Comparison of the Minithek System with Conventional Methods for Identification of Nonfermentative and Oxidase-Positive Fermentative Gram-Negative Bacilli

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Received for publication 1 February 1979

The Minithek system was compared with conventional test methods for identifying a wide range of nonfermentative and oxidase-positive fermentative gram-negative bacilli. A total of 230 isolates representing 33 species and biotypes were tested against 12 Minithek substrates and the corresponding conventional tube media. In addition, supplementary tests were included for 141 (61.3%) of the isolates. Overall, 88% of the positive reactions agreed, and negative reactions agreed 95.5%. Anaerobic dextrose, maltose, lactose, and citrate were responsible for 62.4% of the 93 discrepant positive reactions, and 51.8% of the 83 discrepant negative reactions involved the aerobic dextrose, nitrate, and citrate disks. Some discrepancies were related to specific organisms. The system and supplementary tests correctly identified 88.3% of the isolates to species level and 92.6% to correct genus. No particular organism or substrate was responsible for misidentification errors. In a test challenge with 19 selected organisms, 3 were incorrectly identified to species and 4 were misidentified to genus level. Most of the errors responsible for these incorrect identifications were due to factors other than the Minithek disks and pointed out the importance of using appropriate supplemental data, and the need for a coding manual and a more enriched broth for certain types of isolates.

Nonfermentative and oxidase-positive fermentative gram-negative bacilli have been recognized as significant laboratory isolates both from clinical and epidemiological standpoints. However, these organisms are often very difficult to identify and require a wide variety of test parameters for correct identification to species. The problem is further complicated by the existence of numerous identification schemes based on different types of media and conditions of incubation. Since it is no longer adequate to simply report these isolates as "*Pseudomonas* sp.," the need exists for rapid, accurate, simple, and relatively inexpensive methods to identify them in any clinical laboratory.

In recent years, several kits or systems have become commercially available to facilitate the identification of this challenging and diverse group of organisms. One of these systems is Minithek (BBL Microbiology Systems, Cockeysville, Md.) (M. Slifkin and G. Pouchet, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C88, p. 50), which utilizes disks impregnated with substrates. Minithek is a very versatile system and has been used successfully for the identification of enterics (3), anaerobes (6), *Neisseria* (5), and yeasts (L. Caplan and W. Merz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C91, p. 50).

This study presents data comparing the Min-

itek system with conventional test methods to identify nonfermentative and oxidase-positive fermentative gram-negative bacilli and presents modifications of the manufacturer's scheme.

MATERIALS AND METHODS

Organisms. The 230 isolates tested in this study represented 33 species. Thirty-two reference strains were included, and the remaining 198 organisms were isolated from clinical and environmental specimens received in my laboratory during the past 14 months. Twenty of the reference strains were obtained through the courtesy of BBL Microbiology Systems.

Minithek method. The Minithek system consists of a plastic plate with wells that hold 12 of the 35 different substrate disks available from the manufacturer. A suspension of the organism is made in an inoculum broth, which is dispensed into the wells with a pipette. The following disks were selected for this study: dextrose without nitrate (two disks), xylose, maltose, lactose, mannitol, lysine, ornithine, arginine, nitrate reductase, citrate, and urea. In addition, three samples from the Minithek pipette (total, 0.15 ml) were dispensed into an empty well in a second Minithek plate. A gelatin strip (Key Scientific Products Co., Los Angeles, Calif.) was cut into quarters, and a quarter was placed into this well to test for gelatin hydrolysis. One of the dextrose disks and the lysine, ornithine, arginine, and urea disks were promptly overlaid with mineral oil. The remaining inoculum broth was incubated and used to test for indole production as needed.

	6°	0	33	50	0	0	0	50	0	0	50	67	0	0	42°C-67 Cet-50
<i>Pseudomonas pseudocaligenes</i>															
<i>Pseudomonas putida</i>	8 ^b	13	100	100	0	50	0	13	0	0	88	100	0	0	42°C-0
<i>Pseudomonas putrefaciens</i>	3	0	0	0	67	0	0	100	0	67	0	33	0	67	H ₂ S-100
<i>Pseudomonas stutzeri</i>	3 ^b	0	67	33	0	0	33	67	0	0	33	67	0	0	Starch-0
<i>Pseudomonas testosteroni</i>	3 ^b	0	0	0	0	0	0	100	0	0	0	67	0	0	42°C-0 Cet-0

^a Abbreviations: 42°C, Growth at 42°C; Cet, growth on cetrimide; Ind, indole; H₂S, Hydrogen sulfide.

^b Reference strains included.

