for identification or confirmation. Of the remaining cultures, 47 were received through the courtesy of Analytab Products, Inc., Plainview, N.Y., and 8 were kindly provided by G. L. Gilardi, Hospital for Joint Diseases and Medical Center, New York. In our laboratory, all cultures were either maintained on blood agar slants or lyophilized.

Conventional media and procedures. The media were prepared by the Division's media section as described previously (7). The inoculated media were incubated up to 5 days at 35 to 37°C. The organisms were identified using generally accepted criteria (1, 3, 8-10).

Oxi/Ferm tube system. This is a self-contained plastic tube with eight separate compartments, each containing a substrate chosen so that ultimately nine biochemical tests can be performed. These tests are: OF anaerobic dextrose, arginine dihydrolase, nitrogen gas production, hydrogen sulfide and indole (both read in one compartment), OF xylose, OF aerobic dextrose, urea, and Simmons citrate. By use of an enclosed inoculating needle, a large inoculum is pulled through all eight compartments. The needle is then reinserted into the last three compartments and broken off. At the same time, a Trypticase soy agar plate is inoculated. After a 48-h incubation, the results of the nine tests plus an oxidase test (we used Pathotec strips for cytochrome oxidase) are coded as a four-digit number, called the ID value by the manufacturer. The manual lists the organism corresponding to the ID value. Where two or more organisms are listed for one value, up to eight supplemental tests are given to differen-

tiate among them. A total of 34 supplemental tests are listed. Organisms unable to grow on the agar plate are considered not identifiable by the system. A more detailed description of the Oxi/Ferm tube system is given elsewhere (4).

Methodology and interpretation of results were according to the manufacturer's instructions.

RESULTS

Test reactions. The results of the nine biochemical tests in the Oxi/Ferm tube were compared with the results of the same tests from the conventional method. In comparing the 220 isolates which grew on Trypticase soy agar medium, the method generally agreed. All but two nitrogen production and Simmons citrate tests of the individual tests in the system agreed with the conventional tests most of the time (Table 1). The results of 19 isolates unable to grow on Trypticase soy agar medium were not used in the comparison.

Identification of isolates. The level of identification expected to be attained by the Oxi/Ferm tube system for all 239 isolates was compared with the actual identification achieved (Table 2). Fifty-six percent of the isolates were correctly identified to the expected level by the Oxi/Ferm system. It was interesting to observe that the system correctly identified to genus level 122 (60.4%) of the 202 organisms included in the manufacturer's list and 14 (37.8%) of the 37 which were not included. However, the system misidentified 27.2% of the or-

ganisms included in the Oxi/Ferm charts and 16.7% of those not listed.

An analysis of the reactions and identifications obtained with the Oxi/Ferm tube system for each of the 48 conventionally identified species used in this evaluation is given in Table 3. Of 239 isolates tested, 19 did not grow in Trypticase soy agar; therefore, the Oxi/Ferm tube system could not be used. Of the 220 isolates tested, only 31 isolates needed no supplemental tests for identification. (Of these 31, only 12 were correctly identified.)

Only 41% of the 239 isolates were completely

TABLE 1. Comparison of the nine initial biochemical reactions in the Oxi/Ferm tube system using 220 isolates^a

Test	No. in agreement		No. different	
	+	-	C- OFT+	C+ OFT-
OF anaerobic dextrose	0	208	8	4
Arginine dihydrolase	20	191	4	5
Nitrogen gas	20	169	8	23
Hydrogen sulfide	2	217	0	1
Indole	13	206	1	0
OF xylose	67	137	2	14
OF aerobic dextrose	57	141	0	22
Urea	41	159	8	12
Simmons citrate	102	74	8	36

^a The results for 19 isolates unable to grow on Trypticase soy agar are not included in this comparison. C, Conventional; OFT, Oxi/Ferm tube; +, positive reaction; and -, negative reaction.

