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

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Thirty-one different substrate disks were tested in parallel with comparable, prepared media (BBL) against a minimum of 300 cultures of *Enterobacteriaceae*. An overall correlation of 98% was observed with all the disks tested. In addition, the system was used to identify 461 fresh isolates of *Enterobacteriaceae* in parallel with conventional media using the schema used at the Veterans Administration Hospital, Baltimore. An overall correlation of 97% was observed. Minitek is a time and space saving system. It is accurate and easily adapted to the clinical laboratory. A wide variety of substrates are available, allowing most laboratories to use their own schema. The long shelf life of most disks is a definite advantage.

The BBL Minitek system utilizes paper disks impregnated with appropriate biochemicals to identify the *Enterobacteriaceae* and aid in the identification of clinically significant anaerobes. The system includes instructions, a special disposable plate and lid, dispenser, pipetter, broth and humidor, as well as a color comparator card set to help interpret each reaction. The present study was carried out to determine the accuracy of individual disks, the adaptability to the clinical laboratory, and the reliability for the identification of *Enterobacteriaceae*.

MATERIALS AND METHODS

The microorganisms used in this study were isolated from fresh clinical specimens which included urines, sputa, stools, abscesses, blood, body fluids, drainages, etc. A few stock cultures were used after streaking from a Trypticase agar base tube (BBL) to Levine eosin methylene blue agar and checking for purity. All organisms were identified according to Edwards and Ewing (1). A minimum of 300 cultures of *Enterobacteriaceae* were tested with comparable prepared media for each of the 31 production prototype disks.

BBL Minitek substrates were used following the manufacturer's suggestion. Standard tests were inoculated from the same Levine eosin methylene blue plate at the time Minitek substrates were tested. In addition to inoculating comparable substrates in the conventional manner, the normal schema used in our hospital was also used to identify the organism. The prepared standard media (BBL) included a TSI slant, Trypticase soy broth for indol production, Simmons citrate, malonate, Moeller lysine, and ornithine decarboxylase broths, Trypticase agar base with arabinose, rhamnose, inositol, urea agar slant, and phenylalanine agar. Incubation for all tests was at 37 C. Reagents were added to appropriate disks from a Nalge plastic dropper bottle. One drop of a 10% ferric chloride solution was added to the phenylalanine disk; 2 to 3 drops of Kovac reagent was added to the H_2S disk after the H_2S reaction was recorded; 1 or 2 drops each of nitrate reductase reagents was added to the dextrose/nitrate disk after recording the dextrose reaction; and one or two drops each of Voges-Proskauer reagents was added to the Voges-Proskauer disk. Gas production was recorded when seen in the dextrose/nitrate well.

RESULTS AND DISCUSSION

During the evaluation period Minitek reactions were read and recorded by one person and conventional results by another. We alternated this procedure to prevent bias interpretation. The overall results obtained in the comparison of Minitek production prototype disks are shown in Table 1. The overall correlation for all substrates tested was 97.5%.

Reactions were recorded as correct if they agreed with conventional media after the 18- to 24-h incubation period. The relatively lower correlation with inositol, dulcitol, sucrose, and raffinose does not represent lack of fermentation using the Minitek disk but rather slow fermentation by the conventional method. If fermentation in conventional media was negative in 18-24 h and Minitek was positive, the Minitek reaction was recorded as incorrect. The negative companion conventional tubes were reincubated up to 72 h, and in every case showed positive fermentation with prolonged incuba-

TABLE 1. *Production prototype disks and percentage of correlation with conventional media*

Substrate	Correlation with conventional media (%)	Substrate	Correlation with conventional media (%)
Dextrose	100	Dextrose/nitrate	99.7
			99.7
Sucrose	94.7	Lysine	95.9
ONPG	97.5	H_2S/Indol	96.9
			96.9
Raffinose	95.6	Ornithine	94.7
Malonate	97.7	Lactose	98.1
Rhamnose	97.7	Arginine	97.5
Urea	98.9	Dulcitol	95.9
Inositol	95.6	Trehalose	99.7
Phenylalanine	100	Xylose	95.4
Voges-Proskauer	97.4	Galactose	99.0
Esculin	98.0	Melibiose	98.8
Sorbitol	99.0	Levulose	98.8
Mannitol	99.5	Salicin	98.8
Arabinose	99.0	Maltose	98.8
Adonitol	99.5	Cellobiose	97.9
Citrate	91.2		

tion. The lower correlation of the citrate and ornithine disks using the production prototype was considered a significant problem when compared to the other substrates tested.