Conventional method. All conventional media used in the study were prepared in the laboratory from dehydrated media (Difco). The biochemical tests corresponded to the disks used in the Minitek system and included: OF Basal Medium with 1% dextrose, xylose, maltose, lactose, or mannitol; Moeller lysine, ornithine, and arginine; nitrate broth; Simmons citrate agar; and Christensen urea agar. Growth on cetrimide agar, growth at 42°C in brain heart infusion broth, and deoxyribonuclease activity on deoxyribonuclease test agar with 0.1% toluidine blue were also used as required. Flagella stains were performed according to the method of West et al. (8).

Procedure. Prior to testing, all organisms were streaked on Trypticase soy agar with 5% sheep blood and on MacConkey agar. Colonial and microscopic morphology, pigment production, hemolysis, odor, oxidase production (Pathotec Cytochrome Oxidase Test Strips; Warner-Lambert Co., Morris Plains, N.J.), and catalase production were determined from the appropriate media. A single, well-isolated colony was picked from one of the plates to a Trypticase soy agar slant, which was incubated overnight at room temperature and used as a source of inoculum for all tests and for a flagella stain.

The Minitek tests were inoculated according to the manufacturer's instructions, and conventional media were inoculated in the usual fashion. All tests were incubated at 35°C. After 24 h of incubation, they were read, and a preliminary identification was made. Supplemental tests were set up as needed, and tests were incubated for an additional 24 h. When no reactions were observed or the isolate could not be identified, conventional tests were kept up to 5 days. After 48 h, required reagents were added for indole and nitrate tests, and a final identification was made using data supplied by the manufacturer and acceptable schema (1, 2, 4, 7). When results did not agree, the tests in question were repeated in both systems, and those results were considered final.

RESULTS

The 230 isolates evaluated in this study are listed in Table 1 with results of their biochemical reactions in the Minitek system. Although most reactions (98%) were complete in 24 h, the percentage of positive reactions is based on readings at the end of 48 h of incubation. Percentages, for the most part, correlate with published data for conventional test methods (1, 2, 4, 7) and with data provided by the manufacturer.

However, notable variations did occur with some species. Some strains of *Achromobacter xylosoxidans* varied in their ability to oxidize dextrose and xylose, and these reactions occurred in conventional media as well. One strain of *Aeromonas hydrophila* acidified xylose and decarboxylated lysine; these reactions could not be obtained by conventional methods. Isolates of *Flavobacterium* IIb did not give consistent results for indole production or dextrose utilization.

Carbohydrate disk reactions for *Flavobacterium meningosepticum* and *Pasteurella multocida* were very difficult to interpret. Reactions were not clear because color changes in the disks were very subtle. Disks were only slightly more yellow after incubation, compared to the orange color of the disks when they were initially inoculated. Reactions in the conventional system were also not clear, and sometimes 72 h of incubation was necessary for an accurate interpretation. These isolates had been stored and may have lost some of their activity.

The strains of *Pseudomonas aeruginosa* selected for this study were mostly apyocyanogenic strains or those with unusual antibiograms. Three strains failed to react with arginine in the Minithek system, but conventional results were positive. Some strains of *Pseudomonas cepacia* were slow growers, and reactions were not apparent in conventional media until 72 h of incubation. Only two strains produced acid in mannitol. This is usually a very helpful reaction to distinguish between *P. cepacia* and *Pseudomonas maltophilia*. The single strain of *Pseudomonas paucimobilis* tested was more nonreactive than most isolates of this species. This might be explained by its slow rate of growth.

One or more supplementary biochemical tests were set up on 141 (61.3%) of the isolates. These tests are also indicated in Table 1. However, the most valuable adjunct to identification was a flagella stain. In some instances, this stain was essential for correct identification. It was impossible to distinguish between *Alcaligenes faecalis* and *Pseudomonas alcaligenes* or between *P. alcaligenes* and *Pseudomonas testosteroni* until the flagellar morphology of these isolates was observed.

Table 2 summarizes the results of 2,760 Minithek disks tested in parallel with conventional tube media and shows the number of positive and negative reactions for each substrate in each system as well as the number of positive and negative reactions in agreement. Overall, 615 (88%) of the positive reactions agreed and 1,969 (95.5%) of the negative reactions agreed.

Of the 93 discrepant positive reactions in Minithek, 58 (62.4%) involved the anaerobic dextrose, maltose, lactose, and citrate disks. Aerobic dextrose, nitrate, and citrate disks accounted for 43 (51.8%) of the 83 discrepant negative Minithek reactions. Some of these discrepancies appeared to be related to specific organisms.

Isolates of *Acinetobacter calcoaceticus* subsp. *anitratus* were responsible for 11 of the 14 false-positive anaerobic dextrose disks. Although this organism is not a fermenter, it does produce a positive reaction in this disk. Data accompanying the system took this into account in the identification schema, and these isolates were correctly identified.

