JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1981, p. 370-375
0095-1137/81/100370-06$02.00/0

Vol. 14, No. 4

Clinical Laboratory Evaluation of the AutoMicrobic System *Enterobacteriaceae* Biochemical Card

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Received 7 May 1981/Accepted 21 May 1981

The AutoMicrobic System *Enterobacteriaceae* Biochemical Card (AMS-EBC; Vitek Systems, Inc.) was evaluated in two clinical microbiology laboratories. A total of 502 consecutive clinical isolates representing members of the family *Enterobacteriaceae* were tested in parallel with the AMS-EBC, API 20E, and Enterotube II systems. Discrepancies between systems were resolved with the conventional methods of Edwards and Ewing (P. R. Edwards and W. H. Ewing [ed.], *Identification of Enterobacteriaceae*, 1972) and Ewing and Martin (W. H. Ewing and W. J. Martin, *in Manual of Clinical Microbiology*, 1974) AMS-EBC correctly identified 96.6% and incorrectly identified 3.4% of the isolates. When 12 or more isolates of a species were evaluated, *Serratia marcescens*, *Proteus mirabilis*, and *Enterobacter cloacae* posed the greatest challenge to the system, with 92.6, 95.2, and 95.3%, respectively, being correctly identified. To confirm the accuracy of identification when all systems agreed, 93 randomly selected isolates were identified by conventional methods. The percent agreement was 100%. The reproducibility of triplicate determinations on 93 randomly selected isolates with the AMS-EBC was 99.6%. The AMS-EBC was found to be an easy, rapid, and accurate method for identification of *Enterobacteriaceae*.

Within the past 10 years, there have been many advances in miniaturized and semiautomated techniques for the identification of microorganisms, particularly members of the family *Enterobacteriaceae* (1-5, 8, 9, 11-14). Vitek Systems, Inc., recently introduced the AutoMicrobic System *Enterobacteriaceae* Biochemical Card (AMS-EBC; 10) that allows a fully automated approach to the identification of *Enterobacteriaceae*. This report presents the results of an evaluation of AMS-EBC for its accuracy in identification of fermentative, gram-negative bacilli.

MATERIALS AND METHODS

Organisms. A total of 502 consecutive clinical isolates representing members of the family *Enterobacteriaceae* were obtained from two clinical microbiology laboratories in the Texas Medical Center. All organisms were assigned a numerical code, and the identity of the organisms was unknown during the evaluation.

Identification methods. All organisms were identified in parallel by use of the AMS-EBC (Vitek Systems, Inc., subsidiary of McDonnell-Douglas, Hazelwood, Mo.), API 20E (Analytab Products, Plainview, N.Y.), and Enterotube II systems (Roche Diagnostics, Div. Hoffman-La Roche, Inc., Nutley, N.J.). The API 20E and Enterotube II systems were inoculated, incubated, and read according to the manufacturer's instructions. Since the performance characteristics of the API 20E and Enterotube II systems are well established (1, 2, 5, 9), no further evaluation of these products was made. Isolates that showed discrepancies when tested with the three systems were examined at the Houston City Health Department Laboratories by the conventional methods of Edwards and Ewing (6) and Ewing and Martin (7).

Ninety-three randomly selected isolates that demonstrated complete agreement between the three test systems were identified by conventional methods to confirm their identification. These same 93 isolates were identified by AMS-EBC on three separate occasions to determine the reproducibility of the system.

Inoculum preparation. After approximately 24 h of incubation, several colonies were selected from primary culture plates of blood agar or MacConkey agar and suspended in 1.8 ml of 0.5% NaCl to a density equivalent to a McFarland no. 1 standard. This suspension was used to inoculate the AMS-EBC. A loopful of this suspension was also streaked onto blood agar and incubated overnight at 35°C for use in the inoculation of the API 20E and Enterotube II systems.

AMS-EBC. The AMS-EBC, a 30-compartment card containing 26 dried biochemical broths and a growth control broth, was previously described (10). The AMS-EBC was marked for computer recognition and then attached to the single-barreled card sample

injector. The inoculum was transferred from the injector into the AMS-EBC by the vacuum chamber that is a part of the system. The AMS-EBC was inspected to verify that it filled properly and then placed in the reader/incubator for analysis. Within 8 h, members of the *Enterobacteriaceae* were identified. All biochemical reactions and a probability number for the identification were printed automatically at the 8-h reading. The computer also provided the next-most-probable identification.

