Evaluation of the Enteric Analyzer for Identification of *Enterobacteriaceae*

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The reliability of the Enteric Analyzer for identification of *Enterobacteriaceae* was evaluated using biochemical results previously obtained for 291 organisms with the conventional, R/B, and Minitek systems. The instrument correctly identified 77.3% of the organisms using conventional system results, 74.2% using R/B results, and 60.5% using Minitek results. The low rate of identification with the conventional system occurs primarily because the instrument is not programmed to consider delayed biochemical reactions. The arbitrary use of 90% and 99% probabilities for test reactions also contributes to a lower percentage of identification. The Enteric Analyzer does not replace the judgment of experienced personnel in the identification of atypical bacteria, but it may prove helpful in speeding up final computer identification of typical microorganisms.

Mathematical analysis of data from biochemical reactions is employed by some manufacturers of micromethods for the rapid identification of *Enterobacteriaceae*. The API (Analytab Products Inc.) Profile Register for computer use was recently evaluated by Robertson and MacLowry (11). The Enteric Analyzer, an instrument programmed with Edwards and Ewing percentages (3), was loaned to us by the manufacturer (Diagnostic Research Inc.) for this study. The accuracy of the Enteric Analyzer was evaluated using the results of biochemical reactions in the conventional and two rapid identification systems, R/B and Minitek.

MATERIALS AND METHODS

Biochemical reactions. Biochemical test results for 289 of the 294 isolates (23 species) previously obtained with 14 common tests in the conventional, R/B, and Minitek systems (12) were used in this study. In our original work there was an overall agreement in biochemical tests of 92.6 and 93.1% and correct bacterial identification of 88.4 and 83.6% for the R/B and Minitek systems, respectively (12). To evaluate the Enteric Analyzer extra tests were performed if needed, as required by the manufacturer. Three cultures of *Klebsiella pneumoniae* and two of *Citrobacter diversus* used in a previous report (12) were nonviable and could not be subcultured as needed for additional tests. Two additional isolates of *Citrobacter freundii* were included for a total of 291 isolates. *Shigella sonnei*, which is listed separately from *Shigella* sp. on the indicator panel, was considered correct if both organisms were indicated. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, which appear on the indicator panel, were not tested.

For comparison of the usefulness of the Enteric Analyzer in the three methods, the experimental plan was to perform each test according to its own procedure to impose a uniform intermethod comparison. The inoculated media in the R/B and Minitek systems were read after 18 to 24 h of incubation, whereas conventional tests were read daily for up to 4 days of incubation at 35 to 37 C (3).

Enteric Analyzer. The Enteric Analyzer was designed to be used with the modified R/B system. However, the manufacturer states that it can be used with any other rapid or conventional system that utilizes the same set of specific programmed biochemical reactions.

The Enteric Analyzer comprises an array of input data switches, a read-only memory, and an indicator panel. The memory contains 2,128 possible combinations of biochemical reactions that lead to the identification of the 28 microorganisms listed on the indicator panel. Beside the name of each organism is a small light. Three-position input data switches (positive, negative, and neutral) are used to indicate the results of 14 biochemical reactions in the following sequence: phenylalanine, hydrogen sulfide, gas (from glucose), lysine, lactose, indole, ornithine, motility, citrate, rhamnose, deoxyribonuclease, raffinose, sorbitol, and arabinose. As each biochemical reaction is recorded, the lights on the indicator panel go out for those organisms that have been eliminated, until only one light remains. If, after the first 14 reactions are recorded, more than one light remains, five other parameters may be entered: malonate, urea, inositol, adonitol, and esculin. The additional biochemical reaction(s) that is required to complete the identification may be determined by turning one or more of these five switches and observing the lights on the panel.

The Enteric Analyzer memory has been programmed according to the percentage values de-

rived by Edwards and Ewing from data published by the Center for Disease Control (1-10; these references are given in the manufacturer's pamphlet). The memory is divided into two parts, or pages, for selection of a percentage value of 90 or 99%. The 90% page includes only those organisms which, for any given biochemical reaction (positive or negative), have a $\geq 10\%$ chance of being the unknown, whereas the 99% page includes all those with even a 1% chance. Use of the 90% page limits the number of possibilities; use of the 99% page maximizes them. Thus, the 90% page facilitates the identification of a typical organism and the 99% page, an atypical one.

Criteria. The test results obtained from each of the three systems, the conventional, R/B, and Minitek (12), were used to evaluate the Enteric Analyzer. For some isolates one or more additional tests, as indicated by the instrument, were performed. The following criteria were used to interpret the instrument response.

(i) After the initial 14 reactions were entered with the 90% page, if a single indicator light remained on to identify an organism, the response was recorded, and the 99% page was not used.

