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Evaluation of a Rapid Method to Exclude the Presence of Certain Enteric Pathogens in Stool Specimens

GIUSEPPE TETI,[1] NICHOLAS M. BURDASH,[2]* CESIRA ZAMBONI,[1] CONCETTA FAVA,[1] FRANCESCO TOMASELLO,[1] AND PASQUALE MASTROENI[1]

Institute of Microbiology, University of Messina Medical School, 98100 Messina, Italy,[1] and Department of Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina 29425[2]

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A new commercial method intended to exclude the presence of *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica* and to presumptively identify *Salmonella* isolates within 2 h after primary isolation from stool specimens was evaluated. This system is marketed in Europe as API Z and in the United States as Rapid SST. The strip consists of five pairs of cupules for the screening of five lactose-negative colonies. The first cupule of each pair detects the presence of five enzymatic activities, whereas the second serves to maintain the strain for additional testing if necessary. A total of 197 fresh isolates from stool specimens and 217 stock cultures of *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica* were tested, with the API 20E system as a reference method. In the stool specimens, 77.3% of the bacteria could be excluded from further workup for the presence of these organisms within 2 h. Over 97% of the stock strains and each of three fresh *Salmonella* isolates tested produced a reaction pattern corresponding to a correct presumptive identification. This reaction pattern was not produced by any isolate other than the *Salmonella* isolates. The API Z system can be used as a screen for the presence of *Salmonella* and *Shigella* spp. and can provide an accurate presumptive identification of *Salmonella* isolates within 2 h after primary isolation.

The detection of enteric pathogens is an important task and a major workload for clinical microbiology laboratories. Our knowledge of bacterial enteric pathogens is expanding to include an increasing number of organisms, such as *Campylobacter jejuni* (11), *Aeromonas hydrophila* (4), and *Yersinia enterocolitica* (2). However, classic pathogens such as *Salmonella* and *Shigella* spp. are still important and widespread agents of enteric infection. There is not only a need for rapid techniques for the identification of enteric pathogens, but a need for rapid techniques to exclude the presence of these pathogens, thereby providing valuable clinical information as well as decreasing laboratory workload.

A number of methods have been devised to rapidly screen for the presence of *Salmonella* and *Shigella* spp. Sanders and Okabe described a lactose-sucrose broth intended to initially eliminate the possible presence of these pathogens 2 h after primary isolation (9, 10). Coagglutination from selective broths (8) or primary plates (3) has been shown to reduce the time required for identification of *Salmonella* and *Shigella* spp. Coagglutination has also been used to detect *Salmonella typhi* D, Vi, and d antigens in the urine of patients affected by typhoid fever (6). Recently Stager et al. employed a combination of three conventional media that could be interpreted 4 to 6 h after inoculation (12). Isolates showing suspicious biochemical profiles were processed with an automated system (AutoMicrobic System, Vitek System, Inc., Hazelwood, Mo.) for identification and susceptibility testing. This approach allowed definitive results to be obtained within 12 to 14 h after primary isolation.

The present report describes the evaluation of a new commercial method intended to exclude the presence of *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica* and to presumptively identify *Salmonella* isolates within 2 h after primary isolation. The system is marketed under the name API Z (API Systems, S.A., Montelieu Vercieu, France) in Europe and under the name Rapid SST (DMS Laboratories, Inc., Flemington, N.Y.) in the United States. The system is not intended to be used as an identification system or to exclude the presence of other potential pathogens.

MATERIALS AND METHODS

Bacteria. A total of 197 isolates freshly isolated from stool specimens sent to the clinical laboratory were examined (see Table 1). A total of 217 lyophilized stock strains of *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica* were also tested (see Table 2). These stock strains had been isolated at our institution and in various centers of southern Italy during the last 5 years. They were all identified by the reference centers of the Istituto Superiore di Sanita, Rome, Italy, and the Centro per gli Enterobatteri dell'Italia Meridionale, Palermo, Italy. All stock strains were subcultured at least twice on sheep blood agar plates before testing.

