

Correlation Studies of Entero-Set 20, API 20E, and Conventional Media Systems for *Enterobacteriaceae* Identification

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The Entero-Set kit (Fisher Diagnostics) is a 20-biochemical-test system used in the identification of members of the *Enterobacteriaceae*. This kit was compared with the API 20E (Analytab Products) and conventional media systems, using 505 (303 stock and 202 clinical) strains of *Enterobacteriaceae*. When the Entero-Set and API 20E results were compared with those of the conventional media system, the Entero-Set performed as well as the API 20E in overall identification. Comparison of common biochemical tests among the various systems showed that citrate, arabinose, adonitol, inositol, and malonate gave correlations below 90%. The majority of the discrepancies were found among stock cultures. In addition, most discrepancies occurred with species of *Enterobacter*, *Salmonella*, *Proteus*, *Klebsiella*, and *Serratia*. Reproducibility studies showed the Entero-Set system to perform with a high degree of accuracy and reproducibility.

The majority of the organisms identified in the clinical microbiology laboratory are members of the *Enterobacteriaceae*. Complete identification of these organisms is important in terms of clinical relevance, antibiotic resistance, and epidemiology. For many of the enteric bacteria complete identification entails a variety of biochemical reactions. With the use of conventional tube media, many types of media must be quality controlled and stored. For small-volume laboratories, this can be expensive and cumbersome. Over the past few years, several "kits" have been used in the clinical microbiology laboratory for identification of members of the *Enterobacteriaceae*. The Minitek (BBL Microbiology Systems), API 20E (Analytab Products), Enterotube (Roche Diagnostics), and r/b Enteric (Flow Laboratories, Inc.) systems are widely used kit systems which provide an 18- to 24-h identification of the enteric bacteria (4-6, 8, 10, 11). The Entero-Set 20 (Fisher Diagnostics) is a 20-test kit for overnight identification of members of the *Enterobacteriaceae*. The system is a combination and reformulation of the Entero-Set 1 and Entero-Set 2 (Inolex Corp.) kits, which are used for screening and identification to species, respectively, of enteric isolates (9, 12). This paper presents the results of a study comparing the accuracy and reproducibility of the Entero-Set 20 with those of conventional

tube media and another kit system, the API 20E.

MATERIALS AND METHODS

Organisms. A total of 505 strains of *Enterobacteriaceae* were tested by each of the three systems. Of the total, 202 were consecutive clinical isolates and 303 were stock cultures which had been maintained on nutrient agar slants at room temperature. Before testing, all stock cultures were passed in Trypticase soy broth (BBL Microbiology Systems) with 20% calf serum and plated to MacConkey agar to obtain isolated colonies. All organisms were given a code number, and the identity of the organisms was not known during testing.

Inoculum. All organisms tested, both clinical and stock strains, were taken from MacConkey agar plates incubated overnight at 35°C. To prepare the inoculum, Entero-Set 20 and API 20E recommend picking a single colony and placing it in 5 to 6 ml of saline. To be able to use the same homogeneous inoculum for both kits, we doubled the amount of saline (12 ml; pH 5.5) and to that transferred two to three colonies. This minor modification was felt to have no effect on the results since variation in inoculum density often occurs when the manufacturer's recommendations are used. This suspension was then used to inoculate the Entero-Set 20 and API 20E. All conventional media were inoculated with growth taken directly from the MacConkey agar plate.