(ii) If more than one organism was indicated at this point, additional test results were entered as needed for identification. If a single organism was then shown, the response was recorded.

(iii) If more than one light remained on after all results were entered, the organism was not considered identified. For the purpose of this study, the instrument was switched to the 99% page at this point, and if the organism was among those identified on the panel it was recorded as "included."

(iv) If all organisms were eliminated from the panel (all lights went out) at the 90% page after any number of tests results were entered, the organism was recorded as unidentified at the 90% page, and the instrument was switched to the 99% page. If no lights went on, the organism was recorded as unidentified also at the 99% page. If one light went on, this identification was recorded. If more than one light went on, more test results were entered, leading either to identification (one light on) or no identification (more than one light remaining after all test results were entered).

RESULTS

In this study one person entered the biochemical test results for all 291 known organisms with the three systems and recorded the instrument responses. Then the biochemical reactions of 100 isolates in each system, distributed evenly among the 23 species but randomly selected within each species, were given to another person as unknowns to identify. The responses obtained for the unknowns were in 100% agreement with those for the knowns.

Using the biochemical test reactions obtained with the conventional system, the Enteric Analyzer identified 77.3% of the isolates (Table 1). Since the instrument is programmed

for use with 18- to 24-h test results, delayed positive reactions (up to 4 days in the conventional system) prevented identification of some isolates.

When the test reactions obtained with the R/B system were used (Table 2), 74.2% of the isolates were identified correctly; but about 40% of these (29.2% of the total isolates) required one or more of the additional tests on the panel, which were not included in the R/B system. False lactose-negative results in the R/B system (12) contributed to misidentification of 8 of 15 *K. pneumoniae*.

Significantly fewer isolates (60.5%) were correctly identified from the test reactions obtained with the Minitek system (Table 3). Only 3.8% of the total isolates required one or more of the additional tests not included in the Minitek system. False-positive or -negative hydrogen sulfide test results were a major factor in reducing the percentage of correctly identified organisms.

DISCUSSION

Manual identification (use of charts and judgment of experienced personnel) of the isolates using the same test reactions obtained with the three systems gave consistently higher percentages of identification: 100% for conventional, 88.4% for the R/B, and 83.6% for the Minitek system (12).

The main reasons for the lower rates of identification of the isolates by Enteric Analyzer are:

(i) Delayed test reactions, which are not programmed in the Enteric Analyzer, were responsible for the misidentification of isolates with the conventional method results. This method requires a longer incubation than do the R/B and Minitek systems for most tests. For example, all 17 isolates of *S. sonnei* with delayed positive lactose fermentation and 7 of 15 *Enterobacter hafniae* with delayed positive citrate utilization were misidentified using the conventional test results. The hydrogen sulfide reaction, which presented a problem with manual identification in the Minitek system (12), had a more diverse effect on the identification of the isolates by the Enteric Analyzer. With the R/B system false-negative lactose results were the major problem (see Results).

(ii) The Enteric Analyzer's inability to make a decision outside of its programmed limit caused misidentification of some bacteria using results from all three systems. Table 4 is an example of a misidentified atypical isolate of *E. hafniae*. This particular isolate does not produce gas from glucose and is a late lactose fermenter. The gas-negative reaction alone

TABLE 1. Identification of Enterobacteriaceae by the Enteric Analyzer using the biochemical test reactions obtained with the conventional system^a

Organism	Total no.	90% Page				99% Page ^b			
		C ^c	I ^d	Un ^e	Mis ^f	C ^c	I ^d	Un ^e	Mis ^f
<i>Arizona hinshawii</i>	12	12							
<i>Citrobacter diversus</i>	9	9							
<i>Citrobacter freundii</i>	13	10	1	1	1		2		1
<i>Edwardsiella tarda</i>	4	4							
<i>Enterobacter aerogenes</i>	12	9		3		2	1		
<i>Enterobacter agglomerans</i>	19	17		2		1			1
<i>Enterobacter cloacae</i>	12	8		4		2	2		
<i>Enterobacter hafniae</i>	15	4		11			9		2
<i>Escherichia coli</i>	15	10		5		5			
<i>Klebsiella ozaenae</i>	4	2	1	1			2		
<i>Klebsiella pneumoniae</i>	15	13		2		1	1		
<i>Klebsiella rhinoscleromatis</i>	1	1							
<i>Proteus mirabilis</i>	14	11		3		3			
<i>Proteus morganii</i>	10	10							
<i>Proteus rettgeri</i>	10	4		6		2	4		
<i>Proteus vulgaris</i>	10	8		2		1		1	
<i>Providencia alcalifaciens</i>	7	6		1			1		
<i>Providencia stuartii</i>	12	12							
<i>Salmonella typhi</i>	9	9							
<i>Salmonella</i> sp. (groups B, C, E-I)	21	20		1		1			
<i>Serratia liquefaciens</i>	9	2		6	1	6	1		
<i>Serratia marcescens</i>	13	11		2		1			1
<i>Shigella</i> sp. (groups A, B, C, D)	45	8	21	15	1		37		
Percent grand total		68.8	7.9	22.3	1.0	8.6	20.6	0.3	1.7

