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Identification of *Enterobacteriaceae* by the AutoMicrobic System: *Enterobacteriaceae* Biochemical Cards Versus *Enterobacteriaceae*-Plus Biochemical Cards

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Enterobacteriaceae Biochemical Cards (EBC) may be used in the AutoMicrobic system for identification of enteric bacilli. Recently, the card has been modified to permit identification of enteric and certain nonenteric bacilli. Also, minor modifications have been made in the computer program used for interpretation of tests with the new cards (EBC+). The two types of cards (EBC and EBC+) were tested in parallel and found to be in agreement with 97% of 650 *Enterobacteriaceae*. Most of the discrepancies were resolved when selected strains were retested on 3 separate days. A lack of absolute reproducibility with either system was demonstrated and explained most of the initial discrepancies. Approximately 97% of the AutoMicrobic system identifications agreed with those obtained from standard reference methods, after equivocal AutoMicrobic system results ($P < 0.80$) were excluded. Equivocal responses occurred with 4% of our EBC tests and 7% of our EBC+ tests; additional tests are needed before such strains can be identified with confidence.

The AutoMicrobic system (AMS) of Vitek Systems, Inc. (Hazelwood, Mo.), provides the opportunity for nearly complete automation of several microbiological procedures. By using the *Enterobacteriaceae* Biochemical Card (EBC), most *Enterobacteriaceae* can be identified within 8 h, expending a minimum amount of the technologist's time. Other investigators have found this system to be quite satisfactory (2, 4–7).

More recently, Vitek Systems, Inc. has introduced the *Enterobacteriaceae*-plus Biochemical Card (EBC+), which is said to be capable of identifying certain nonenteric gram-negative bacilli as well as the *Enterobacteriaceae*. The test reagents included in the EBC are DP-300 (3,4,4′-trichloro-2′-hydroxydiphenyl ether [4.0 μg], growth control, cetrimide, plant indican, urea, citrate, malonate, tryptophane deaminase, raffinose, rhamnose, maltose, sorbitol, melibiose, mannitol, xylose, sucrose, inositol, adonitol, H_2S, *o*-nitrophenyl-β-D-galactopyranoside, lactose, arabinose, glucose (fermentation), arginine, lysine, ornithine, and a decarboxylase control. The EBC+ contains all of the test reagents included in the EBC, but three additional tests were added to help identify certain nonenteric bacilli: glucose (oxidative), acetimide, and *p*-coumaric acid. When the EBC+ was introduced, a slightly modified computer program was also developed for interpreting test results with the new card. The species that are included in the two computer programs (EBC and EBC+) are listed in Table 1. The EBC program attempts to identify *Salmonella cholera-suis*, *Salmonella enteritidis*, *Shigella boydii*, and *Shigella flexneri*, whereas the EBC+ program identifies those species as *Salmonella* species or *Shigella* species. In addition, the EBC+ system recognizes three additional species that were not included in the EBC program.

A more complete evaluation of the AMS, using the EBC+, is presented in an accompanying manuscript. The present report compares the identifications of 650 *Enterobacteriaceae* with both types of cards and their appropriate computer programs. Triplicate retesting of 45 selected strains was then carried out to determine whether discrepancies were repeatable; most discrepancies were resolved with retesting, but new discrepancies occurred.

MATERIALS AND METHODS

Bacterial strains. The 650 stock cultures used in this study were identified by using standard reference methods as described by Ewing and Davis (3) and incorporating nomenclatural changes suggested by Brenner et al. (1). Eighteen tests performed with all isolates included: the oxidase spot test with 1% tetramethyl-*p*-phenylenediamine, H_2S production in triple sugar iron agar, the *o*-nitrophenyl-β-D-galactopyranoside test for β-D-galactosidase activity, DNase activity, lysine and ornithine decarboxylase, phenylalanine