The computerized data base for the AMS-EBC was developed independently of the classical reactions of Edwards and Ewing (6). The development of an independent data base was necessary since the formulation of some of the media in the AMS-EBC, as well as inoculum density, volume within each well, passage of atmospheric gases, and length of incubation, differed from that of conventional methods. Consequently, the results obtained from the AMS-EBC are not expected or required to correlate always with those produced by conventional methods. Therefore, a test-by-test comparison of individual reactions appeared to be of limited value and thus was not performed. The evaluation was designed and conducted to determine the overall accuracy of identification by AMS-EBC.

RESULTS

A total of 502 members of the family *Enterobacteriaceae* representing 10 genera were tested. The distribution of these clinical isolates is shown in Table 1 by organism identification.

AMS-EBC correctly identified 96.6% of the clinical isolates tested in this evaluation (Table 1). Seventeen organisms (3.4%) were incorrectly identified. None of the organisms studied yielded inconclusive results.

Escherichia coli and *Klebsiella pneumoniae* were the predominant organisms and were correctly identified at 98.9 and 100%, respectively. When 12 or more isolates of a species were evaluated, *Serratia marcescens*, *Proteus mirabilis*, and *Enterobacter cloacae* posed the greatest challenge to the system, with 92.6, 95.2, and 95.3% of the strains, respectively, being correctly identified. These isolates represented 41.2% of the total identification errors. Two isolates of *Klebsiella ozaenae* and one each of *Enterobacter agglomerans*, *Enterobacter sakazakii*, *Citrobacter amalonaticus*, and *Shigella flexneri* biotype 6 were incorrectly identified. It should be pointed out that *E. sakazakii* and *C. amalonaticus* were not in the AMS-EBC data base, and, when the cultures were evaluated with the expanded AMS-EBC plus data base, they were correctly identified.

TABLE 1. *Accuracy of AMS-EBC identification of Enterobacteriaceae*

Organism	No. correct/no. tested	% Correct
Klebsiella pneumoniae	76/76	100
Enterobacter aerogenes	22/22	100
Citrobacter diversus	13/13	100
Morganella morganii	11/11	100
Providencia stuartii	5/5	100
Providencia rettgeri	4/4	100
Proteus vulgaris	3/3	100
Klebsiella oxytoca	3/3	100
Salmonella sp.	1/1	100
Escherichia coli	210/212	98.9
Enterobacter cloacae	41/43	95.3
Proteus mirabilis	59/62	95.2
Serratia marcescens	25/27	92.6
Citrobacter freundii	8/10	80
Enterobacter agglomerans	2/3	67
Shigella sp.	2/3	67
Klebsiella ozaenae	0/2	0
Citrobacter amalonaticus	0/1	0
Enterobacter sakazakii	0/1	0

The accuracy of the AMS-EBC (96.6%) was equivalent to those of the API 20E and Enterotube II systems at 97.2 and 97.8%, respectively. At the genus and species level of identification, the three methods agreed in 473 of the 502 isolates, or 94.2% (Table 2). Two of the three methods agreed in a further 4.4% of isolates (98.6%). When identification was taken only to the genus level, there was complete agreement with 95.2% of the isolates.

When two of the three systems produced the same identification, AMS-EBC was found to disagree 12 out of 22 times (Table 3). In all but three cases, when the results of any two systems yielded the same genus and species, the identification agreed with that obtained by conventional methods. The three exceptions were two *K. pneumoniae* and one *E. agglomerans*. In these three cases, none of the three systems gave the correct answer.

Discrepancies in AMS-EBC identification are shown in Table 4. Three *P. mirabilis* were identified as *Morganella morganii*. All reactions necessary to identify these organisms as *P. mirabilis* correlated with conventional testing, except for a negative xylose reaction by AMS-EBC.