^a Total correct for both pages, 225 (77.3%).^b Results in 99% phage are the organisms not correctly identified as a single organism in 90% page.^c C, Correct identification as a single organism.^d I, Correct organism included among other organisms (not considered as identified).^e Un, Unidentified (all organisms eliminated).^f Mis, Misidentified.

TABLE 2. Identification of Enterobacteriaceae by the Enteric Analyzer using the biochemical test reactions obtained with the R/B system^a

Organism	Total no.	90% page					99% page ^b				
		C ^c	Cx ^d	I ^e	Un ^f	Mis ^g	C ^c	Cx ^d	I ^e	Un ^f	Mis ^g
<i>Arizona hinshawii</i>	12		11		1			1			
<i>Citrobacter diversus</i>	9		8		1			1			
<i>Citrobacter freundii</i>	13	8	1	1	2	1		1	2		1
<i>Edwardsiella tarda</i>	4	3			1						1
<i>Enterobacter aerogenes</i>	12		3		2	7		7	2		
<i>Enterobacter agglomerans</i>	19	8	4		6	1	1	2			4
<i>Enterobacter cloacae</i>	12	1	7		3	1			2		2
<i>Enterobacter hafniae</i>	15	8			6	1			5		2
<i>Escherichia coli</i>	15	8			5	2	3		3		1
<i>Klebsiella ozaenae</i>	4				3	1			3		1
<i>Klebsiella pneumoniae</i>	15		5		10					1	9
<i>Klebsiella rhinoscleromatis</i>	1		1								
<i>Proteus mirabilis</i>	14	7			7		2			5	
<i>Proteus morgani</i>	10	10									
<i>Proteus rettgeri</i>	10		2		8			2	4	1	1
<i>Proteus vulgaris</i>	10	7			3		2			1	
<i>Providencia alcalifaciens</i>	7		3		4				2		2
<i>Providencia stuartii</i>	12	4	3		5						5
<i>Salmonella typhi</i>	9	3			6						6
<i>Salmonella</i> sp. (groups B, C, E-I)	21		20		1			1			
<i>Serratia liquefaciens</i>	9	2			5	2	2	2	2		1
<i>Serratia marcescens</i>	13	7			6				1		5
<i>Shigella</i> sp. (groups A, B, C, D)	45	30		13	1	1	15				
Percent grand total		36.5	23.4	4.8	29.5	5.8	8.6	5.8	8.9	2.7	14.1

^a Total correct for both pages, 216 (74.2%).^b Results in 99% page are the organisms not correctly identified as a single organism in 90% page.^c C, Correct identification as a single organism.^d Cx, Correct identification as a single organism by using one or more extra tests.^e I, Correct organism included among other organisms (not considered correctly identified).^f Un, Unidentified (all organisms eliminated).^g Mis, Misidentified.

TABLE 3. Identification of Enterobacteriaceae by the Enteric Analyzer using the biochemical test reactions obtained with the Minitek system^a

Organism	Total no.	90% page					99% page ^b				
		C ^c	Cx ^d	I ^e	Un ^f	Mis ^g	C ^c	Cx ^d	I ^e	Un ^f	Mis ^g
<i>Arizona hinshawii</i>	12	3			5	4					9
<i>Citrobacter diversus</i>	9	6			1	2					3
<i>Citrobacter freundii</i>	13	9			3	1			4		
<i>Edwardsiella tarda</i>	4	2			1	1					2
<i>Enterobacter aerogenes</i>	12	11			1						1
<i>Enterobacter agglomerans</i>	19	11	6			2					2
<i>Enterobacter cloacae</i>	12	4		1	3	4		1	2		5
<i>Enterobacter hafniae</i>	15	2			12	1		1	7		5
<i>Escherichia coli</i>	15	8			5	2	4		2		1
<i>Klebsiella ozaenae</i>	4			2		2			4		
<i>Klebsiella pneumoniae</i>	15	8	1		5	1			1	4	1
<i>Klebsiella rhinoscleromatis</i>	1	1									
<i>Proteus mirabilis</i>	14	12			2		2				
<i>Proteus morgani</i>	10	5			5				5		
<i>Proteus rettgeri</i>	10	5			5				3		2
<i>Proteus vulgaris</i>	10	7			3		2	1			
<i>Providencia alcalifaciens</i>	7	5			2						2
<i>Providencia stuartii</i>	12	12									
<i>Salmonella typhi</i>	9				9				7		2
<i>Salmonella</i> sp. (groups B, C, E-I)	21	8			9	4			12	1	
<i>Serratia liquefaciens</i>	9	1			8		5	1			2
<i>Serratia marcescens</i>	13	11			2						2
<i>Shigella</i> sp. (groups A, B, C, D)	45	21		24					24		
Percent grand total		52.3	2.4	9.3	27.8	8.2	4.5	1.3	24.4	1.7	13.4