TABLE 1. Species identified by EBC and EBC+ computer programs for the AMS

Species	Identified by:	
	EBC	EBC+
Arizona hinshawii	+	+
Citrobacter diversus	+	+
C. freundii	+	+
C. amalonaticus	−	+
Edwardsiella tarda	+	+
Enterobacter cloacae	+	+
E. aerogenes	+	+
E. agglomerans	+	+
E. gergoviae	−	+
E. sakazakii	−	+
Escherichia coli	+	+
Hafnia alvei	+	+
Klebsiella ozaenae	+	+
K. pneumoniae	+	+
K. rhinoscleromatis	+	+
Morganella morganii	+	+
Proteus mirabilis	+	+
Proteus vulgaris	+	+
Providencia stuartii	+	+
P. stuartii (urea positive)	+	+
P. rettgeri	+	+
P. alcalifaciens	+	+
Salmonella spp.	−	+
S. typhi	+	+
S. cholerae-suis	+	−
S. enteritidis	+	−
Serratia rubidaea	+	+
S. marcescens	+	+
S. liquefaciens	+	+
Shigella spp.	−	+
S. sonnei	+	+
S. dysenteriae	+	+
S. boydii	+	−
S. flexneri	+	−
Yersinia enterocolitica	+	+
Y. pseudotuberculosis	+	+
Aeromonas hydrophila	−	+
Plesiomonas shigelloides	−	+
Acinetobacter calcoaceticus	−	+
Pseudomonas aeruginosa	−	+
P. cepacia	−	+
P. fluorescens-putida	−	+
P. maltophilia	−	+
Vibrio cholerae	−	+
V. parahaemolyticus	−	+

deaminase, urease activity, malonate utilization, indole production, Voges-Proskauer reaction, motility, and fermentation of adonitol, arabinose, lactose, salicin, sucrose, and xylose. Additional tests were performed as needed to confirm the species identification. The isolates were stock cultures of clinical isolates selected to represent most, but not all, species or biotypes of the *Enterobacteriaceae* found in clinical material. The challenge set of cultures represented a disproportionately large number of atypical or uncommon strains; they are not representative of isolates routinely encountered in most clinical laboratories.

AMS. The AMS was utilized according to the manufacturer's instructions. A freshly isolated colony was selected from an 18- to 24-h blood agar plate culture and suspended in 1.8 ml of saline. The turbidity was adjusted, if necessary, to match that of a McFarland no. 1 standard. Both types of cards were then filled, using the Vitek injector. Near the end of this study, cards were filled with Vitek transfer tubes, reducing the number of filling failures from approximately 5 to <1%. Both cards were then transferred to the reader-incubator, which automatically examined each card at hourly intervals for 8 h or for 13 h if glucose was not fermented in the card. The test results were then interpreted by the AMS computer, and a report was printed. The printed report listed the reactions in each test well, the first- and second-choice identifications, and the probability (*P* value) that each identification was the correct one. The *P* value represents an estimate of the confidence that can be given to each identification. The manufacturer does not provide guidelines for deciding when a response can be accepted or when additional confirmatory tests may be needed to increase the confidence that can be given to an identification. In this report, AMS identifications with low *P* values were considered equivocal identifications; i.e., those for which additional tests were required before a report could be issued with reasonable confidence. On rare occasions, the AMS printed a report of an unidentified organism or nonviable (unsatisfactory growth in the control well); such responses were also considered equivocal. In all cases, direct comparisons of EBC and EBC+ identifications represent parallel tests performed at the same time and interpreted by the appropriate AMS computer program.

RESULTS

The overall agreement with reference identifications (accuracy) of both AMS test systems is summarized in Table 2. All first-choice identifications obtained with the EBC were 94.2% accurate, whereas those obtained with the EBC+ were 93.1% accurate. Many of the discrepant strains had first-choice identifications with relatively low *P* values and thus could be considered equivocal responses; i.e., there was a low probability that the response was a correct one. If one accepted only those first-choice identifications with $P \geq 0.90$, agreement with reference methods increased to 96.6% for the EBC and 97.1% for the EBC+. However, that would exclude 8.3% of the EBC tests and 8.8% of the EBC+ tests. By accepting responses with somewhat lower *P* values, the overall accuracy of both tests diminished slightly, but the percentage of strains excluded also decreased. We arbitrarily elected to consider all first-choice responses with $P < 0.80$ as being equivocal for either test system. At that level, 4.3% of the EBC responses and 6.9% of the EBC+ responses would be excluded as being equivocal tests. Identifications with $P \geq 0.80$ were 96.3 and 96.7% accurate for the EBC and EBC+, respec-