Upon completion of the prototype study, a total of 461 fresh isolates of *Enterobacteriaceae* were identified using the normal schema used at the Veterans Administration (V.A.) Hospital and compared with production Minitek disks, which included improved citrate and ornithine disks (Table 2).

The results of this study are summarized in Table 3. Using the Minitek system, 14 of 461 organisms were misidentified or unable to be identified. The vast majority of isolates were in the *Escherichia*, *Klebsiella*, and *Proteus* groups, and 98% of these were correctly identified.

The 461 strains tested represented a total of 5,993 reactions, of which 5,930 were correct. Of the 63 incorrect reactions, 19 led to the misidentification of the 14 organisms. Table 4 lists the incorrect reactions for the organisms missed.

In every case the production disks performed as well as, if not better than, the production prototype. In fact, the production citrate and ornithine improved appreciably over the prototype disks (Table 5).

There were a total of nine false-positive H_2S reactions out of 20 cultures using the first production lot no. 402019 disks. We then requested and tested a replacement lot of H_2S disks, and for the remaining 441 cultures there were no false positives.

TABLE 2. *Biochemicals for identification of Enterobacteriaceae*

Normal schema V.A. Hospital	Minitek substrates
TSI	Dextrose/nitrate
	ONPG
	H_2S
Indol	Indol
Citrate	Citrate
Malonate	Malonate
Moeller lysine	Lysine
Moeller ornithine	Ornithine
Phenylalanine	Phenylalanine
Urea slants	Urea
Arabinose	Arabinose
Rhamnose	Rhamnose
Inositol	Inositol

TABLE 3. *Fresh isolates of Enterobacteriaceae*

Organism	Minitek correct	Correctly identified (%)
Escherichia coli	155	98.7
Shigella	4	100.0
Salmonella	6	100.0
S. typhi	1	50.0
Arizona	1	100.0
Citrobacter freundii	2	66.6
C. diversus	19	100.0
Klebsiella pneumoniae	96	95.0
Enterobacter cloacae	16	88.9
E. aerogenes	7	100.0
E. agglomerans	2	66.6
Serratia marcescens	12	100.0
S. liquefaciens	2	100.0
Proteus vulgaris	5	100.0
P. mirabilis	88	97.7
P. morganii	5	100.0
P. rettgeri	10	100.0
Providencia stuartii	11	100.0
Acinetobacter calcoaceticus	1	
Pseudomonas species (Oxidase-positive strains)	25	

Color changes for carbohydrate fermentation were clear and easy to read. Gas production with the dextrose/nitrate disk overlaid with mineral oil was not a reliable reaction. The color comparator card was necessary until one became familiar with the color changes. The carbohydrates, citrate, malonate, urea, and phenylalanine gave strong positive reactions in all cases when positive. The o-nitrophenyl-β-D-galactopyranoside (ONPG) and H_2S disks gave intermediate to strong positive reactions in our hands. Reaction color changes with the lysine

TABLE 4. *Organisms not identified by Minitek and incorrect reactions*

Organism	Minitek	Conventional
Escherichia coli (2 strains)	Indol−	Indol+
Salmonella typhi	H_2S−	H_2S+ (slow)
Citrobacter freundii	H_2S−	H_2S+
Klebsiella pneumoniae	Lysine− Ornithine+ Urea−	Lysine+ Ornithine− Urea+
K. pneumoniae (4 strains)	Lysine−	Lysine+
Enterobacter cloacae (2 strains)	Ornithine− Malonate−	Ornithine+ Malonate+
E. agglomerans	Citrate− Arabinose−	Citrate+ Arabinose+
Proteus mirabilis	Urea−	Urea+
P. mirabilis	Ornithine−	Ornithine+

TABLE 5. *Minitek substrates percentage of correlation*

Substrate	Production prototype	Production disks
Dextrose	99.7	99.6
Indol	96.9	98.9
Citrate	91.2	96.3
Lysine	95.9	98.2
Ornithine	94.7	98.0
ONPG	97.5	99.6
Malonate	97.7	99.7
Phenylalanine	100.0	99.3
Urea	98.9	99.6
Arabinose	99.0	99.6
Rhamnose	97.7	99.6
Inositol	95.6	99.6
H_2S	96.9	98.0