^a Total correct for both pages, 176 (60.5%).^b Results in 99% page are the organisms not correctly identified as a single organism in 90% page.^c C, Correct identification as a single organism.^d Cx, Correct identification as a single organism by using one or more extra tests.^e I, Correct organism included among other organisms (not considered correctly identified).^f Un, Unidentified (all organisms eliminated).^g Mis, Misidentified.

TABLE 4. Data for misidentification by Enteric Analyzer of an atypical isolate of *E. hafniae* as *E. aerogenes*

Biochemical parameter ^a	<i>E. hafniae</i> ^b		<i>E. aerogenes</i> ^b		Isolate (E863)
	Sign	% +	Sign	% +	
Primary					
Phenylalaninedeaminase	—	0	—	0	—
Hydrogen sulfide	—	0	—	0	—
Gas (from glucose)	+	98.9	+	95.9	—
Lysine	+	99.6	+	97.5	+
Lactose	—	2.8	+	92.5	+
		(11.9) ^c			(2 days)
Indole	—	0	—	0.8	—
Ornithine	+	98.6	+	95.9	+
Motility	+	94.1	+	91.7	+
Citrate	—	5.6	+	92.6	—
Rhamnose	+	95.4	+	99.2	+
DNase	—	0	—	0	—
Raffinose	—	3.8	+	96.7	—
Sorbitol	—	0	+	98.3	—
Arabinose	+	99.3	+	100	+
Additional					
Malonate	+	67.2	+	74.7	+
Urea	—	6.6	—	5	—
Inositol	—	0	+	96.7	—
Adonitol	—	0	+	97.5	—
Esculin	—	6	+	98	—

^a In the sequence in which they appear on Enteric Analyzer panel. DNase, Deoxyribonuclease.

^b Reactions and percentages used in programming the Enteric Analyzer, taken from Center for Disease Control publications (6, 7).

^c Delayed reactions are not programmed in the Enteric Analyzer.

caused the Enteric Analyzer to eliminate *E. hafniae* from both the 90 and 99% pages. This isolate was identified as *E. aerogenes* at the 99% page, even though the isolate does not utilize citrate and does not ferment raffinose, sorbitol, inositol, adonitol, and esculin. In manual identification with the use of charts, no competent technician would make such an error.

(iii) In many instances the Enteric Analyzer could narrow the identification of an isolate down to a few organisms, including the correct one, but not far enough to identify it (Table 5). When such instrument responses are included, the percentage of identification comes very close to that achieved manually (12). The instrument thus narrows down the possibilities effectively, but its identifications are frequently incomplete. It performs best with results obtained with the conventional method and with the R/B system, for which it was originally designed. However, it lags approximately 23% behind manual interpretation of either conventional method or Minitek results and only 14% behind manual interpretation of R/B results (Table 5).

It appears that personal judgment in interpreting the identification charts is necessary to

TABLE 5. Comparison between identification of Enterobacteriaceae by the Enteric Analyzer and manually

System	Enteric Analyzer		Manual, using charts (%) ^c
	Correctly identified (%) ^a	Correctly identified and included among those remaining (%) ^b	
Conventional	77.3	97.9	100
R/B	74.2	83.1	88.4
Minitek	60.5	84.9	83.6

^a Correct identification to a single organism (C and Cx from Tables 1–3).

^b Correctly identified or among those organisms still indicated after all test results were entered (C, Cx, and I from Tables 1–3).

^c Reference 12.

identify the more difficult atypical isolates. Use of the Enteric Analyzer to identify *Enterobacteriaceae* would be helpful in laboratories with less experienced personnel, provided a reference laboratory is available for organisms unidentified by this method. Experienced personnel should

use the percentage charts specifically for reactions with low or variable percentages, indicating atypical bacteria.

The Enteric Analyzer might also be a useful training tool to demonstrate the value of each test in the differentiation of enteric bacteria.

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