

Clinical Evaluation of the Minitek Differential System for Identification of *Enterobacteriaceae*

PATRICIA J. FINKLEA,* MARSHA S. COLE, AND THOMAS M. SODEMAN

Department of Pathology, Microbiology Section, University Hospital, Ann Arbor, Michigan 48109

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Forty-one stock organisms and 581 fresh clinical isolates were used in comparing the Minitek system to conventional tubed media to determine if this system is feasible and accurate for a high-volume clinical microbiology laboratory. In addition to comparison with tubed media, the following parameters were tested: (i) reproducibility of disks, (ii) the effect of variation in inoculum size, (iii) the effect of the age of culture, and (iv) the effect of predispending disks. A total of 5,947 disks were compared with tube reactions; 95.9% agreed on the initial reading. After repeating questionable tube and disk reactions, this agreement rose to 98.2%. Using the disk reactions and the identification schema currently in use with tubed media, 94.9% of the Minitek final identifications agreed with those of tubed media; 1.5% gave differing identifications and 3.6% were inconclusive. This system is accurate, inexpensive, flexible, and convenient.

Members of *Enterobacteriaceae* are among the most common organisms isolated in the clinical laboratory. Several miniaturized systems have been marketed to facilitate identification of these organisms. Minitek (Bioquest, Div. of Becton, Dickinson and Co., Cockeysville, Md.), based upon substrate impregnated disks, is such a system. It was evaluated for its accuracy of both organism identification and individual biochemical reactions.

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MATERIALS AND METHODS

The Minitek system consists of a small plastic plate with wells, cartridges of substrate disks which are dispensed into the wells, an inoculum broth, and a pipette gun set to deliver 0.05 ml of the inoculated broth onto each disk. Some inoculated disks are overlaid with sterile mineral oil, and the plates are incubated in a humidifier. Both sterile and nonsterile tips were used with the pipette gun. No evidence of contamination was found with the nonsterile tips, and they were easier and less expensive to use than the sterile tips. The manufacturer's directions were followed with these modifications. (i) The inoculum was homogenized with the pipette gun rather than being vortexed. (ii) Oil was dispensed with an autoclavable Oxford dispenser model S, set to deliver 0.15 ml of oil. (iii) A triple sugar iron slant and a motility tube were inoculated in addition to Minitek disks. (iv) Indole production was tested by (a) using the H₂S disk—both H₂S and indole reactions are read from the H₂S disk, and (b) inoculating the broth into an empty well for incubation (open well).

Before comparing the Minitek with the conventional tube system, preliminary work was performed to evaluate sensitivity of Minitek to a variety of parameters. The conventional media used in this comparison included: (i) commercially prepared media from BBL (Bioquest, Division of Becton, Dickinson & Co., Cockeysville, Md.) consisting of triple sugar iron, 2% tryptone, citrate, urea broth; phenylalanine, lysine, arginine, ornithine, adonitol, arabinose, raffinose, rhamnose, sorbitol, inositol, xylose, and maltose; and (ii) homemade media prepared from Difco dehydrated media consisting of MR-VP, motility, and malonate. Reproducibility of disk reactions was checked by testing 47 substrate lots (a total of 3,859 disks) with known organisms. Up to 100 disks of each lot available were inoculated, half with an organism giving a positive reaction and half with an organism giving a negative reaction.

Potential variation of results due to differing inoculum size was tested using one colony versus six to eight colonies per diluent broth for eight organisms in 182 tests. Effect of culture age was tested using 1-, 2-, 3-, 4-, and 5-day-old cultures of four organisms in 156 tests. Disks were predisposed to the plastic plates, refrigerated at 8°C from 1 to 7 days before use, and evaluated for reproducibility by a total of 462 tests with three organisms.

Comparisons of biochemical reactions and organism identifications were made between the Minitek and conventional tube systems. All clinical isolates were inoculated into the Minitek broth from a 24-h MacConkey plate and taken to one of three biochemical patterns depending on the colony morphology (Table 1).

Conventional tubed media were run in parallel according to routine procedures. Both tubed media and Minitek were incubated for 18 to 24 h at 35°C. Reagents were added, results were read, and orga-

nism identification was made according to the charts of Edwards and Ewing (1). If the results of the two systems did not agree, the test was repeated in both systems.