Two *S. marcescens* were identified as *Serratia*

TABLE 2. *Overall comparison of organism identification*

No. of systems in agreement	Organisms in agreement			
	Genus and species level		Genus level	
	No.	%	No.	%
3	473	94.2	478	95.2
2	22	4.4	21	4.2
0	7	1.4	3	0.6

TABLE 3. *Correlation of systems in agreement with organism identification to genus and species level*[a]

Organism identification	No. of systems in agreement		Systems in disagreement		
	3	2	AMS-EBC	API 20E	Entero-tube II
Escherichia coli	210	2	2	0	0
Klebsiella pneumoniae	76	2	2[a]	0	0
Proteus mirabilis	59	3	3	0	0
Enterobacter cloacae	36	6	1	1	4
Enterobacter aerogenes	19	2	0	0	2
Serratia marcescens	25	2	2	0	0
Citrobacter diversus	13	0	0	0	0
Citrobacter freundii	8	1	1	0	0
Morganella morganii	10	1	0	1	0
Providencia stuartii	5	0	0	0	0
Providencia rettgeri	4	0	0	0	0
Proteus vulgaris	3	0	0	0	0
Klebsiella oxytoca	3	0	0	0	0
Enterobacter agglomerans	0	2	0	2[a]	0
Enterobacter sakazakii	0	1	1	0	0
Shigella sp.	1	0	0	0	0
Salmonella sp.	1	0	0	0	0

[a] When the results of any two systems yielded the same genus and species, the identification agreed with that obtained by conventional methods with the exception of two *K. pneumoniae* and one *E. agglomerans*. In these three cases, none of the three systems gave the correct answer.

TABLE 4. *Identification errors in the AMS-EBC*

Organism	AMS-EBC identification	Reasons[a]
Proteus mirabilis	*Morganella morganii* (3)[b]	Xylose negative
Serratia marcescens	*Serratia liquefaciens* (2)	Raffinose positive
Enterobacter sakazakii	*S. liquefaciens*	Rhamnose, lactose, and arginine negative
Enterobacter cloacae	*S. liquefaciens*	Arginine negative; lysine positive
	Enterobacter agglomerans	Urea, raffinose, and adonitol negative; malonate positive
Enterobacter agglomerans	*Enterobacter cloacae*	Ornithine and arginine positive
Citrobacter freundii	*E. agglomerans*	H_2S, arginine, adonitol, and urea negative; malonate positive
	E. cloacae	Arginine, inositol, rhamnose, and DP 300 negative; plant indican positive
Klebsiella ozaenae	*E. agglomerans*	Malonate, lysine, inositol, and xylose negative
	Klebsiella pneumoniae	Malonate, urea, and citrate positive
Escherichia coli	*Citrobacter freundii*	Urea, citrate, and H_2S positive
	Salmonella typhi	Sorbitol, rhamnose, and raffinose negative
Citrobacter amalonaticus	*Salmonella* sp.	*o*-Nitrophenyl-β-D-galactopyranoside, lactose, urea, arginine, plant indican, and DP 300 negative
Shigella flexneri biotype 6	*Shigella dysenteriae*	Mannitol negative[c]

[a] Reasons for inaccurate AMS-EBC reaction.

[b] Number in parentheses indicates number of isolates.

[c] AMS-EBC reaction agreed with conventional result; AMS-EBC data base not capable of yielding correct identification with negative mannitol reaction.

liquefaciens. These two isolates were arabinose negative by both AMS-EBC and conventional testing. This result would favor *S. marcescens* since arabinose is negative in 99% of *S. marcescens* and in only 5% of *S. liquefaciens*. However, false-positive raffinose utilization was responsible for these misidentifications. Ninety-one percent of *S. liquefaciens* are raffinose positive versus only 1% for *S. marcescens*, this single reaction resulting in misidentification.

One *E. sakazakii* was identified as *S. liquefaciens*. False-negative rhamnose, lactose, and

arginine dihydrolase reactions were primarily responsible for the identification as *S. liquefaciens* by AMS-EBC. *E. sakazakii* was not in the AMS-EBC data base at the time of this evaluation. Repeat testing of this isolate with the expanded AMS-EBC plus data base correctly identified this isolate as *E. sakazakii*.

Two *E. cloacae* showed discrepancies in the AMS-EBC analysis. False-negative arginine and false-positive lysine decarboxylase reactions led to the designation of *S. liquefaciens*, which is characteristically negative for arginine at a 99% level and positive for lysine 64% of the time. Arginine should be positive in 60% of reactions with *E. cloacae*, whereas lysine should be negative in 99% of reactions.

