

## Evaluation of the Minitek System for Identification of *Enterobacteriaceae*

T. E. KIEHN, K. BRENNAN, AND P. D. ELLNER

*Diagnostic Microbiology Service, Columbia-Presbyterian Medical Center; and the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032*

Received for publication 15 August 1974

Clinical isolates (869) and stock cultures (35) of *Enterobacteriaceae* were tested in parallel with the Minitek and conventional systems. The Minitek correctly identified 822 of 904 cultures. When a deoxyribonuclease plate was inoculated along with the Minitek, it was possible to speciate *Enterobacteriaceae* within 24 h. False-positive hydrogen sulfide reactions were the major fault with this system. Reactions were clear-cut and easy for technologists to read.

In recent years, a variety of multimedia systems and devices designed to facilitate bacterial identification have become commercially available. Many of these proprietary systems consist of combinations of substrates in more or less fixed form, obliging the user to utilize the biochemical reactions selected by the manufacturer.

Recently, a new system (Minitek; BioQuest) has become available which offers the microbiologist considerable flexibility in selecting the tests to be used for bacterial identification. The Minitek system utilizes paper disks impregnated with individual substrates. These disks are placed in wells in a plastic plate and inoculated with a broth suspension of the isolate. Subsequent identification is based upon color reactions occurring in the disks following incubation. Thirty-five different disks are presently available from the manufacturer permitting the user to select those tests appropriate for his scheme of identification.

### MATERIALS AND METHODS

Organisms tested in this study were recovered from clinical specimens submitted to the Diagnostic Microbiology Service of Presbyterian Hospital, or they were obtained from the National Center for Disease Control.

Clinical isolates (869) and stock cultures (35) were tested in parallel by the conventional system employed in this laboratory, and by Minitek production material as marketed by the manufacturer. Bacteria were first identified by a technologist using the conventional system described below and then were given to a second technologist as blind coded subcultures on eosin-methylene blue or blood agar plates for identification by the Minitek system.

**Conventional system.** Isolates were inoculated onto Christensen urea agar, Moeller ornithine and

lysine decarboxylase broth, Simmon citrate, 1% adonitol and 1% arabinose in phenol red broth base, phenylalanine agar, malonate broth, deoxyribonuclease (DNase) agar, and sulfide-indole-motility agar. Reactions were read after 24 h. All organisms were tested for cytochrome oxidase and, when appropriate, for beta-galactosidase with *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) tablets. Bacteria were identified according to the schema of Edwards and Ewing (1).

**Minitek system.** The Minitek system consists of a covered, rectangular, plastic plate containing 12 wells. The manufacturer also supplied a multiple disk dispenser, a pipetter with disposable tips, and vials containing 1.0 ml of inoculum broth. For use, the plate was placed in the disk dispenser and a preselected set of disks was released into the wells. Disks employed in our study included: urea, ornithine, lysine, citrate, adonitol, arabinose, phenylalanine, malonate, hydrogen sulfide-indole, ONPG, inositol, and Voges-Proskauer.

With the exception of Voges-Proskauer, inositol, and motility, all tests were employed in both systems. Since the Minitek does not provide for the determination of motility, identification schemes were devised which utilized Voges-Proskauer and inositol reactions instead. Organisms giving a positive oxidase test were excluded from the evaluation. Isolates showing discrepant reactions between the two systems were retested with both systems. A single colony of the isolate was picked with a loop and emulsified in a vial of the inoculum broth. One milliliter of the inoculum was withdrawn from the vial by means of the pipetter and distributed into each well in portions of 0.05 ml. Two-tenths milliliters of sterile mineral oil was placed into wells containing hydrogen sulfide-indole, urea, lysine, ornithine, adonitol, arabinose, and inositol disks. The lid was replaced and the plate was placed in a humidior which was incubated at 35 C for 18 to 24 h. Reactions were read either by observing color changes in the disks or, in certain cases (indole, Voges-Proskauer, and phenylalanine), the color produced in the well after the addition of the appropriate

reagents. *Enterobacteriaceae* were identified according to the scheme shown in Fig. 1 and Tables 1-6, which utilize the data of Edwards and Ewing (1).

**RESULTS**

False-positive hydrogen sulfide reactions (123) occurred with the Minithek; these were not associated with any particular species. On retesting with a new lot of hydrogen sulfide disks, 38% of them still gave false-positive reactions.

Twenty-five discrepancies occurred with the urea reaction of the Minithek system. These 25 involved false-negative Minithek reactions with *Serratia*, *Enterobacter*, or *Citrobacter*. On retesting, all of these reactions remained negative.