RESULTS

Parameter testing. Thirteen of the 17 different disks (comprising 38 lots and 2,674 disks) were evaluated for reproducibility and gave 100% appropriately negative or positive reactions. The remaining four substrates, arginine (two lots tested), citrate (two lots tested), ornithine (four lots tested), and xylose (one lot tested), gave less than 100% appropriate results (Table 2). The other three parameters tested, i.e., the effects of culture age, inoculum size, and predispensed disks, caused no detectable variations from the expected reaction patterns.

Comparison of tube versus disk. A total of 5,947 disks were compared with the tube reactions. When initial disk and tube reactions dis-

agreed, both were repeated and a confirmed tube reaction was considered correct. Of the total disks and tubes compared, 95.9% agreed on initial reading. After repeating questionable results, this figure rose to 98.2%.

Table 3 indicates the initial and final correlation of each type of disk with the tubed media. Fifteen of the 20 reactions tested gave an initial agreement of 95% or higher. The five exceptions were H₂S lot 402019, disk indole, citrate, ornithine, and xylose. Table 4 demonstrates that most initially disagreeing disks were negative when the corresponding tube was positive.

The H₂S lot 402019 was the first production lot released by the manufacturer and appeared too sensitive, giving false-positive reactions. The false-positive H₂S reaction was corrected in subsequent lots of H₂S disks, which gave a 98.0% initial correlation with the tubed media and a 99.4% final agreement. For this reason

TABLE 1. *Minitek biochemical patterns used*

Pattern 1 ^a	Pattern 2 ^b	Pattern 3 ^c
H ₂ S	H ₂ S	H ₂ S
Disk indole	Disk indole	Disk indole
Open indole	Open indole	VP
VP ^d	Citrate	Citrate
Citrate	Adonitol	Malonate
Malonate	Urea	Lysine
Lysine	Phenylalanine	Arginine
Adonitol	Ornithine	Ornithine
Urea	Inositol	Adonitol
Phenylalanine	Xylose	Arabinose
	Maltose	Raffinose
		Rhamnose
		Sorbitol

^a Pattern 1, Flat lactose positive; flat lactose negative, non-*Proteus*.

^b Pattern 2, Flat lactose negative, i.e., *Proteus*.

^c Pattern 3, Mucoid, lactose positive, lactose variable.

^d VP, Voges-Proskauer.

TABLE 2. *Disk lot variability*

Substrate	Lot no.	No. of disks tested per lot	% Agreement with appropriate reaction	
			Positive	Negative
Arginine	311062	100	92	100
	403078	75	98	100
Citrate	402014	150	84	98
	404108	150	94	100
Ornithine	402013	100	100	100
	404019	100	100	100
	407029	28	100	100
	311072	32	50	100
Xylose	311075	150	100	68

TABLE 3. *Disk agreement*

Test disk	No. tested	% Agreement	
		Final	Initial
H ₂ S (lot 402019)	301	No repeat	88.7
H ₂ S (lot 405017)	352	99.4	98.0
Indole (open well)	433	96.8	95.8
Indole (disk)	632	96.7	89.9
Voges-Proskauer	445	99.8	99.1
Citrate	573	98.8	94.6
Malonate	281	99.6	98.9
Lysine	390	99.2	98.5
Arginine	156	99.4	98.1
Ornithine	435	92.0	91.5
Adonitol	402	99.5	98.8
Arabinose	171	100.0	99.4
Raffinose	194	99.5	98.4
Rhamnose	194	100.0	98.4
Sorbitol	194	99.5	97.4
Urea	319	98.7	98.4
Phenylalanine	246		100.0
Inositol	129		100.0
Xylose	74	95.9	85.1
Maltose	26	100.0	96.2
Total	5,947	98.2	95.9

TABLE 4. *Problem disks*

Test disk	No. disagree/total	Disagreeing results (%)	
		+ with Minitek, - with tube	- with Minitek, + with tube
Indole (open well)	18/433	5.6	94.4
Indole (disk)	64/632	3.1	96.9
Citrate	31/573	12.9	87.1
Ornithine	39/435	5.1	94.9
Xylose	11/74	90.9	9.1

the results of the H₂S testing are divided into lot 402019 and lot 405017.