The other isolate of *E. cloacae* was identified as an *E. agglomerans*. Arginine was positive by AMS-EBC and conventional tests, thus favoring *E. cloacae*, since only 1% of *E. agglomerans* are arginine positive. Sorbitol and melibiose reactions were negative by AMS-EBC and conventional testing, and this result would favor *E. agglomerans*. False-negative raffinose fermentation was probably the pivotal reaction resulting in misidentification, since 90% of *E. cloacae* are raffinose-positive versus only 24% for *E. agglomerans*.

One strain of *E. agglomerans* was identified by AMS-EBC as an *E. cloacae*. The substrates involved in this misidentification were positive reactions for ornithine decarboxylase and arginine. Therefore, the reaction file in AMS-EBC yielded an identification of *E. cloacae* which is positive for ornithine and arginine at a 93 and 90% level, respectively. Ornithine and arginine are negative at a level of 99% for *E. agglomerans*.

There were two misidentifications of *C. freundii*. One isolate of *C. freundii* was identified as *E. agglomerans* by AMS-EBC. Five incorrect reactions were found when compared with conventional biochemicals. However, false-negative H_2S and arginine reactions were key reactions that led to the incorrect identification of *E. agglomerans*, with a probability of 57% as compared with 42% for *C. freundii*. Only 1% of *E. agglomerans* are positive for these two substrates, whereas *C. freundii* is positive for H_2S and arginine at 79 and 46%, respectively.

The other isolate of *C. freundii* was identified by AMS-EBC as *E. cloacae*. When compared with conventional biochemicals, false-negative arginine, inositol, and rhamnose reactions were noted. These reactions were not critical to the identification of *C. freundii*. A positive plant indican and a negative DP 300 reaction were probably responsible for the misidentification as *E. cloacae*. The three isolates were H_2S negative and xylose negative by AMS-EBC but were H_2S negative and xylose positive by conventional testing. The false-negative xylose reaction by AMS-EBC, which according to the AMS-EBC data base is 99% negative for *M. morganii*, was the pivotal reaction resulting in misidentification.

Two isolates of *K. ozaenae* were misidentified by AMS-EBC. In one instance, the organism was identified as an *E. agglomerans* with a probability of 63%. *K. ozaenae* was listed as a second choice at 32%. False-negative malonate, lysine, inositol, and xylose reactions by AMS-EBC were found when compared with conventional biochemicals.

In the second instance, *K. ozaenae* was identified as *K. pneumoniae*. This isolate produced false-positive malonate, urea, and citrate reactions when compared with the reactions obtained by conventional biochemical testing. Ninety-two and 50% of *K. pneumoniae* are malonate positive and urea positive, respectively, versus only 4 and 1%, respectively, for *K. ozaenae*; these two reactions were primarily responsible for this misidentification.

There were two misidentifications of *E. coli* by AMS-EBC. One of these isolates was identified as *C. freundii*. False-positive DP 300, urea, citrate, and H_2S reactions resulted in this misidentification. The other isolate of *E. coli* was identified as *Salmonella typhi*. This strain of *E. coli* was lactose and *o*-nitrophenyl-β-D-galactopyranoside negative by both AMS and conventional biochemicals. However, false-negative sorbitol, rhamnose, and raffinose reactions by AMS-EBC resulted in misidentification as *S. typhi*. Sorbitol, rhamnose, and raffinose were positive in only 1% of *S. typhi* versus 93, 83, and 49%, respectively, for *E. coli* All three carbohydrates were positive at 48 h when tested with conventional biochemicals.

One strain of *C. amalonaticus* was identified by AMS-EBC as a *Salmonella* species as a result of false-negative *o*-nitrophenyl-β-D-galactopyranoside, lactose, urea, and arginine reactions. The first two biochemicals were key reactions that resulted in misidentification. In addition, the plant indican and DP 300 reactions were negative. Only 1% of *Salmonella* species are positive for these latter two reactions. *C. amalonaticus* was not in the AMS-EBC data base. Repeat of this isolate with the AMS-EBC plus data base correctly identified the organism as *C. amalonaticus*.