Entero-Set 20 system. Each of the substrate-indicator combinations is contained within a capillary chamber on a plastic tray. The tests provided are: malonate utilization, glucose, phenylalanine deaminase, β -galactosidase (ONPG [*o*-nitrophenyl- β -D-galactopyranoside]), indole, H₂S, lysine and ornithine decarboxylases, urease, sucrose, arginine dihydrolase,

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citrate, salicin, adonitol, inositol, sorbitol, arabinose, maltose, trehalose and xylose. Each capillary tube in the Entero-Set 20 (Fig. 1) was filled by placing 3 to 5 drops of the inoculum suspension into the upper tube opening. The capillary tubes for indole, lysine and ornithine decarboxylases, and urease were filled minus 1 drop. To the lysine and ornithine decarboxylase and urease wells, 1 to 2 drops of sterile mineral oil were added. A cover was placed over the strip, which was then incubated for 20 to 24 h at 35°C. One drop each of ferric chloride and Kovac reagent was then added to the phenylalanine deaminase and indole wells, respectively. The reaction in each well was determined according to the manufacturer's recommendations and recorded on a work form. A seven-digit octal code number was derived from the reaction results, and the organism identity was determined from the code book. In a small percentage of the cases, the code numbers were not in the code book and were referred to the manufacturer's computer service for identification.

API 20E system. A detailed description of the procedure for inoculating and reading the API 20E has been published (1). Briefly, the inoculum prepared above was inoculated into all wells; the arginine dihydrolase, lysine and ornithine decarboxylase, and urease tests were overlaid with mineral oil, and the strip was incubated for 20 to 24 h. Reagents were added when necessary, the reactions were read according to the manufacturer's recommendations, and the organism identification was determined from the computer profile codon. In a small percentage of the cases, the codon numbers were not in the code book and were referred to the manufacturer's computer service for identification.

Conventional tube media. Each organism was set up on a battery of conventional media which included triple sugar iron agar, lysine iron agar, motility-indole-ornithine decarboxylase agar, phenylalanine-urea broth, β -galactosidase (ONPG), citrate agar, the Voges-Proskauer test, and deoxyribonuclease. All media were incubated at 35°C for 18 to 24 h. Reagents were added to the indole, phenylalanine deaminase, urease, and Voges-Proskauer tests, and the reactions were read. When necessary (indicated by "+" and "-" in Table 3), additional tube media tests were performed to give complete identification. These included adonitol, malonate, arabinose, inositol, sorbitol, xylose, rhamnose, and sucrose. Deoxyribonuclease agar (Difco Labora-

tories) containing toluidine blue was inoculated and read after 24 h of incubation for deoxyribonucleic acid hydrolysis by the appearance of a pink halo around the bacterial growth. This is a modification of the method of Lachica et al. (7). β -Galactoside disks (Difco Laboratories) were placed in 0.2 ml of organism suspension in saline and read after 4 or 24 h or both. The organisms were identified by examining the sets of biochemical reactions, using the charts of Edwards and Ewing (3).

Reproducibility study. To determine the reproducibility of each of the biochemical reactions and identifications, Entero-Set strips were set up on 7 consecutive days with the following organisms: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 13315), and *Salmonella typhimurium* (ATCC 14028). Each organism was passed daily on a sheep blood agar plate to ensure young colonies. The inoculum was prepared by transferring a single colony to 5 ml of sterile saline, pH 5.5. Each suspension was then inoculated to an Entero-Set 20 strip, incubated, and read according to the manufacturer's recommendations.

Identification manual. The identification manual that accompanies the Entero-Set strip contains a statistical analysis of the observed biochemical reactions which have been translated to a seven-digit octal codon. Each codon relates to an organism, its expected frequency, recommended confirmation reactions, and improbable test results observed. All reactions in the strip carry equal importance in the statistical analysis. Only organisms with an expected frequency of 1 in 5,000 or less are listed in the identification manual. Unlisted codons can be statistically analyzed up to a frequency of 1 in 1,000,000 by using the computer service offered by the manufacturer.