TABLE 2. Identification of 239 isolates by the Oxi/Ferm tube system

Oxi/Ferm's expected level of identification	No. of isolates	Oxi/Ferm identification [no. (%)]				Complete identification (as required by our reference laboratory) [no. (%)]
		To expected level	Correct only to genus	Misidentified	Unidentified	
Genus ^a	15	15 (100)	— ^b	0	0	
Species ^a	158	89 (56.3)	7 (4.4)	45 (28.5)	17 (10.8)	89 (56.3)
Biotype	29	10 (34.5)	1 (3.4)	10 (34.5)	8 (27.6)	10 (34.5)
Total	202	114 (56.4)	8 (4.0)	55 (27.2)	25 (12.4)	99 (49.0)
No expected identification (not included in system)						
Growth on TSA, ^c not on Oxi/Ferm list	18		14 (77.8)	3 (16.7)	1 (5.5)	0
No growth on TSA	19		0	0	19 (100)	0
Total	239	114 (47.7)	22 (9.2)	58 (24.3)	20 (8.4)	99 (41.4)

^a Unnamed organisms were included in species and biotype whenever applicable.

^b For the 15 isolates in this category, "to genus only" was the expected level of identification.

^c TSA, Trypticase soy agar.

TABLE 3. Results of the Oxi/Ferm system identification of nonfermentative gram-negative rods

Organism	Oxi/Ferm's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:		Incorrect identifications ^a	Reaction errors ^b	
				Genus	Species		Initial tests	Supplemental tests
<i>Achromobacter xylosoxidans</i> IIIA	Species	10	10 (4)		10			
<i>A. xylosoxidans</i> IIIB	Species	7	3 (3), 1 (4)		4	1 Unidentified 1 Unidentified 1 <i>Pseudomonas stutzeri</i>	No ID value (citrate -)	AKX + BB -
<i>Achromobacter</i> sp. biotype 1	Biotype	3			1	1 Unidentified	No ID value (N ₂ -, urea -, citrate -)	
<i>Achromobacter</i> sp. biotype 2	Biotype	5	1 (3)	1		1 Group M-4F 2 Unidentified 1 Unidentified	N ₂ -, citrate - No ID value (citrate -) No ID value (OF dextrose +, urea -, citrate -)	
<i>Acinetobacter calcoaceticus</i> subsp. anitratus	Species (subspecies)	10	8 (1)		10	1 Group Va-2		SS -
<i>A. calcoaceticus</i> subsp. <i>luoffii</i>	Species (subspecies)	10	7 (1), 3 (2)		10			
<i>Alcaligenes denitrificans</i>	Species	5	1 (1), 3 (3), 1 (6)		3	1 Unidentified 1 <i>A. faecalis</i> 2 <i>A. denitrificans</i> 1 <i>Pseudomonas alcaligenes</i>	OF xylose + N ₂ - N ₂ +	
<i>A. faecalis</i>	Species	5	1 (3), 3 (6)		2			42°C -
<i>A. odorans</i>	Species	4	3 (6), 1 (8)		2	1 Unidentified	Citrate -	
<i>Alcaligenes</i> -like group IVe	Species	6	1 (1), 5 (3)		4	1 Unidentified 1 <i>A. denitrificans</i> 1 <i>Moraxella phenylpyruvica</i>	N ₂ +, citrate - Citrate -	42°C -
<i>Bordetella bronchiseptica</i>	Species	7	7 (3)		7			
<i>Eikenella corrodens</i>	Not included	6		0		6 Unidentified	(No growth on TSA)	
<i>Flavobacterium meningosepticum</i>	Genus	4		4				
<i>Flavobacterium</i> sp. biotype IIb	Genus	4	3 (4)	1		3 Unidentified		Pen +
Group TM-1	Not included	1		0		1 Unidentified	(No growth on TSA)	
Group M-1	Not included	3		0		3 Unidentified	(No growth on TSA)	
Group IIj	Species	4	4 (4)		4			
Group IIj	Species	1	1 (4)		0	1 <i>Pasteurella multocida</i>	Urea +	
<i>Moraxella bovis</i>	Genus	1	1 (8)	1				
<i>M. lacunata</i>	Genus	1	1 (8)	1				