TABLE 2. Effect of excluding equivocal responses with low probability of accurate results from data obtained with the EBC and EBC+ in the AMS

% Probability values excluded	% Excluded at each P value[a]		% Agreement with reference tests[a]	
	EBC	EBC+	EBC	EBC+
None[b]			94.2	93.1
<0.90	8.3	8.8	96.6	97.1
<0.80	4.3	6.9	96.3	96.7
<0.70	2.9	4.8	95.9	95.8
<0.60	1.2	2.5	94.9	94.3
<0.50	0.6	1.1	94.6	93.8

[a] Based on tests with 650 *Enterobacteriaceae*, using both identification cards.
[b] Evaluation of all first-choice identifications without excluding responses with low P values.

tively, and that was considered to be very acceptable. Only 23 of 623 unequivocal EBC responses and 20 of 605 EBC+ responses disagreed with the reference tests. The 650 strains provided 27 EBC responses and 45 EBC+ responses that were considered equivocal; over half of these equivocal identifications were incorrect.

Because of the greater number of species that can be potentially identified with EBC+ (41

TABLE 3. Direct comparison of EBC and EBC+ identifications of 650 *Enterobacteriaceae* before and after excluding equivocal responses

Reference identification	No. of strains tested	No. of positive identifications after excluding equivocal responses[a]			% Agreement[b]	
		EBC	EBC+	EBC and EBC+	After excluding equivocal responses	All first-choice responses
Arizona hinshawii	6	6	6	6	100	100
Citrobacter diversus	23	23	22	22	100	100
Citrobacter freundii	29	29	27	27	89	90
Edwardsiella tarda	4	4	3	3	100	75
Enterobacter cloacae	58	56	52	52	100	97
Enterobacter aerogenes	52	51	50	49	98	98
Enterobacter agglomerans	17	13	12	11	100	94
Enterobacter gergoviae	3	3	3	3	0	0
Enterobacter sakazakii	3	3	1	1	0	0
Escherichia coli	102	97	96	94	99	94
Hafnia alvei	8	7	8	7	100	100
Klebsiella ozaenae	3	3	3	3	100	100
Klebsiella pneumoniae	72	70	68	68	94	92
Morganella morganii	26	26	26	26	100	100
Proteus mirabilis	72	66	65	63	100	94
Proteus vulgaris	28	27	23	23	96	86
Providencia stuartii	15	14	14	14	100	100
Providencia stuartii (urea positive)	6	5	6	5	100	100
Providencia rettgeri	12	12	12	12	92	92
Providencia alcalifaciens	4	4	4	4	100	100
Salmonella spp.	10	10	10	10	100	100
Salmonella typhi	8	8	8	8	88	88
Serratia rubidaea	3	3	3	3	100	100
Serratia marcescens	48	46	47	45	100	98
Serratia liquefaciens	2	1	1	1	100	50
Shigella spp.	6	6	6	6	100	100
Shigella sonnei	19	19	19	19	100	100
Yersinia enterocolitica	10	10	9	9	89	80
Yersinia pseudotuberculosis	1	1	1	1	100	100

[a] Identifications were considered to be equivocal if the probability of an accurate response was <0.80 or if "unidentified organism" was reported by the AMS.
[b] The percent agreement was 97% when equivocal results were excluded and 94% for all first-choice responses, with no exclusions.

TABLE 4. Reproducibility of EBC and EBC+ responses when 45 selected strains were retested on 3 separate days with both types of cards[a]

AMS response and type of card	No. of data pairs which agreed/ total no.[b]	Reproducibility index[c]
First-choice responses		
EBC	109/135	0.81
EBC+	123/135	0.91
Excluding equivocal responses[d]		
EBC	87/102	0.85
EBC+	102/106	0.96

[a] Strains represent those that initially demonstrated discrepancies among the EBC, EBC+ and reference identifications.