One strain of *S. flexneri* biotype 6 was identified as a *Shigella dysenteriae* by AMS-EBC. The misidentification was based on a negative

mannitol reaction. Mannitol utilization was also negative by conventional testing. *S. flexneri* biotype 6 is unusual in that it is mannitol negative. A negative mannitol reaction in the reaction file resulted in the AMS-EBC computer yielding an identification of *S. dysenteriae*.

Ninety-three randomly selected isolates demonstrating complete agreement between AMS-EBC, API 20E, and Enterotube II were identified by conventional methods to confirm their identification. The percent agreement was 100%.

The reproducibility of triplicate determinations on 93 randomly selected isolates with the AMS-EBC was 99.6%. The 0.4% error represents 1 of 279 determinations. An *E. coli*, identified in two runs with a probability of 99%, was identified as an *E. aerogenes* at a probability of 44%.

DISCUSSION

The concept of automation is not new to microbiology laboratories, but the development and application of instruments for microbiological techniques have only recently become available. The increased productivity and efficiency of automation are widely recognized. The availability of an automated system for the identification of *Enterobacteriaceae* is a significant development for microbiology laboratories because no other group of organisms is more frequently encountered or more difficult to identify to the species level. The ability of the AMS-EBC to perform identification of the *Enterobacteriaceae* at an acceptable level was established by Isenberg et al. (10). In that collaborative study of 5,450 representatives of the *Enterobacteriaceae*, the AMS-EBC performed at an accuracy of 96.4%, compared with the routine methods used in the participating laboratories. The AMS-EBC correctly identified 96.6% of the 502 clinical isolates in the study reported here and compared favorably with the API 20E and Enterotube II systems at 97.2 and 97.8%, respectively.

A detailed discussion of the performance characteristics of the AMS-EBC was presented by Isenberg et al. (10) and will not be repeated here. We did, however, make the following relevant observations in our evaluation. Two or more false-negative biochemical reactions on AMS-EBC were noted with single isolates of *E. sakazakii*, *E. cloacae*, *C. freundii*, *K. ozaenae*, *E. coli*, and *C. amalonaticus* and resulted in misidentification of these organisms. Two or more false-positive biochemical reactions on AMS-EBC were noted with single isolates of *E. agglomerans*, *K. ozaenae*, and *E. coli* and resulted in misidentification of these organisms. These discrepancies were not common to any species and probably were the result of either too light or heavy inocula, respectively. Therefore, 9 of the 17 discrepancies observed with AMS-EBC were probably the result of inoculum error.

S. flexneri biotype 6, which is mannitol negative, would be identified as *S. dysenteriae* with the current AMS-EBC data base. Inclusion of dulcitol in the reaction file would have allowed identification of this organism as either *S. flexneri* biotype 6 or *S. dysenteriae* biotype 5.

The criterion used in this study for correct identification was 100% agreement for the three systems. To establish the validity of this assumption, 93 such isolates were identified by conventional biochemicals. The identifications obtained by conventional testing agreed with the three test systems in all cases. In fact, the identification of 19 of 22 isolates for which only two of the three systems agreed was substantiated by conventional testing (86.4%).

The reproducibility of the AMS-EBC in 279 determinations on 93 isolates was 99.6%. The one error observed was probably due to a bad EBC, rather than any inherent problem within either the equipment or the computer program.

The results produced by the three commercial systems were essentially equivalent. The AMS-EBC significantly reduces the time required to identify the *Enterobacteriaceae* and requires no technologist time for interpretation and recording of results, as do the other two systems. The automation of these functions also reduces the opportunity for human error. The ability of the AMS-EBC to yield the same result on repeat testing was outstanding at 99.6%. The capability of the AMS-EBC to identify accurately members of the *Enterobacteriaceae* coupled with the ability of the AMS to provide other automated functions such as urine microbiology, identification of nonfermentative gram-negative bacilli, antimicrobial susceptibility testing, and yeast identification, indicate that the AMS is a major advancement in automation of clinical microbiology.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Fran Schaeffer, Steven Johnson, Debbie Boyd, Laura Stout, and Loraine Vienn for their technical assistance in this evaluation. We also thank J. Vernon Knight and Robert P. Williams for their critical review of the manuscript and Gina Geyer for secretarial assistance.

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