TABLE 3—Continued

Organism	Ori/Ferm's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:		Incorrect identifications ^a	Reaction errors ^b	
				Genus	Species		Initial tests	Supplemental tests
<i>M. nonliquefaciens</i>	Genus	3	3 (8)	3				
<i>M. osloensis</i>	Genus	4	4 (8)	4				
<i>M. phenylpyruvica</i>	Species	2	1 (3)		0	1 <i>A. denitrificans</i> 1 <i>Pasteurella ureae</i>	N ₂ +, urea –	ANG +
<i>M. urethralis</i>	Genus	2	2 (8)	2				
<i>Moraxella</i> -like group M-3	Not included	6	1 (8)	0		5 Unidentified 1 <i>Pasteurella haemolytica</i>	(No growth on TSA)	ANG + (for <i>Moraxella</i>)
<i>Moraxella</i> -like group M-4	Species	6	1 (6), 5 (8)	5	1			
<i>Moraxella</i> -like group M-4f	Species	8	5 (3), 2 (8)	1	2	4 <i>A. odorans</i> 1 Group Ilk-1 2 <i>Pasteurella haemolytica</i>	Citrate + Urea –	ANG + (for <i>Moraxella</i>)
<i>Moraxella</i> -like group M-5	Not included	9	9 (8)	7		1 Unidentified	(No growth on TSA)	
<i>Moraxella</i> -like group M-6	Not included	8	7 (8)	7				
<i>Pseudomonas acidovorans</i>	Species	5	5 (6)		1	1 Unidentified 3 <i>Pseudomonas alcaligenes</i>		RTO –, FRU – RTO –, FRU –
<i>P. aeruginosa</i>	Species	9	8 (3), 1 (5)		9			
<i>P. alcaligenes</i>	Species	4	1 (6), 2 (8)		0	1 <i>A. denitrificans</i> 1 <i>P. acidovorans</i> 2 <i>P. diminuta</i> 1 <i>A. anitratus</i> 2 <i>P. fluorescens</i>	N ₂ + Citrate – Oxidase – Urea –	RTO +
<i>P. cepacia</i>	Species	3	2 (3)		0			
<i>P. diminuta</i>	Species	7	7 (8)		7			
<i>P. fluorescens</i>	Species	3	1 (5)		0			
<i>P. maltophilia</i>	Species	8	2 (1), 5 (2)		7	1 Unidentified 1 <i>P. pseudomallei</i> 1 <i>P. putida</i>	No ID value (citrate –) Citrate –	GEL –
<i>P. pseudocalcaligenes</i>	Species	9	1 (2), 5 (6), 3 (8)		0	1 Unidentified 2 <i>A. faecalis</i> 3 <i>P. alcaligenes</i> 1 <i>P. fluorescens</i> 1 <i>P. testosteroni</i> 2 <i>P. testosteroni</i>	No ID value (N ₂ +) Arginine +	FRU – 42°C –, FRU – FRU – 42°C –, FRU –
<i>P. putida</i>	Species	7	1 (2), 1 (3), 4 (5)		0	1 Unidentified 1 Group Ve-2 4 Group Ve-2 1 <i>P. fluorescens</i>	No ID value (citrate –) OF xylose –, N ₂ + Arginine –	CET –