The data base for the Entero-Set strip is primarily that of Edwards and Ewing (3). Included with the identification manual is a biochemical reaction chart which reflects the above data base and can be used for identification in lieu of the octal codon. The nomenclature of the system reflects recent taxon changes to include *Citrobacter amalonaticus*, *Enterobacter gergoviae*, *Enterobacter sakazakii*, *Hafnia alvei*, *Klebsiella oxytoca*, *Morganella morganii*, *Providencia rettgeri*, and *Salmonella arizonae*-Arizona. However, the identification manual identified some isolates as

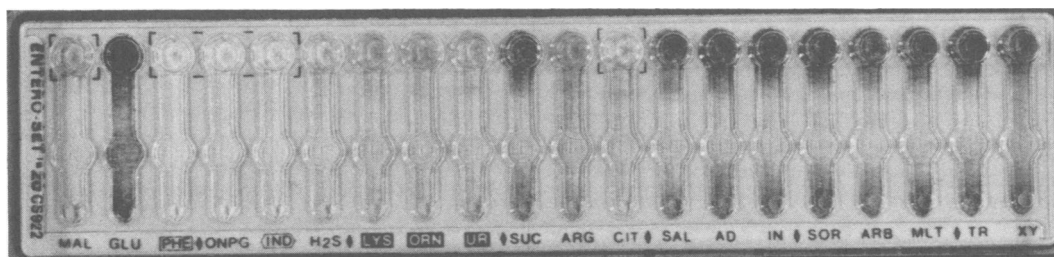


FIG. 1. Entero-Set 20 biochemical strip. Each test is inoculated through the upper opening in each capillary tube. The bottom small venting hole allows the inoculum to flow down the tube by capillary action. The upper portions of the bracketed tests are read for color change, whereas the middle portions of the remaining tests are read.

Erwinia species that we identified as *Enterobacter agglomerans*.

RESULTS

Overall identification correlation. Table 1 lists the name and number of each organism tested. As expected, the majority of the clinical isolates tested fell into the *E. coli-Proteus mirabilis-K. pneumoniae* group, which are by far the most commonly isolated members of the *Enterobacteriaceae*. However, the less commonly isolated strains of this family were well represented among the stock organisms. Table 2 shows the overall correlation percentages of

TABLE 1. *Organisms tested*

Organism	No. of clinical isolates	No. of stock cultures
<i>Escherichia coli</i>	113	30
<i>Klebsiella pneumoniae</i>	15	16
<i>K. oxytoca</i>	2	12
<i>K. ozaenae</i>	0	5
<i>Edwardsiella tarda</i>	0	8
<i>Arizona hinshawii</i>	0	9
<i>Citrobacter freundii</i>	3	8
<i>C. diversus</i>	1	10
<i>Shigella dysenteriae</i>	0	11
<i>S. flexneri</i>	0	7
<i>S. boydii</i>	0	13
<i>S. sonnei</i>	0	10
<i>Proteus mirabilis</i>	38	19
<i>P. vulgaris</i>	4	6
<i>Morganella morganii</i>	2	6
<i>Providencia stuartii</i>	7	22
<i>P. alcalifaciens</i>	0	8
<i>P. rettgeri</i>	1	4
<i>Enterobacter cloacae</i>	8	15
<i>E. aerogenes</i>	4	6
<i>E. agglomerans</i>	2	13
<i>Hafnia alvei</i>	0	12
<i>Serratia marcescens</i>	2	14
<i>S. liquefaciens</i>	0	6
<i>Yersinia enterocolitica</i>	0	8
<i>Salmonella typhi</i>	0	10
<i>S. cholerae-suis</i>	0	5
<i>S. enteritidis</i>	0	10

TABLE 2. *Correlation percentages of organism identification among the various systems*

Systems	% of isolates (no. correct/total) identified		
	Combined	Clinical	Stock
Entero-Set vs tube	97 (488/505)	98 (198/202)	96 (290/303)
API vs tube	95 (478/505)	97 (195/202)	93 (283/303)
Entero-Set vs API	92 (464/505)	95 (191/202)	90 (273/303)
Entero-Set vs API vs conventional	91 (461/505)	94 (190/202)	89 (271/303)

organism identification among the various systems. When the Entero-Set and API were compared with the conventional tube media, the Entero-Set and API performed equally well. When the Entero-Set and API were compared, they both gave the same identification 92% of the time. Overall, all three systems correlated on organism identification on only 461 of the 505 strains tested.