[b] Each triplicate test provided three data pairs (first and second, first and third, and second and third tests).

[c] Reproducibility index, number of data pairs in agreement divided by the total number of pairs compared.

[d] Test results were considered equivocal if $P < 0.80$ or if response given read "unidentified organism" or "nonviable."

versus 31), the computer program for interpretation of EBC+ results generated more first-choice identifications with relatively lower P values, and thus more equivocal identifications were obtained with EBC+ than with EBC. The EBC system provided 27 isolates which were considered equivocal, but 45 isolates were equivocal with the EBC+ system. If we had accepted all EBC+ identifications with $P \geq 0.70$, only 4.8% would have been excluded, and the overall accuracy would have been 95.8%. These differences were not considered sufficient to permit acceptance of EBC+ responses with lower P values.

Table 3 lists the species that were included in the 650 *Enterobacteriaceae* used to challenge both types of AMS cards. *Proteus mirabilis* and *Enterobacter agglomerans* provided the largest proportion of equivocal test results with both systems. Equivocal results were found for 54 strains with one or both systems; 97% of the remaining 596 strains displayed agreement between EBC and EBC+ identifications. If all first-choice identifications were accepted without excluding equivocal responses, the overall agreement between EBC and EBC+ was 94%. Six disagreements involved tests with *Enterobacter gergoviae* or *Enterobacter sakazakii*, which are not recognized by the EBC program but are included in the EBC+ program. With one exception, the EBC system identified the

TABLE 5. Repeated tests with strains initially yielding discrepancies between reference, EBC, and EBC+ identifications and variable AMS results when retested in triplicate

Reference identification	AMS card	First-choice identification (probability of accurate response[a])			
		Result from initial tests	Result from repeated tests in triplicate on separate days in trial no.:		
			1	2	3
Escherichia coli	EBC	*Enterobacter cloacae* (0.50)	*E. coli* (0.99)	*E. coli* (0.99)	*Arizona hinshawii* (0.94)
	EBC+	*E. coli* (0.99)	*E. coli* (0.99)	*E. coli* (0.99)	*E. coli* (0.99)
E. coli	EBC	*Edwardsiella tarda* (0.90)	*Shigella dysenteriae* (0.59)	*Shigella* sp. (0.39)	*E. tarda* (0.90)
	EBC+	*Vibrio cholerae* (0.77)	*V. cholerae* (0.77)	*V. cholerae* (0.98)	*V. cholerae* (0.99)
Edwardsiella tarda	EBC	*E. tarda* (0.99)	*Morganella morganii* (0.88)	*E. tarda* (0.98)	*M. morganii* (0.88)
	EBC+	*V. cholerae* (0.69)	*V. cholerae* (0.69)	*V. cholerae* (0.69)	*V. cholerae* (0.69)
Enterobacter agglomerans	EBC	*Klebsiella ozaenae* (0.67)	*K. ozaenae* (0.67)	*K. ozaenae* (0.67)	*K. ozaenae* (0.67)
	EBC+	*E. agglomerans* (0.59)	*K. ozaenae* (0.66)	*E. agglomerans* (0.59)	*K. ozaenae* (0.66)
Klebsiella pneumoniae	EBC	*Enterobacter aerogenes* (0.88)	*K. pneumoniae* (0.91)	*E. aerogenes* (0.98)	*K. pneumoniae* (0.91)
	EBC+	*K. pneumoniae* (0.91)	*K. pneumoniae* (0.91)	*K. pneumoniae* (0.91)	*K. pneumoniae* (0.91)