Thirteen of the 15 false-positive maltose disks and 9 of the 16 discrepant positive lactose disks were also caused by *A. calcoaceticus* subsp. *anitratus*. These reactions are often delayed for this organism in conventional media (7) and may simply occur more rapidly in Minithek.

For the aerobic dextrose disk, 8 of the 15 false-negative reactions occurred with isolates of *P. maltophilia*. The conventional media always required at least 48 h of incubation for a positive reaction to occur.

Positive reactions for the ornithine and lysine disks showed the lowest agreement of all (64.7 and 78.1%, respectively). Three strains of *Eikenella corrodens*, two *P. maltophilia*, one *P.*

TABLE 2. Comparison of biochemical tests in Minithek and conventional systems^a

Substrate	No. of tests +		No. of tests -		No. + in agreement (%)	No. - in agreement (%)
	Conv	Mini	Conv	Mini		
Dextrose (anaerobic)	21	34	209	196	20 (95.2)	195 (93.3)
Dextrose	116	104	114	126	101 (87.1)	111 (97.4)
Xylose	90	96	140	134	87 (96.7)	131 (93.6)
Maltose	44	54	186	176	39 (88.6)	171 (91.9)
Lactose	30	43	200	187	27 (90.0)	184 (92.0)
Mannitol	27	28	203	202	23 (85.2)	198 (97.5)
Nitrate	93	88	137	142	81 (87.1)	130 (94.9)
Lysine	32	32	198	198	25 (78.1)	191 (96.5)
Ornithine	17	11	213	219	11 (64.7)	213 (100)
Arginine	45	40	185	190	38 (84.4)	183 (98.9)
Citrate	156	153	74	77	140 (89.7)	61 (82.4)
Urea	27	25	203	205	23 (85.2)	201 (99.0)
Total	698	708	2,062	2,052	615 (88)	1,969 (95.5)

^a +, Positive reaction; -, negative reaction; Conv, conventional systems; Mini, Minithek system.

cepacia, and one *A. hydrophila* produced the seven false-positive lysine reactions. However, with the exception of *A. hydrophila*, positive Minitek reactions agreed with published data (2, 4, 7) for these organisms. Discrepancies with ornithine were caused by five strains of *E. corrodens* and one strain of *Pseudomonas putrefaciens*. Minitek failed to produce positive reactions in these cases. Strains of *E. corrodens* were also responsible for five of the seven negative Minitek lysine reactions that disagreed with conventional methods.

Eleven of the 13 false-positive Minitek citrate disks occurred with isolates of *P. maltophilia*, and 7 of the 16 false-negative citrate disks occurred with strains of *A. calcoaceticus* subsp. *anitratu*s. Citrate had the lowest agreement for negative reactions (82.4%).

Discrepancies in the xylose, mannitol, nitrate, arginine, and urea disks did not appear to be organism related and were distributed among the various species.

A total of 93 (40%) of the 230 isolates tested were involved in the 176 discrepant reactions. Most discrepancies, however, did not affect correct identification, and Minitek actually identified 203 (88.3%) of the strains to species and placed a total of 213 (92.6%) in the correct genus. No particular disk or species was a major source of error (Table 3).

To evaluate how well the system would perform in the hands of technologists who would be using it, 19 strains were submitted to them as unknown. Both common and rare isolates were

included as well as reference and clinical strains. Some had been misidentified in the initial study or were problem isolates.

Seven strains were misidentified. A strain of *Alcaligenes odorans* lost its fruity odor and was identified as *A. faecalis*. *Flavobacterium odoratum* appeared to be motile in a hanging-drop preparation and was called CDC group IVe. Misidentification of *P. multocida* resulted from misinterpretation of the subtle color changes in the carbohydrate disks. A mannitol-negative strain of *Pseudomonas acidovorans* was confused with *A. faecalis* because a flagella stain had not been performed. An oxidase-, dextrose-, and mannitol-negative strain of *P. cepacia* was designated as *P. maltophilia*. Minitek also failed to recognize a maltose- and starch-negative strain of *Pseudomonas stutzeri* and confused a strain of *Pseudomonas fluorescens* that failed to hydrolyze gelatin with *Pseudomonas putida*.

Five of the errors responsible for misidentification of the challenge isolates involved supplementary tests or procedures, loss of identifying characteristics upon storage of the organism, technologist failure to interpret test results accurately, or failure to set up appropriate supplementary tests. The remaining two misidentifications were attributed to false-negative reactions in the Minitek substrate disks.