Clinical isolates correlated better among all systems than did the stock isolates. Since stock organisms become somewhat "metabolically inactive," these results are not surprising.

Common test correlation by system. Table 3 lists the tests in each system and also indicates which tests are common among the systems. Table 4 indicates the correlation percentage among the systems by test. For all three systems, the citrate test gave the lowest correlations, all below 90%. When API was compared with conventional media, inositol, adonitol, and malonate, in addition to citrate, had correlation

TABLE 3. *Biochemical tests in each identification system*

Test	Result with:		
	Entero-set 20	API 20E	Conventional tubes
D-Glucose	+	+	+
ONPG	+	+	+
Indole	+	+	+
H ₂ S	+	+	+
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	+
Urease	+	+	+
Citrate	+	+	+
Phenylalanine deaminase	+	+ ^a	+
Sucrose	+	+	+
Arabinose	+	+	+
Inositol	+	+	+
Sorbitol	+	+	+
Adonitol	+	-	+
Malonate	+	-	+
Voges-Praskauer	-	+	+
Xylose	+	-	+
Arginine dihydrolase	+	+	-
Rhamnose	-	+	+
Gelatin	-	+	-
Mannitol	-	+	-
Melibiose	-	+	-
Amygdalin	-	+	-
Motility	-	-	+
Deoxyribonuclease	-	-	+
Salicin	+	-	-
Maltose	+	-	-
Trehalose	+	-	-

^a In the API 20E tryptophane deaminase is detected instead of phenylalanine deaminase.

TABLE 4. Comparison of the three systems by common test

Test	% Correlation (no. correct/total) with:		
	Enteroto-Set vs API	Enteroto-Set vs tube	API vs tube
Glucose	93 (469/505)	93 (469/505)	99 (504/505)
Phenylalanine deaminase	98 (496/505)	99 (500/505)	99 (500/505)
ONPG	94 (475/505)	93 (468/505)	95 (480/505)
Indole	97 (492/505)	98 (493/505)	97 (490/505)
H ₂ S	98 (495/505)	98 (494/505)	97 (488/505)
Lysine decarboxylase	95 (479/505)	98 (498/505)	95 (481/505)
Ornithine decarboxylase	95 (479/505)	94 (473/505)	96 (487/505)
Urease	93 (471/505)	95 (479/505)	93 (469/505)
Sucrose	97 (490/505)	94 (257/273)	94 (257/273)
Citrate	85 (430/505)	86 (433/505)	83 (417/505)
Inositol	95 (477/505)	87 (13/15)	93 (14/15)
Sorbitol	96 (485/505)	100 (1/1)	100 (1/1)
Arabinose	93 (470/505)	100 (33/33)	82 (27/33)
Arginine dihydrolase	95 (478/505)	— ^a	—
Adonitol	—	86 (12/14)	—
Xylose	—	94 (15/16)	—
Voges-Praskauer	—	—	93 (469/505)
Malonate	—	89 (270/303)	—
Rhamnose	—	—	100 (4/4)

^a —, Not done.

percentages of <90%. Only citrate was below 90% correlation when the Enteroto-Set and API were compared.