K. pneumoniae	EBC	*K. pneumoniae* (0.99)	*S. rubidaea* (0.99)	*K. pneumoniae* (0.86)	*S. rubidaea* (0.99)
	EBC+	*Serratia rubidaea* (0.96)	*S. rubidaea* (0.97)	*K. pneumoniae* (0.83)	*S. rubidaea* (0.87)
K. pneumoniae	EBC	*K. pneumoniae* (0.89)	*E. aerogenes* (0.99)	*K. pneumoniae* (0.89)	*K. pneumoniae* (0.99)
	EBC+	*S. rubidaea* (0.67)	*K. pneumoniae* (0.88)	*K. pneumoniae* (0.88)	*K. pneumoniae* (0.89)
K. pneumoniae	EBC	*K. pneumoniae* (0.99)	*E. cloacae* (0.89)	*K. pneumoniae* (0.98)	*K. pneumoniae* (0.98)
	EBC+	*E. cloacae* (0.87)	*K. pneumoniae* (0.99)	*K. pneumoniae* (0.97)	*K. pneumoniae* (0.97)
K. pneumoniae	EBC	*K. pneumoniae* (0.93)	*K. pneumoniae* (0.99)	*K. pneumoniae* (0.99)	*E. aerogenes* (0.99)
	EBC+	*E. aerogenes* (0.88)	*K. pneumoniae* (0.92)	*K. pneumoniae* (0.99)	*K. pneumoniae* (0.92)
Serratia liquefaciens	EBC	*Serratia marcescens* (0.64)	*Yersinia enterocolitica* (0.98)	*Y. enterocolitica* (0.99)	*Y. enterocolitica* (0.82)
	EBC	*E. agglomerans* (0.59)	*Y. enterocolitica* (0.98)	*E. agglomerans* (0.59)	*E. agglomerans* (0.59)
Proteus mirabilis	EBC	*P. mirabilis* (0.73)	*P. mirabilis* (0.70)	*M. morganii* (0.93)	*M. morganii* (0.69)
	EBC+	*M. morganii* (0.63)	*M. morganii* (0.89)	*M. morganii* (0.99)	*M. morganii* (0.99)
Providencia rettgeri	EBC	*P. rettgeri* (0.99)	*P. rettgeri* (0.99)	*P. rettgeri* (0.99)	*P. rettgeri* (0.99)
	EBC+	*Providencia stuartii*, urea positive (0.91)	*P. stuartii*, urea positive (0.91)	*P. stuartii* (0.99)	*P. stuartii*, urea positive (0.91)
Y. enterocolitica	EBC	*Y. enterocolitica* (0.94)	*Y. enterocolitica* (0.80)	*Y. enterocolitica* (0.55)	*Shigella* sp. (0.95)
	EBC+	*Shigella* sp. (0.74)	*Y. enterocolitica* (0.55)	*Shigella* sp. (0.74)	*Shigella* sp. (0.74)
Acinetobacter calcoaceticus	EBC	*P. stuartii* (0.76)	*P. stuartii* (0.76)	Glucose negative	Glucose negative
	EBC+	*A. calcoaceticus* (0.99)	*A. calcoaceticus* (0.99)	*A. calcoaceticus* (0.99)	*A. calcoaceticus* (0.99)
Citrobacter freundii	EBC	*E. coli* (0.82)	*C. freundii* (0.88)	*E. coli* (0.89)	*E. coli* (0.89)
	EBC+	*E. coli* (0.83)	*E. coli* (0.82)	*E. coli* (0.82)	*E. coli* (0.82)
C. freundii	EBC	*E. agglomerans* (0.94)	*C. freundii* (0.77)	*C. freundii* (0.77)	*E. agglomerans* (0.89)
	EBC+	*E. agglomerans* (0.99)	*E. coli* (0.40)	*C. freundii* (0.46)	*E. agglomerans* (0.88)
C. freundii	EBC	*E. agglomerans* (0.98)	*E. agglomerans* (0.99)	*E. agglomerans* (0.99)	*E. agglomerans* (0.99)
	EBC+	*E. agglomerans* (0.78)	Unidentified organism	*C. freundii* (0.80)	*C. freundii* (0.80)

[a] Probability that the first-choice identification is accurate. Tests were considered equivocal if $P < 0.80$.

latter species as *Enterobacter cloacae*, as might be expected. The other discrepancies were not as easily explained, since they occurred sporadically, with strains belonging to a variety of species.