Two *Proteus vulgaris* isolates gave false-negative indole reactions with Minithek. On retesting,

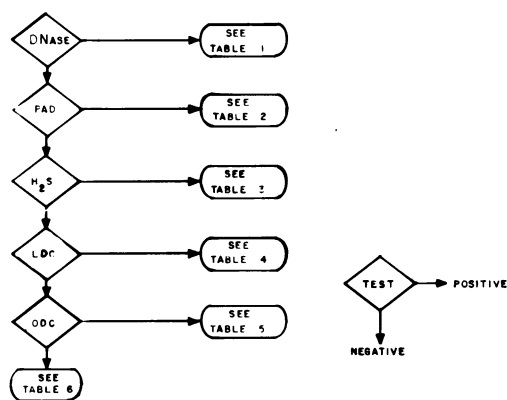


FIG. 1. Basic reactions for grouping *Enterobacteriaceae*.

TABLE 1. Identification of DNase-positive organisms

Organisms	Phenylalanine	Ornithine	Arabinose
<i>Serratia marcescens</i>	-	+	-
<i>Serratia liquefaciens</i>	-	+	+
<i>Serratia rubidaea</i>	-	-	-
<i>Proteus vulgaris</i>	+	-	-

TABLE 2. Identification of DNase-negative and phenylalanine-positive organisms

Organisms	ONPG	Urea	Adonitol	H <sub>2</sub> S	Ornithine	Indole
<i>Proteus vulgaris</i>	-	+	-	+	-	-
<i>Proteus mirabilis</i>	-	+	-	V <sup>a</sup>	+	-
<i>Proteusmorganii</i>	-	+	-	-	+	+
<i>Proteus rettgeri</i>	-	+	-	-	-	-
<i>Providencia stuartii</i>	-	-	+	-	-	-
<i>Providencia alcalifaciens</i>	-	-	-	-	-	-
<i>Enterobacter agglomerans</i>	+	-	-	-	-	-

<sup>a</sup> V, Variable reactions.

TABLE 3. Identification of DNase- and phenylalanine-negative and H<sub>2</sub>S-positive organisms

Organisms	Indole	ONPG	Lysine
<i>Salmonella</i>	-	-	+
<i>Arizona hinshawii</i>	-	+	+
<i>Citrobacter freundii</i>	-	-	-
<i>Escherichia coli</i>	+	+	-
<i>Edwardsiella tarda</i>	+	-	-

the indole reaction remained negative.

A single isolate of *Klebsiella pneumoniae* repeatedly gave a false-negative citrate reaction with Minithek. *Proteus* isolates gave variable results for citrate utilization with both the conventional and Minithek systems, and consequently this reaction was not utilized in the identification of this genus.

On initial testing, some discrepancies between Minithek and conventional reactions were noted which were resolved on retesting.

The results of the comparison of Minithek tests with the conventional tests are shown in Table 7.

The organisms tested are shown in Table 8. Minithek provided correct identification of 822 of 904 organisms. All 87 errors in identification were the result of the false-positive hydrogen sulfide reaction of the Minithek.

Three *Proteusmorganii* strains were initially identified as *Proteus rettgeri* because of false-negative ornithine reactions. A *Citrobacter diversus* was incorrectly identified as a *Citrobacter freundii* because of an initial false-negative indole reaction. A *Salmonella* group B was mistaken for an *Enterobacter aerogenes* because of a false-negative hydrogen sulfide reaction. On retesting, each of these organisms were correctly identified.

**DISCUSSION**

The appearance of false-positive hydrogen sulfide reactions is a major fault we have found with this system. These reactions were weaker than a true positive at 18 h, but could, and

TABLE 4. Identification of DNase-, phenylalanine-, and H<sub>2</sub>S-negative and lysine-positive organisms

Organisms	Indole	Ornithine	Urea	Voges-Proskauer	Adonitol	ONPG
<i>Klebsiella pneumoniae</i>	V	-	V	+	-	-
<i>Klebsiella ozoenae</i>	-	-	-	-	+	-
<i>Enterobacter aerogenes</i>	-	+	-	-	+	-
<i>Enterobacter hafniae</i>	-	+	-	+	-	-
<i>Escherichia coli</i>	V	V	-	-	-	+
<i>Salmonella typhi</i>	-	-	-	-	-	-

TABLE 5. Identification of DNase-, phenylalanine-, H<sub>2</sub>S-, and lysine-negative and ornithine-positive organisms

Organisms	Citrate	Indole	Urea	ONPG	adonitol	Voges-Proskauer
<i>Citrobacter freundii</i>	+	V	-	-	-	-
<i>Citrobacter diversus</i>	+	+	-	-	+	-
<i>Enterobacter cloacae</i>	+	-	-	-	-	+
<i>Yersinia enterocolytica</i>	-	-	+	-	-	-
<i>Escherichia coli</i>	-	+	-	-	-	-
<i>Shigella sonnei</i>	-	-	-	+	-	-
<i>Salmonella paratyphi A</i>	-	-	-	-	-	-

TABLE 6. Identification of DNase-, phenylalanine-, H<sub>2</sub>S-, lysine-, and ornithine-negative organisms

Organisms	Voges-Proskauer	Inositol	Adonitol	Malonate	Citrate	Urea
<i>Klebsiella pneumoniae</i>	+	+	-	-	-	-
<i>Enterobacter agglomerans</i>	V	-	-	-	V	-
<i>Klebsiella ozoenae</i>	-	-	+	-	-	-
<i>Klebsiella rhinoscleromatis</i>	-	-	+	+	-	-
<i>Citrobacter freundii</i>	-	-	-	-	+	-
<i>Yersinia pseudotuberculosis</i>	-	-	-	-	-	+
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>Shigella</i> (not group D) <sup>a</sup>	-	-	-	-	-	-

<sup>a</sup> Distinguished from *Escherichia coli* by agglutination reaction.