Common test by organism. Further analysis of the common reactions showed that certain groups of organisms, both clinical and stock, were responsible for lowering the correlation of some of the common reactions. Organisms responsible for low correlations of lysine decarboxylase were *Salmonella* species, *K. pneumoniae*, and *E. agglomerans*; for ONPG, *E. agglomerans* and *H. alvei*; for urease, *Citrobacter* species, *Klebsiella* species, *Enterobacter cloacae*, and *Serratia marcescens*; for glucose, *Shigella* species and *Edwardsiella tarda*; for citrate, *Salmonella* species, *Proteus* species, *H. alvei*, *E. agglomerans*, *Citrobacter freundii*, and *E. tarda*; and for ornithine decarboxylase, *E. coli*. Even though the stock cultures were passed in serum-containing medium, they produced the majority of the discrepancies. However, certain fresh clinical isolates produced a significant number of aberrant reactions in certain tests. For example, *P. mirabilis* clinical isolates were responsible for 35 to 46 discrepancies involving the citrate reaction, *K. pneumoniae* isolates were responsible for 8 of 12 urease discrepancies, and *E. coli* isolates were responsible for 14 or 22 ornithine decarboxylase discrepancies.

Misidentifications. Table 5 shows the distribution and frequency of misidentifications by

each system. Since the conventional tube media were used as the standard, no misidentifications are listed for that system. Using the API system, we found that in nine cases *Shigella sonnei* was called *C. freundii*. However, in each case serology was suggested for confirmation and *Shigella* species was the second choice for identification; therefore, these cases are not included in the overall correlation. In seven cases, *Serratia liquefaciens* was called *S. marcescens* due to false-negative arabinose reactions. The majority of the misidentifications involved stock cultures, but a significant number of fresh clinical isolates were also misidentified. Misidentifications with the Enteroto-Set were widely distributed among the various species, with no clusters of misidentifications occurring. Again, stock cultures caused the majority of the misidentifications but fresh clinical strains were also involved.

The misidentifications can be divided into major and minor discrepancies. A major discrepancy is considered identifying an organism by the wrong genus. A minor discrepancy involves placing the organism in the correct genus but in the wrong species. Analysis of the present data indicates that the Enteroto-Set had 15 major and 3 minor discrepancies, whereas the API had 12 major and 13 minor discrepancies.

Reproducibility studies. Using American Type Culture Collection quality control strains, we examined the reproducibility of the Enteroto-Set to give the same set of identifications and biochemical reactions over a 7-day period. The results were identical each day for *S. typhimurium*, *E. coli*, and *K. pneumoniae*. The only discrepancies found were with *P. vulgaris*. On day 4 of the study, the glucose was negative and on day 6 the maltose was negative. However, these discrepancies did not interfere with the correct identification of the organism.

DISCUSSION

In recent years, several kit methods have been developed as substitutes for conventional tube media. The Enterotube, API 20E, and r/b systems have all been widely used in the identification of members of the *Enterobacteriaceae*. In the present study, the Enteroto-Set 20 system was examined for its efficiency in identification of members of the *Enterobacteriaceae*. The configuration of the Enteroto-Set test strip is similar to that of the API 20E in that there are 20 biochemical tests impregnated into a plastic strip. Inoculation of the strip was easy. Three to five drops of inoculum are added to the top of each tube, which is filled by capillary action due to a small "venting" hole at the opposite end of the tube. Inoculum preparation, normally one