Reproducibility studies were performed with 45 selected isolates, including 39 strains with discrepancies between the EBC and EBC+ identifications and 6 strains with which the two AMS cards agreed, but both disagreed with the reference identification. These 45 isolates were repeatedly tested in both AMS cards on 3 separate days to determine whether the discrepancies between the EBC and EBC+ identifications were repeatable and to establish reproducibility of the two systems. The relative precision of the two systems was expressed as reproducibility indexes, which permits comparison of the two systems before and after exclusion of equivocal responses (Table 4). Since each isolate was tested on 3 separate days, three pairs of data were generated (first and second, first and third, and second and third trials). The 45 strains thus generated 135 pairs of data that could agree or disagree, if all first-choice identifications were compared. After excluding equivocal responses, there were 102 pairs of EBC identifications and 106 pairs of EBC+ identifications that could be compared. Each reproducibility index was calculated by dividing the number of pairs in agreement by the total number of pairs available for comparison; a ratio of 1.0 indicates absolute reproducibility. The EBC+ system appeared to be more reproducible than the EBC system ($P < 0.02$), and both were slightly more reproducible ($P < 0.01$) after equivocal responses were excluded (Table 4).

Repeatability of discrepancies between the EBC and EBC+ responses was investigated by examining the reproducibility data described above; these data included 39 strains which initially gave discrepancies between EBC and EBC+ responses. Upon retesting on 3 separate days, consistent results were obtained with 28 of 45 strains; 21 now showed agreement between EBC and EBC+ responses. Table 5 shows the results of repeated tests with the 17 strains that produced different first-choice identifications on 3 separate days. Six strains initially produced the same identification with the EBC and EBC+ systems, but three of these six strains became discrepant when retested on 3 separate days. The remaining 39 strains initially demonstrated discrepancies between EBC and EBC+ responses; only 12 were truly discrepant, since 27 of these 39 strains initially would have been considered equivocal in one or both systems. When retested in triplicate, 28 of the 39 initially discrepant strains demonstrated at least one agreement between EBC and EBC+ responses. Of the 11 strains which consistently showed discrepancies between EBC and EBC+ responses, 4 represented *E. gergoviae* and *E. sakazakii* isolates that were identified as *E. cloacae* by the EBC program, an expected result easily explained by limitations included in the EBC computer program.

Relative accuracy of the two systems with the 45 strains used for reproducibility studies was evaluated by calculating the number of agreements with the reference identification. Four discrepancies involved *E. gergoviae* or *E. sakazakii* misidentified as *E. cloacae* by the EBC system, and one other discrepancy involved *Salmonella typhi*, which was consistently reported to be a *Salmonella* species (other than *Salmonella typhi*) by both systems. The 40 remaining strains initially yielded 21 discrepancies between the EBC system and reference tests, but 11 of these discrepancies involved equivocal EBC responses. Discrepancies between the EBC+ system and reference tests occurred with 29 strains, 11 after exclusion of equivocal responses. When the AMS tests were repeated on 3 separate days, only 10 of the 40 strains consistently gave discrepancies between the EBC system and reference tests, compared with 21 strains in the initial trials. With the EBC+ system, 29 strains were initially discrepant, but only 13 consistently disagreed with the reference tests.

DISCUSSION

Since the EBC and EBC+ systems contain the same test reagents for identification of the *Enterobacteriaceae*, one would expect the two cards to give identical results. However, we observed a few unexpected discrepancies between the two systems; some are due to minor differences in the computer programs for interpretation of EBC and EBC+ results. In our initial tests with 650 *Enterobacteriaceae*, 6% of the strains were discrepant, but when equivocal responses were excluded, the discrepancies were reduced to 3%.