Since indole production was tested with the H₂S disk and open well, these results are divided into two sections. The open-well indole (inoculum plus reagent) gave consistently good reactions throughout the study with only a 1% improvement upon repeating questionable reactions. As Table 4 demonstrates, 94.4% of the 18 disagreeing results were due to negative Minitex indoles as opposed to positive tube reactions. However, five of these indole tube reactions were positive only after 48 h of incubation. Fourteen of the 17 strains giving negative Minitex indole were species of *Proteus*. The disk indole test had an initial agreement with tubed media of only 89.9%. However, since the disk indole reaction is read from the H₂S disk, it is possible that lot 402019 H₂S disks also gave misleading indole reactions. When the tests were repeated with a new lot a final 96.7% disk agreement compared well with the 96.8% final open well. As with the open-well indole (Table 4), 96.9% of the 64 disagreeing Minitex disk indoles were negative when the tube indole was positive. Forty of the 59 affected strains were *Proteus*. The pattern of negative Minitex indoles was inconsistent: some strains had a negative disk and a positive open-well indole; some strains had positive disk and a negative open-well indole; some had negative open-well and disk indole with a weakly positive tube indole. Two strains of *Proteus* that were indole positive by Minitex and negative by tube had other biochemical reactions that suggested that a positive indole was the expected result.

Citrate was another problem disk with some organisms. Minitex disks most often gave initially negative results when tube results were positive (Table 4). The 87.1% includes 11 *Citrobacter*, 5 *Proteus*, and 11 *Enterobacter*. After the citrate was repeated, seven organisms remained citrate negative. As with the H₂S, the earlier lots of citrate had more disagreeing results than the later lots when the problem strains were retested.

Ornithine follows the pattern of indole and citrate (Table 4) in that 94% of the 39 disagreeing reactions are negative with Minitex and positive in 24 h with tubed media. Thirty-four out of 37 (94.9%) of these false-negative readings were *Proteus*, and 31 of these were *Proteus morganii*. Even after incubating the ornithine disk for 48 h, and retesting these organisms with different lots of ornithine, 29 of 35 *P. morganii* strains continued to give persistently negative Minitex results.

The ornithine disks have been modified since

this study was completed (D. Mayfield, Bioquest, personal communication), and the 31 strains of *P. morganii* do give a positive reaction in 24 h using the modified disk.

The 11 disagreeing disk of xylose, in contrast to the other disks discussed, were 90.9% positive when the tubes were negative. This is a result of heat sensitivity of this sugar with a loss of activity during production. The necessary changes are being made to correct this (Mayfield, personal communication).

Comparison of final identifications. Table 5 shows a 92.1% initial agreement and a 94.9% final agreement, with 1.3% wrong identifications and 3.8% inconclusive results needing further testing. Table 6 provides more specific information about the strains studied. Of the 19 species included in this study, 11 had identifications agreeing 95 to 100% with the tubed-media identifications. For an identification to agree, we required that all Minitex disks crucial to identification agree with the tube. A dichotomous schema was not used and, depending on a particular laboratory's requirements for identification, the percentage of final correlation using our disk results could be higher or lower.

Table 6 shows that *Citrobacter freundii* had a 83.7% initial and 93.0% final identification agreement. The seven initial and two final inconclusive identifications of *C. freundii* were based upon citrate-negative disk reactions and positive tube reactions. It should also be noted that the *Citrobacter* species sometimes gives a strongly positive urea on the Minitex when a urea broth is negative. Since urease production is expected in some citrobacters such a reaction suggests that the Minitex urea disks are more sensitive than urea broth.

The *P. morganii* presented the single largest group of problem identifications. Because 29 of 35 strains were ornithine disk negative, the H₂S, indole, citrate, adonitol, and inositol reactions were used to identify this species. Even using these five reactions, only 77.1% were finally identified as *P. morganii* when compared with the results from tubed media. Six *P. morganii* were also indole negative on the Minitex. Since others (2, 3) have not reported a similar problem, it is possible that this reaction pattern is due to a strain variation.

Proteus vulgaris and *Providencia* both fall

TABLE 5. Organism identification summary

Minitex readings	Agreement (%)	Wrong identification (%)	Inconclusive (%)
Initial	92.1	0.6	7.3
Final	94.9	1.3	3.8

below the 95% final agreement because of indole disk-negative strains: 1 out of 19 strains of *P. vulgaris* and 2 out of 7 *Providencia*.

There were a variety of reasons that the six species of *Klebsiella*, *Serratia*, and *Enterobacter* fell below 95% final agreement with the tube identification (Table 6). There were no trends

within a species and no single disk was a predominant cause of inconclusive or wrong identifications. Table 7 shows the seven wrong identifications out of the 622 strains studied.