TABLE 5. Misidentifications by each system

System	Organism	Was called:	No. of times		
			Stock	Clinical	
API	<i>Salmonella arizonae</i>	<i>Citrobacter freundii</i>	1		
	<i>Salmonella typhi</i>	<i>Shigella flexneri</i>	3		
	<i>Salmonella enteritidis</i>	<i>Citrobacter freundii</i>	1		
	<i>Salmonella cholerae-suis</i>	<i>Salmonella paratyphi A</i>	1		
	<i>Salmonella dysenteriae</i>	<i>Pseudomonas cepacia</i>	1		
	<i>Shigella sonnei</i>	<i>Citrobacter freundii</i>	9		
	<i>Shigella flexneri</i>	CDC V	1		
	<i>S. flexneri</i>	<i>Shigella boydii</i>	1		
	<i>Serratia marcescens</i>	<i>Serratia liquefaciens</i>	5	2	
	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	1	2	
	<i>Hafnia alvei</i>	<i>Klebsiella ozaenae</i>	1		
	<i>Enterobacter agglomerans</i>	<i>K. ozaenae</i>	2		
	<i>Providencia stuartii</i>	<i>Providencia alcalifaciens</i>	1		
	<i>Citrobacter diversus</i>	<i>Citrobacter freundii</i>	1		
	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>		2	
	<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae</i>		1	
	<i>Enterobacter cloacae</i>	<i>Enterobacter aerogenes</i>		1	
	<i>Proteus vulgaris</i>	<i>Providencia stuartii</i>		1	
	Entero-Set	<i>Salmonella typhi</i>	<i>Salmonella enteritidis</i>	1	
		<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae</i>	1	1
<i>Klebsiella ozaenae</i>		<i>Klebsiella species</i>	1		
<i>K. ozaenae</i>		<i>Citrobacter amalonaticus</i>	1		
<i>Enterobacter cloacae</i>		<i>Citrobacter diversus</i>	2		
<i>E. cloacae</i>		<i>Serratia liquefaciens</i>	1		
<i>Enterobacter agglomerans</i>		<i>Klebsiella pneumoniae</i>	1		
<i>E. agglomerans</i>		<i>Klebsiella ozaenae</i>	1	1	
<i>E. agglomerans</i>		<i>Shigella species</i>	1		
<i>Yersinia enterocolitica</i>		No listing	1		
<i>Citrobacter diversus</i>		<i>Enterobacter cloacae</i>	1	1	
<i>Citrobacter freundii</i>		<i>Salmonella arizonae</i>	1		
<i>Escherichia coli</i>		<i>Citrobacter diversus</i>		1	
<i>Enterobacter aerogenes</i>		<i>Klebsiella pneumoniae</i>		1	
<i>Morganella morganii</i>		<i>Proteus mirabilis</i>		1	

colony per 5 ml of saline, was best accomplished by picking isolated colonies with sterile applicator sticks instead of an inoculating loop. Mucoid colonies tend to give less homogeneous suspensions when the latter is used.

In overall identification, the Entero-Set 20 and API 20E systems performed equally well when each was compared with conventional tube media. When the two kit methods were compared, the correlation percentage proved to be the sum of the errors when each system was compared with the standard conventional method; this occurs when such comparisons are made (2). Such was not the case when common tests of the kits were compared. A comparison of the common reactions among the various systems showed that the citrate test was the least accurate of all of the tests performed. API performed better in the glucose and inositol tests, whereas the Entero-Set excelled in the arabinose test, when each was compared with

the conventional tube media. Analysis of the remaining common reactions showed no significant difference in the performance of the kit systems when compared with the conventional tube media.

The majority of the discrepancies seen in identification and common reactions involved stock strains which had been stored on agar slants. This is not surprising in view of the "inactive metabolic machinery" in organisms stored for any length of time. Passing the organisms in serum-containing media is generally accepted as a method to reactivate stock cultures. However, certain groups of organisms, clinical and stock, appear to be responsible for the majority of the biochemical discrepancies. *Salmonella*, *Enterobacter*, *Proteus*, *Klebsiella*, and *Serratia* species showed the greatest incidence of aberrant reactions. These differences may result from the sensitivity of the substrate/indicator ratio in that low levels of end products may not be

detected, resulting in false-negative reactions. Alternatively, nonspecific shifts of the indicator may result in false-positive reactions. The color reactions involved in each test were for the most part clear-cut in terms of positive or negative. Occasionally, a weak positive H₂S, ONPG, or lysine or ornithine decarboxylase test presented some problem and a certain amount of subjectivity had to be used. Reproducibility studies showed a high degree of reliability of the Entero-Set in identification and biochemical reactions.

In conclusion, the present study has shown that the Entero-Set performed with a high degree of accuracy and reproducibility when compared with conventional tube media. When compared with another widely used kit, the API 20E, the Entero-Set performed equally well and offered the advantages of being easier to inoculate and requiring less reagents.

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