TABLE 3—Continued

Organism	Oxi/Ferm's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:			Incorrect identifications ^a	Reaction errors ^b	
				Genus	Species	Bio-type		Initial tests	Supplemental tests
<i>P. putrefaciens</i>	Species	3	1 (3)		2		1 Unidentified	H ₂ S –	
<i>P. stutzeri</i>	Species	4	1 (1), 2 (3)		3		1 Unidentified	OF dextrose +, indole +, citrate –	
<i>P. testosteroni</i>	Species	7	1 (3), 5 (8)		1		1 Unidentified		42°C +
							2 Unidentified		ANG +, 42°C +
<i>Pseudomonas</i> -like group IIk, biotype 1	Biotype	7	3 (1), 1 (2), 1 (3), 2 (8)		4		1 <i>A. denitrificans</i>	N ₂ +	
							1 <i>P. alcaligenes</i>	Urea +	
							1 <i>P. diminuta</i>		NA –
							1 Unidentified	Urea +	
							1 <i>P. diminuta</i>		
<i>Pseudomonas</i> -like group IIk, biotype 2	Biotype	4	2 (1), 2 (3)		2		1 <i>P. maltophilia</i>	Urea +	
<i>Pseudomonas</i> -like group Va, biotype 1	Biotype	2	1 (1), 1 (3)		0		2 <i>P. fluorescens</i>	Urea –	
							1 <i>A. denitrificans</i>	Arginine –, OF dextrose –, citrate –	
							1 <i>P. alcaligenes</i>	Arginine –, N ₂ –, OF dextrose –, OF xylose –, citrate –	
<i>Pseudomonas</i> -like group Va, biotype 2	Biotype	1	1 (3)		0		1 <i>P. pseudomallei</i>	Arginine –	
<i>Pseudomonas</i> -like group Ve, biotype 1	Biotype	4	1 (1)		1		2 Unidentified	No ID value (OF dextrose +)	
<i>Pseudomonas</i> -like group Ve, biotype 2	Biotype	3	2 (1)		2		1 Group Ve-2	Arginine –	
<i>Comphylobacter fetus</i> (<i>Vibrio fetus</i>)	Not included	4	1 (8)	0			1 Unidentified	No ID value (N ₂ +)	
							3 Unidentified	No ID value (No growth on TSA)	

^a Incorrect identifications found by the system combined with the supplemental tests.^b –, Negative reaction; +, positive reaction; AKX, alkaline xylose; ANG, anaerobic growth; BB, barred from broth; CET, cetrimide; FRU, fructose; GEL, gelatin hydrolysis; H₂S, hydrogen sulfide; MOT, motility; NA, nalidixic acid; N₂, nitrogen gas; Pen, penicillin; RTO, room temperature optimum; SS, *Salmonella-Shigella* agar; TSA, Trypticase soy agar; YEP, yellow pigment; and 42°C, growth at 42°C.

identified to species and biotype. However, all isolates of both varieties of *Acinetobacter calcoaceticus* and all isolates of *Bordetella bronchiseptica*, *P. aeruginosa*, and *P. diminuta* were correctly identified by the Oxi/Ferm tube system.

DISCUSSION

With increasing frequency, clinical and reference laboratories are receiving specimens for identification of nonfermentative gram-negative bacteria. Finding a simplified, accurate procedure for this usually complicated task has become important to clinical bacteriologists. The introduction by Roche Diagnostics of the Oxi/Ferm tube system, specially designed for identification of OF gram-negative rods, prompted us to evaluate the system for the complete identification of nonfermenters as required by a reference laboratory.

We used 239 isolates which belong to 48 species and biotypes of nonfermenters, only 7 of which are not included in the manufacturer's scheme. Eighty-six percent of the 220 isolates which grew on Trypticase soy agar medium required one or more of the supplemental tests suggested by the manufacturer. Of the errors in identification, 15% could be traced to incorrect citrate and nitrogen production tests.

Our results confirmed the suitability of the Oxi/Ferm system for identification of the commonly encountered nonfermenters such as *P. aeruginosa* and *Acinetobacter* species. The system failed, however, to meet the need of reference laboratories as it could only identify completely 41% of the isolates examined. The percentage is even less (33%) if the organisms which

can be diagnosed by the clinical laboratories are excluded.

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