DISCUSSION

The parameter testing with the Minitek dem-

TABLE 6. Identification comparison^a

Organism	No. of strains	Minitek readings	Agreement (%)	Wrong identification (%)	Inconclusive (%)
<i>Escherichia coli</i>	116	Initial	99.1		0.9
		Final	99.1		0.9
<i>Citrobacter freundii</i>	43	Initial	83.7		16.3
		Final	93.0	2.4	4.6
<i>Proteus mirabilis</i>	121	Initial	96.7		3.3
		Final	97.5	0.9	1.6
<i>P. morgani</i>	35	Initial	71.4		28.6
		Final	77.1	2.9	20.0
<i>P. vulgaris</i>	19	Initial	89.5		10.5
		Final	91.7		8.3
<i>Providencia</i>	7	Initial	71.5		28.5
		Final	71.5		28.5
<i>Klebsiella pneumoniae</i>	72	Initial	94.4	1.4	4.2
		Final	94.4	2.8	2.8
<i>Serratia marcescens</i>	51	Initial	92.2		7.8
		Final	98.0		2.0
<i>Enterobacter aerogenes</i>	37	Initial	91.9	5.4	2.7
		Final	94.6	5.4	
<i>E. cloacae</i>	53	Initial	83.0		17.0
		Final	90.6	1.9	7.5
<i>E. agglomerans</i>	8	Initial	62.5		37.5
		Final	87.5		12.5
<i>E. hafniae</i>	5	Initial	60.0		40.0
		Final	100.0		

^a This table does not include the following organisms because the initial Minitek and tube identifications agreed 100%: *Edwardsiella* (3 strains), *Shigella* (5), *Salmonella* (7), *Arizona* (3), *Citrobacter diversus* (16), *Proteus rettgeri* (16), and *Serratia liquefaciens* (2).

TABLE 7. Identification errors in the Minitek system

Organism	Minitek identification	Reasons ^a
<i>C. freundii</i>	<i>E. coli</i>	Disk indole positive, citrate negative
<i>P. mirabilis</i>	<i>P. morgani</i>	Open indole positive, H ₂ S negative
<i>P. morgani</i>	<i>P. mirabilis</i>	Indole negative
<i>K. pneumoniae</i>	<i>E. aerogenes</i>	Ornithine positive
<i>K. pneumoniae</i>	<i>E. agglomerans</i>	Lysine and citrate negative
<i>E. aerogenes</i>	<i>E. hafniae</i>	Voges-Proskauer, citrate, sorbitol, and raffinose negative
<i>E. cloacae</i>	<i>E. agglomerans</i>	Arginine and ornithine negative

^a Inaccurate Minitek reaction.

onstrated that reasonable variation in inoculum size and age of culture when inoculated into broth had no effect on the results. Disks may be dispensed to the plastic plates on a weekly basis, refrigerated, and used as needed. Some lot to lot variation in the disks, especially with earlier lots, dictates that quality control must be done on all new lots of disks. No deterioration of any of the lots over the 6-month period was noticed. The disks have a manufacturer's 2-year outdate.

For work with this system, we recommend using one, rather than three, disk patterns to eliminate the possibility of inoculating an organism to the wrong pattern. Our suggested pattern includes a tube motility and the following disks: H₂S-indole, Voges-Proskauer, citrate, *o*-nitrophenyl- β -D-galactopyranoside, phenylalanine, urea, lysine, ornithine, adonitol, inositol, arabinose and sorbitol. Of 116 additional organisms inoculated using this pattern, only two required additional work on the second day to complete the identifications.

Minitek is a convenient system to use. The plates were inoculated in a fraction of the time needed to inoculate equivalent tubed media, and the inoculum was more uniform to each substrate. The relatively large number of tests

per plate (up to 12) helps eliminate possible misidentifications and shortens the number of days needed to identify an organism. Because of the flexibility of this system, it has been expanded to the identification of anaerobes (4) and pathogenic *Neisseria* (D. C. Mayfield, G. L. Wans, and C. A. Fien, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C18, p. 29). It also has the potential for expansion to the identification of nonfermenters and yeasts. The fact that the system is accurate, and in addition compact and reliable, presents a distinct advantage over the conventional tube system.

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