DISCUSSION

Results of this study indicate that the Minitek system correlates well with conventional meth-

TABLE 3. *Organisms misidentified by the Minitek system*

Organism	No. misidentified	Incorrect identification ^a	Incorrect reactions ^b
<i>Achromobacter xylosoxidans</i>	2	<i>Alcaligenes faecalis</i>	D-, X-
<i>Aeromonas hydrophila</i>	1	<i>Pseudomonas cepacia</i>	LY+, X+
CDC Group IVe	1	CDC Group IV C-2	N-
CDC Group IV C-2	1	CDC Group IVe	C-
<i>Eikenella corrodens</i>	4	3 CDC Group TM-1	LY-, O-
		1 unidentifiable	LY-, N-
<i>Flavobacterium IIb</i>	1	<i>Moraxella</i> sp.	D(A)-, D-, I-
<i>Pseudomonas aeruginosa</i>	4	<i>P. pseudoalcaligenes</i>	3 A-; 1 D-, X-
<i>Pseudomonas acidovorans</i>	2	<i>P. testosteroni</i>	MN-
<i>Pseudomonas cepacia</i>	3	<i>P. stutzeri</i>	LY-, L-
		Enteric	OX-, D+
		<i>P. maltophilia</i>	OX-, D-, MN-
<i>Pseudomonas maltophilia</i>	3	<i>P. cepacia</i>	1 DNase-, 1 MN+
		Unidentifiable	D-, M-, LY-
<i>Pseudomonas paucimobilis</i>	1	Unidentifiable	D-, M-, L-, M-
<i>Pseudomonas putida</i>	2	<i>P. pseudoalcaligenes</i>	1 A-; 1 no discrepancies, atypical strain
<i>Pseudomonas stutzeri</i>	2	<i>P. pseudoalcaligenes</i>	No discrepancies, atypical strain

^a Incorrect identifications include those found by Minitek or supplementary tests.

^b D, dextrose; X, xylose; LY, lysine; N, nitrate; C, citrate; O, ornithine; D(A), anaerobic dextrose; I, indole; A, arginine; MN, mannitol; L, lactose; OX, oxidase; DNase, deoxyribonuclease; M, maltose.

ods. When used in conjunction with the oxidase test, flagellar stain, susceptibility data, and other simple tests, it offers a rapid, reliable, and relatively inexpensive way to identify a wide range of nonfermentative and oxidase-positive fermentative organisms.

One of the major advantages of Minitek over other commercially available systems is its flexibility. The user is not locked into any one particular battery of tests. Substrates may be selected and varied to meet the needs of the individual laboratory and can take into consideration the types of organisms most frequently encountered as well as the extent of identification that must be provided by that laboratory. As previously mentioned, there are 35 substrates to choose from.

The Minitek system also takes less time to set up and is easier to handle than tubed media. A Minitek battery was completely set up in 1 min, compared to 2.5 min needed to completely inoculate the corresponding conventional media.

Minitek reactions are as rapid as or more rapid than conventional test methods. Tests are designed to be read within 48 h. The key to rapid results is usually a very dense inoculum. A correct identification can be made within a relatively short time and, thereby, provide relevant clinical or epidemiological information in a timely manner. Some conventional procedures require 7 to 14 days of incubation before an identification can be made.

Since the substrate disks and inoculum broth have a long shelf life, it is possible to maintain the numerous tests needed for identification. This is especially convenient for the smaller laboratory. The problems of outdated media, media preparation, and the quality control of media are also avoided.

One of the obvious inconveniences of the system, as it currently exists, is the lack of enough wells on a single plate. If more than 12 tests are desired, a second plate is required.

It would also be helpful to have a more enriched inoculum broth for weak or questionable fermenters and for more fastidious organisms such as *Pasteurella*, *Flavobacterium*, and *Actinobacillus*. The *Neisseria* Minitek broth might be suitable for this purpose. In a limited experiment using *Neisseria* broth, isolates of these organisms showed carbohydrate reactions that were clear and easy to interpret. Positive car-

bohydrate disks turned a bright yellow, and negative reactions were red. Some of these were the same isolates that had equivocal carbohydrate reactions in the initial study.

Since there was no coding system or identification manual available at the time of the study, all identifications were based on pattern recognition and correlation of all supplementary characteristics to arrive at a final identification. The decision to set up additional tests, and the specific tests needed to distinguish between closely related organisms or atypical isolates, rested solely with the operator.

Although a numerical coding system should not and cannot be relied on exclusively for identification, it might have provided a guide for less experienced personnel to improve accuracy of identification, especially of the less commonly encountered organisms.

Overall, the Minitek system was found to be a useful alternative to conventional methods and expands the identification capabilities of a routine clinical laboratory of any size.

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