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API System: a Multitube Micromethod for Identification of *Enterobacteriaceae*

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The API system for identification of *Enterobacteriaceae* was evaluated with 366 cultures. Overall accuracy of identification was 96.4%; of the 13 cultures misidentified, 7 were atypical strains.

One of the several devices available commercially for identification of *Enterobacteriaceae* is the API system, a plastic strip holding 20 miniaturized compartments, or cupules, each containing a dehydrated substrate for a different test. This device is based on work by Buissiere and Nardon (1) who established many of the physical and chemical requirements of such micromethods. The technique is basically a modification of one of the many "little tube" methods, as enumerated by Hartman (7). The biochemical tests used in the identification of enteric bacteria have long been a prime target of investigators interested in miniaturizing microbiological methods, for few other groups of procedures are so widely used or so well standardized. A variety of investigators, including Weaver et al. (11), have established that miniaturized techniques may be as sensitive as corresponding macrotechniques. However, many problems which are inconsequential in macrotechniques assume important roles in microtechniques. Some of these problems concern carry-over of substrate in the inoculum, age of inoculum, concentration of inoculum, and oxidation-reduction potentials. The simple proportions required of inoculum to substrate present new problems, as do requirements of the various systems for buffers, indicators, etc. Obviously, these and other factors which may influence results must be carefully considered. The API system employs a series of plastic cupules fixed to a plastic strip. More than one such method exists, and these methods may produce quite different results with the same bacterial strain (J. Buissiere, *personal communication*).

The API system has been used to identify bacteria in more than one taxonomic group. Paule (R. Paule, Ph.D. thesis, Univ. of Lyon, France, 1971) employed this device in an extensive study of the genus *Lactobacillus* by micromethods. Several investigators have applied it to identification of the *Enterobacteriaceae*. In France, Guillermet and Desbresles (6) reported that the "API *Enterobacteriaceae* system" was very useful in the study of 522 enteric and 79 *Moraxella* and *Pseudomonas* cultures. In Denmark, Nielsen (9) found that he could differentiate 78 *Salmonella* or *Arizona* cultures from 22 non-*Salmonella* cultures without difficulty with this system. Washington et al. (10), in the United States, tested the API system with 128 cultures of *Enterobacteriaceae* from clinical sources and obtained nearly 90% accuracy of identification on initial testing and 93% accuracy on repeat testing. They noted that about 3 min were required to prepare and inoculate the 20 cupules of the API system, and they considered this expenditure of time its greatest disadvantage. The present study of 366 cultures from different sources provides additional information on the clinical value of this system.

MATERIALS AND METHODS

The API strips used were supplied by Analytab Products, Inc., New York. The API system is comprised of the following tests: *o*-nitrophenyl-β-D-galactosidase (ONPG), arginine dihydrolase, lysine and ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, acetoin production, gelatinase, and fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, and arabinose. Each of these tests was duplicated by conventional methods with these exceptions: the ONPG and amygdaline fermentation tests were not used, and the phenylalanine deaminase was used instead of the tryptophan deaminase test. The conventional methods employed also included tests for acid production (methyl red test), growth in potassium cyanide broth, reaction on triple sugar iron agar, motility, and fermentation of raffinose. These conven-

tional tests were those recommended by Ewing (4) and Edwards and Ewing (3), but serology was not employed as an aid in identification or for confirmation of results.

Bacterial cultures used were documented strains of *Enterobacteriaceae* which had been submitted to the Center for Disease Control (CDC) for identification. They were supplied for this study as coded unknowns on plain agar slants. Each culture was streaked onto a MacConkey agar plate and then transferred into the various media required for identification by conventional means. Another technician then inoculated the API system, by using the following technique: a small loopful of culture from the agar slant was suspended in 4.5 ml of sterile, distilled water at *p*H 7.0 to a density of approximately 10^6 organisms per milliliter; McFarland barium sulfate standards were used for comparison. The cupules of the API system were then filled by using a Pasteur pipette. The strip containing the inoculated cupules was then incubated overnight at 37 C in the plastic container supplied by the manufacturer and with a small amount of water to maintain humidity. After 18 to 24 hr, results were recorded and identifications made. The API system was then discarded. Conventional tests were incubated for the recommended periods of time before results were recorded and identifications made. All results were submitted to a third party who compared them and indicated if any additional or repeat tests were necessary. The reference laboratory used, when required, was the CDC Enterobacteriology Unit.