TABLE 7. Comparison of Minitek and conventional tests

Test <sup>a</sup>	No. tested	No. agreeing (%)	Minitek +/ conventional -	Minitek -/ conventional +
H <sub>2</sub> S	904	781 (86.4)	123	-
Urea	904	879 (97.2)	-	25
Indole	904	902 (99.8)	-	2
Citrate	752	751 (99.9)	-	1
PAD	904	904 (100)	-	-
LDC	904	904 (100)	-	-
ODC	904	904 (100)	-	-
ONPG	202	202 (100)	-	-
Adonitol	93	93 (100)	-	-
Arabinose	42	42 (100)	-	-
Malonate	213	213 (100)	-	-

<sup>a</sup> PAD, Phenylalanine deaminase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase.

sometimes did, lead to false identification. If this deficiency were corrected, all organisms in our study would have been correctly identified.

The false-negative urea reactions with the *Serratia*, *Enterobacter*, and *Citrobacter* species were not entirely unexpected. Christensen urea, used in the conventional system, is known to be a very sensitive indicator of urease activity. These false-negative reactions did not lead to false identification.

The rare aberrant indole and citrate reactions did not lead to incorrect identification.

During the initial phase of the study, it was observed that erroneous results with the Minitek could result from an inadvertent oil seal created as a result of spillage. It was also noted that a delay in excess of 30 min in adding the oil was responsible for a number of inaccurate results. Prompt and careful addition of the oil on retesting was found to markedly reduce these incorrect reactions.

Technologists in our laboratory were able to learn the Minitek method quickly and easily. The inoculum is prepared from a single colony and reduces the possibility of error due to a mixed culture. Reactions are clear-cut and

TABLE 8. Accuracy of identification using the Minithek system

Organism	No. tested	No. correct
<i>Escherichia coli</i>	298	298
<i>Klebsiella pneumoniae</i>	214	174
<i>Proteus mirabilis</i>	133	133
<i>Enterobacter cloacae</i>	50	34
<i>Enterobacter aerogenes</i>	43	29
<i>Serratia marcescens</i>	41	32
<i>Proteus morgani</i>	20	20
<i>Providencia stuartii</i>	16	16
<i>Salmonella</i> group B	12	12
<i>Proteus rettgeri</i>	11	11
<i>Citrobacter freundii</i>	11	11
<i>Citrobacter diversus</i>	11	8
<i>Shigella</i> group D	9	9
<i>Proteus vulgaris</i>	5	5
<i>Enterobacter agglomerans</i>	4	4
<i>Salmonella typhi</i>	4	4
<i>Salmonella</i> group C <sub>1</sub>	4	4
<i>Shigella</i> group B	3	3
<i>Salmonella</i> group D	3	3
<i>Klebsiella ozoenae</i>	2	2
<i>Edwardsiella tarda</i>	2	2
<i>Yersinia enterocolytica</i>	2	2
<i>Serratia liquefaciens</i>	1	1
<i>Serratia rubidaea</i>	1	1
<i>Providencia alcalifaciens</i>	1	1
<i>Salmonella paratyphi</i> A	1	1
<i>Arizona hinshawii</i>	1	1
<i>Yersinia pseudotuberculosis</i>	1	1

readily discernable. Inoculation of the conventional system takes twice as long as with the Minithek system. One considerable advantage of the Minithek over the other systems is the flexibility which allows the user to select tests for his own system. When a DNase plate was inoculated together with the Minithek, it was possible to routinely speciate members of the *Enterobacteriaceae* within 24 h. We consider the use of the DNase reaction essential for identification by our scheme. The Minithek system utilizes significantly less refrigerator and incubator space than the tubes of a conventional system. The manufacturer claims a 2-year shelf life for the disks.

We found the Minithek system to provide a most convenient means of performing biochemical tests on the *Enterobacteriaceae*, and if the hydrogen sulfide problem is corrected, it would provide an acceptable alternative to conventional methods.

#### ACKNOWLEDGMENTS

We thank Clyde Thornsberry of the National Center for Disease Control in Atlanta for providing a number of cultures used in this study. We also gratefully acknowledge the assistance of Helen Ssinegurski for her help in preparing the tables.

#### LITERATURE CITED

1. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minn.