and ornithine disks tended to be very confusing. The color comparator cards for the lysine and ornithine disks were identical. However, our experience using the system showed that the lysine positive was never as intense in color as was the ornithine positive. Positive reactions with the lysine disk were in the yellow-orange to orange range and seldom in the red range. Negative reactions were always yellow. The ornithine positives were in the orange red to dark-red range and negatives in the yellow to yellow-orange range. Our data reflects the use of our modification of the color comparator card for interpretation of the lysine reaction. Indol production, phenylalanine deaminase, and nitrate reduction was determined after the addition of appropriate reagents. The phenylalanine deaminase reaction was a rapid one and should be recorded immediately, as the color disappears upon standing. The nitrate reaction was also rapid, especially when mixed in the well with a wooden applicator stick. Indol production was also easy to interpret. After the addition of Kovac reagent if no pink color developed, mixing with a wooden applicator stick hastened the reaction if positive. Upon standing, the indol reaction tends to fade. Any pink to red color should be regarded as positive, even if the color later fades.

The *Acinetobacter* and *Pseudomonas* species mentioned in Table 3 represent nonfermenters often encountered in the clinical laboratory. Four of five isolates of *Acinetobacter* gave apparent fermentation reactions with dextrose. Subsequent nitrate tests, however, were negative indicating the organisms were not members of the *Enterobacteriaceae*. We also ran 25 strains of oxidase-positive *Pseudomonas* species and none of these gave erroneous dextrose reactions.

The increased need to rapidly and accurately differentiate members of the family *Enterobacteriaceae* has initiated the use of miniaturized, compact, ready-to-use biochemical systems. In recent years many such systems have become commercially available.

The major criteria for evaluating an identification system in our laboratory are accuracy of identification, adaptability to the laboratory, ease in interpretation of reactions, flexibility of use and, ideally, time and space saving.

It has been our experience and others' that several such systems demonstrate a high degree of accuracy when compared with conventional media (2, 3, 4). The overall accuracy of the Minitek in our study was 96.9%. An accuracy of 98% was noted with members of the *Escherichia*, *Klebsiella*, and *Proteus* groups. Identification of organisms in our laboratory depends not only on biochemical reactions, but also on colony morphology on primary media and the sensitivity pattern of the organism. We did not take these factors into consideration in our evaluation. If we had, four strains of *Klebsiella pneumoniae* and two strains each of *Escherichia coli* and *Proteus mirabilis* would not have been misidentified.

Ease of adaptability in the laboratory is dependent on two things: clarity in instructions as stated by the manufacturer, and willingness of the technical staff to fairly evaluate the system. Instructions for use of the Minitek are lengthy, but once understood and followed flow smoothly and easily. It was our experience in using the system that the amount of oil overlay used was a critical point. Too little would give

aberrent reactions; too much was not a problem unless the oil spilled onto the lid and created a seal that can prevent aerobic reactions from occurring in the ONPG, citrate, and malonate disks. It was also important to overlay the urea disk within 10 min after inoculation. An entire colony should be picked to the inoculum broth. It was our experience that an excess never interferred with proper results, but too sparse a suspension of organisms may not allow the reactions to occur in the recommended incubation period. It was our procedure to complete seeding of all broths before inoculation of the Minitek plates. We utilized both sterile and nonsterile pipet tips and did not find contamination to be a problem. It was essential to maintain an adequate reservoir of water in the humidor, or dehydration of unlayered disks occurred. After incubation reagents should be added and read one plate at a time. For those disks with an oil overlay, mixing with a wooden applicator stick often hastens the reaction.

The only reaction that is difficult to interpret is the lysine decarboxylase. The lysine and ornithine reactions should not be compared to each other. We believe that a different color comparator card should be made available for the interpretation of the lysine and ornithine reactions. The manufacturer agrees and at this writing is in the process of distributing corrected color comparator cards for the lysine and ornithine reactions. The urea disk appeared to be very sensitive. *K. pneumoniae* species gave strong positive reactions when often the conventional urea agar slant yielded slower or delayed reactions.

The Minitek system offers complete flexibility. The choice of substrates is left to the discretion of the user. The schema used in this study was determined by the needs of our laboratory. Though it is not always necessary to use 12 biochemicals for identification of each organism, it was found advantageous to do so because it eliminated the need for additional subculturing and consequently saved considerable time and cost.

The 2 to 3 min required to prepare and inoculate a Minitek plate is a definite advantage. By filling the plates in advance and allowing them to sit at room temperature, even less time is required to inoculate a plate. No mechanical problems were encountered with either the dispenser or the pipetter. The minimal space required to store supplies, the long shelf life of most of the substrates, the accuracy, ease in interpretation of reactions, and flexibility have satisfied the needs of this laboratory for rapid and reliable identification of *Enterobacteriaceae*.

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