RESULTS AND DISCUSSION

Three hundred and sixty-six cultures were tested by both the API and conventional systems. Table 1 shows the agreement of results of 18 of the tests in the API system and their corresponding conventional tests. The results of the 18 pairs of tests showed good agreement. The urea test employed in the API system is basically that of Ferguson and Hook (5), in which only buffered urea and an indicator are used, whereas our conventional urease test, which is somewhat more sensitive, is that of Christensen (2). The different mechanisms involved could account for the level of urease test agreement obtained. The API system lysine decarboxylase test is buffered to *p*H 6.2 initially with phenol red indicator and contains no glucose, whereas our conventional test is that of Moeller (8). The initial directions and descriptive literature of the manufacturer stated that the development of a red color indicated a positive lysine decarboxylase test. Subsequent literature and directions prepared by the manufacturer stated that a red or orange color was indicative of a positive test for lysine decarboxylase. By adhering to the latest directions supplied by the manufacturer, we obtained an agreement for lysine decarboxylase of 97.8%. This percentage includes those organisms that gave a strong (red) positive reaction and those that gave an orange or weakly positive reaction. Table 1 does not further delineate the strongly and weakly positive tests. Overall, 96.5% agreement of individual test results was obtained.

The major criterion for evaluating an identification procedure must be accuracy of identification, and these results are presented in Table 2. Results 100% correct were obtained with the API system for cultures of *Edwardsiella*, *Klebsiella*, *Providencia*, *Salmonella*, *Shigella*, three of the four species of *Proteus*, and two species of *Enterobacter*. One culture was missed of each of *Enterobacter aerogenes* and *Proteus rettgeri*, and two cultures were missed of each of *Arizona*, *Escherichia coli*, *Citrobacter*, and *Enterobacter liquefaciens*. Only the cultures of *Serratia* were identified with less than 90% accuracy, but even these identifications were 88.5% correct. Of the 366 cultures tested, 96.4% of them (all but 13) were identified correctly with the API system.

Errors in identification were caused both by aberrant reactions in the API system and by atypical strains. These errors are listed in Table 3, and atypical strains are denoted. Of the 13 errors, seven were primarily caused by atypical strains rather than by false positive or negative reactions in the API system. On the other hand, the API system relies upon ONPG

TABLE 1. *Comparative test results with the API and conventional systems*

Test	A/T[a]	Agreement (%)
Glucose	366/366	100%
Sorbitol	365/366	99.7%
Phenylalanine	365/366	99.7%
Sucrose	364/366	99.5%
Ornithine	363/366	99.2%
Mannitol	362/366	98.9%
Arginine	361/366	98.6%
Rhamnose	361/366	98.6%
Lysine	358/366	97.8%
Indole	357/366	97.5%
Arabinose	355/366	97.0%
H_2S	350/366	95.6%
Gelatin	345/366	94.3%
Inositol	344/366	93.8%
Melebiose	338/366	92.3%
Acetoin	338/366	92.3%
Citrate	337/366	91.2%
Urea	331/366	90.4%
		Avg: 96.5%

[a] A/T, No. of results in agreement per no. tested.