Reproducibility studies demonstrated that most of the discrepancies were not repeatable. For 17 of 45 selected strains, the first-choice identification changed when the tests were repeated on 3 separate days. With repeated testing, one or more reactions might vary from day to day for technical reasons. Occasionally, a variable reaction might represent a key test which could shift the computer's interpretation from one species to another, closely related species. However, when this occurs, the patterns of reactions are often atypical enough to produce low P values, suggesting that confirmatory tests are needed. For the same reason, parallel tests performed in two essentially identi-

cal cards (EBC and EBC+) occasionally gave discrepant interpretations which were resolved by repeated testing. Overall, the reproducibility of both systems was excellent, but the EBC+ program appeared to be somewhat more reproducible than the older EBC system (Table 4). Although variability in tests with one or both cards occasionally produced discrepant interpretations, we concluded that the two cards produced essentially identical results when challenged with *Enterobacteriaceae*. The few species that are not included in the EBC computer program (*Citrobacter amalonaticus*, *E. gergoviae*, and *E. sakazakii*) represent obvious exceptions to this generalization.

The accuracy of the AMS was expressed as the overall agreement with the standard reference system. In a larger, separate study (Barry et al., submitted for publication), we demonstrated that the standard reference system used in this study was approximately 96% accurate. Accuracy of AMS responses was expressed as percent agreement with the reference identifications. The EBC system was found to be 94% accurate, and the EBC+ system was 93% accurate, but when identifications with probabilities of <0.80 were excluded, the two systems were approximately 96 to 97% accurate (comparable to the reference method). When the AMS tests were repeated three times, most strains that were initially discrepant gave at least one response that agreed with the reference tests. With the few tests which were consistently discrepant, it is entirely possible that some of the reference identifications were actually erroneous. Most discrepancies involved identification of closely related species that are difficult to separate with a high degree of confidence. In summary, we concluded that both the EBC and EBC+ systems are comparable and both are perfectly acceptable for identification of the *Enterobacteriaceae*, providing that strains with equivocal responses ($P < 0.80$) are subjected to additional tests before a final report is issued. The accuracy of EBC+ tests with nonenteric gram-negative bacilli remains to be documented.

LITERATURE CITED

1. **Brenner, D. J., J. J. Farmer, F. W. Hickman, M. A. Asbury, and A. G. Steigerwalt.** 1977. Taxonomic and nomenclatural changes in *Enterobacteriaceae*. Department of Health, Education and Welfare Publication (CDC) 78-8356, Centers for Disease Control, Atlanta, Ga.
2. **Burdash, N. M., G. Teti, M. E. West, E. R. Bannister, and J. P. Manos.** 1981. Evaluation of an automated, computerized system (AutoMicrobic system) for *Enterobacteriaceae* identification. J. Clin. Microbiol. **13**:331–334.
3. **Ewing, W. H., and B. R. Davis.** 1975. Media and tests for differentiation of *Enterobacteriaceae*. Department of Health, Education and Welfare, National Communicable Diseases Center, Atlanta, Ga.
4. **Freeman, J. W., R. W. Rowland, S. B. Overman, and N. L. Goodman.** 1981. Laboratory evaluation of the AutoMicrobic system for identification of *Enterobacteriaceae*. J. Clin. Microbiol. **13**:895–898.
5. **Hasyn, J. J., K. R. Cundy, C. C. Dietz, and W. Wong.** 1981. Clinical laboratory evaluation of the AutoMicrobic system for rapid identification of *Enterobacteriaceae*. J. Clin. Microbiol. **13**:491–497.
6. **Isenberg, H. D., T. L. Gavan, P. B. Smith, A. Sonnenwirth, W. Taylor, W. J. Martin, D. Rhoden, and A. Balows.** 1980. Collaborative investigation of the AutoMicrobic system *Enterobacteriaceae* biochemical card. J. Clin. Microbiol. **11**:694–702.
7. **Kelly, M. T., and J. M. Latimer.** 1980. Comparison of the AutoMicrobic system with API, Enterotube, Micro-ID, Micro-Media Systems, and conventional methods for identification of *Enterobacteriaceae*. J. Clin. Microbiol. **12**:659–662.