and gelatin tests to differentiate *Arizona* and *Salmonella*, with *Arizona* being positive in both and *Salmonella* negative. In our tests, none of the 29 *Arizona* strains tested were gelatin positive, so differentiation between these two genera was based solely on the ONPG reaction. Similarly, in the API system, *Arizona* and *Citrobacter* are differentiated on the basis of differences in decarboxylase reactions and on fermentation of amygdaline and sucrose by some strains of *Citrobacter*. Unfortunately, none of our *Arizona* or *Citrobacter* strains fermented amygdaline, and only two cultures of *Citrobacter* fermented sucrose. Thus, these two genera could be differentiated only on the basis of decarboxylase reactions in the API system. In actual use, however, only the failure of the gelatin test to perform properly was important; the sucrose and amygdaline reactions were not critical in the identification of unknown cultures (Table 3). As previously stated, serology was not employed in this study. If all identifications of *Salmonella*, *Shigella*, and *Arizona* had been confirmed serologically, some of the misidentifications probably would have been detected and corrected.

TABLE 2. *Agreement between API system and conventional identification*

Organism	C/T[a]	% Correct
Enterobacter cloacae	25/25	100
Enterobacter hafniae	19/19	100
Edwardsiella	18/18	100
Klebsiella	21/21	100
Proteus mirabilis	16/16	100
Proteus morganii	20/20	100
Proteus vulgaris	11/11	100
Providencia	28/28	100
Salmonella	28/28	100
Shigella	12/12	100
Enterobacter aerogenes	21/22	95.5
Proteus rettgeri	18/19	94.7
Arizona	27/29	93.1
Escherichia coli	26/28	92.9
Citrobacter	21/23	91.3
Enterobacter liquefaciens	19/21	90.5
Serratia	23/26	88.5
	Avg:	96.4

[a] C/T, No. correct per no. tested.

The 3 min (approximately) required to prepare and use the inoculum for the API system was not a disadvantage in this study because of the number of tests performed. Moderate drying out of some cupules during overnight incubation caused a slight problem, but this could be controlled by carefully adding exactly the proper amount of water to the plastic container in which the strips were incubated. This was, in fact, the most serious problem encountered, with the exception of the previously mentioned tests which did not perform as expected. The advantages of the API system, however, far outweigh these minor disadvantages. We found the API system to be easy to use, accompanied by complete instructions, accurate in the identification of unknown cultures, readily disposable after use, easily stored at either room or refrigerator temperatures, and most economical in terms of the information provided. Some of the tests used in the system might be replaced by tests more familiar to laboratorians in this country; i.e., the

TABLE 3. *API system identification errors*

Organism	API identification	Reasons
Proteus rettgeri	*Providencia*	Urea negative
Escherichia coli	*Shigella*[a]	Lysine, arginine, and melibiose negative
E. coli	*Shigella*[a]	Lysine, arginine, ornithine, and indole negative
Enterobacter aerogenes	*Enterobacter cloacae*[a]	Arginine and lysine negative
Enterobacter liquefaciens[b]	*E. aerogenes* (2)[a]	Rhamnose and sucrose positive; corn oil needed
Serratia	*E. liquefaciens* (3)	Arabinose positive
Citrobacter	*Salmonella*	Arginine and ornithine positive; ONPG negative
Citrobacter	*Arizona*	Arginine and ONPG positive
Arizona	*Salmonella* (2)[a]	ONPG and gelatin negative

[a] Misidentification due to strain characteristics.

[b] These two cultures were misidentified with the API and conventional systems. Only by observing their reactions in corn oil can they be identified as *E. liquefaciens*.

amygdaline test might be replaced by the dulcitol or malonate test. However, even in its present form, the API system appears to offer a reasonable alternative to conventional systems for identifying *Enterobacteriaceae*, provided one is willing to accept the indicated degree of error. Obviously, the user must adhere rigidly to the manufacturer's instructions, and he must use the system within its intended limits. Thus, the API system should be used only on suspected cultures of *Enterobacteriaceae*, and other information gained from observing colony morphology, growth on selective media, and results of serological and other tests must also be considered. Under these conditions, the user can expect a highly accurate identification of enteric bacteria with the API system.

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