API Z. The API Z system was used in this study. Although the API Z and Rapid SST systems are identical (G. V. Bartoni, API Systems, personal communication), the instructions contained in the package inserts are slightly different with regard to color changes. Briefly, the system consists of a strip with five pairs of cupules and allows five lactose-negative colonies to be screened within 2 h. The compounds in the first cupule (cupule A) of each pair detect five enzymatic activities. Phenylalanine deaminase, β-xylosidase, and lipase are not possessed by *Salmonella* spp., *Shigella* spp., or *Y. enterocolitica* and are used to exclude the presence of these organisms. β-Galactosidase excludes most *Salmonella* and *Shigella* species, but it is possessed by a significant percentage of *Salmonella arizonae*, *Shigella sonnei*, and *Y. enterocolitica* strains, so that a positive reaction indicates further testing is necessary. The fifth enzyme, an esterase which cleaves a naphthol derivative, is characteristic of *Salmonella* spp., according to the manufac-

* Corresponding author.

turer, and again indicates further testing. The second cupule (cupule B) of each pair is used to maintain the viability of the isolate in case further testing is needed.

A drop of sterile distilled water was dispensed into cupule A and cupule B of an API Z strip. One lactose-negative colony, as determined from the primary plate, was picked from MacConkey or Hecktoen plates with a wooden applicator stick. This was rubbed with a rotary motion on the fibrous supports in the base of each cupule. After inoculation, cupule A ranged from colorless to a pale beige. The strip was incubated at 35°C for 2 h, and any spontaneous change in color of cupule A (A_{1-} result) was noted. The development of a yellow, green, or brown-orange color was considered to indicate the presence of at least one of the three enzymes not possessed by *Salmonella* spp., *Shigella* spp., or *Y. enterocolitica* and thus excluded the presence of these microorganisms. A blue color, caused by the enzyme β-galactosidase, does not exclude the presence of *Shigella sonnei*, *Salmonella arizonae*, or *Y. enterocolitica* and indicates further testing is needed. In the package insert of API Z, but not of Rapid SST, the possibility of a spontaneous blue-green reaction in cupule A is also mentioned. This blue-green reaction should exclude the presence of enteric pathogens, according to the manufacturer. We found it difficult in preliminary tests to distinguish between blue and blue-green, even after the addition of a drop of 0.1 N HCl, as suggested in the API Z package instructions. Thus, for the purpose of this study any color change showing a blue component was considered blue, as per the instructions in the package insert of Rapid SST. If cupule A did not show any change in color (A_{1-} result), a drop of Fast Violet B (API Systems) was added to reveal the hydrolysis of a naphthol derivative. A pink-red color developing within 2 to 3 min corresponds to a positive reaction (A_{2-} result). In this case an oxidase test (API OX reagent) was performed on cupule B, after removing its contents. An A_{2-} result in the presence of a negative oxidase test was taken as a strong indication of *Salmonella* spp. Performing an oxidase test in the case of an A_{2-} result is necessary since some oxidase-positive nonfermentative bacilli may mimic the reaction of *Salmonella* spp. In the case of either a blue A_{1+} or an A_{1-} reaction, it is recommended that the contents of cupule B be used to perform additional tests, since the presence of the three aforementioned enteric pathogens cannot be excluded.

Reference identification. For the purpose of this study any isolate used to inoculate the API Z system was subcultured on a MacConkey plate with the contents of cupule B after the 2-h incubation period. Pure cultures were always obtained and used to inoculate the API 20E system for the identification of clinical strains. Identification of appropriate enteric pathogens was always confirmed by serology. Quality control for API Z included testing each lot with stock cultures of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella enteriditis*, *Shigella flexneri*, and *Y. enterocolitica*. Quality control for the API 20E system included testing each lot with stock cultures of *K. pneumoniae*, *Enterobacter cloacae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*.

RESULTS

Table 1 shows that the API Z reaction patterns produced by 197 non-lactose fermenters freshly isolated from stool specimens. Only three enteric pathogens (two from *Salmonella* group B and one from *Salmonella* group D) were isolated during the period. All three strains produced a reaction pattern (A_{1-}, A_{2-}, oxidase negative) indicating a strong suspicion of *Salmonella* spp. Overall, 150 of 194 (77.3%) clinically irrelevant organisms showed a yellow-brown or green A_{1+} reaction, indicating that the presence of *Salmonella* spp., *Shigella* spp., or *Y. enterocolitica* could be excluded. The majority of these isolates were represented by members of the tribe of *Proteeae* (Table 1). All *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia rettgeri* strains and 14 of 26 *Morganella morganii* strains produced a yellow-brown reaction, probably due to the presence of phenylalanine deaminase. The remaining 12 *M. morganii* strains gave a yellow A_{1+} reaction. Thus, all the members of the tribe of *Proteeae* gave reactions that allowed their elimination from further testing. A total of 13 of 30 *Serratia marcescens* and all 4 *Serratia liquefaciens* strains tested gave a green A_{1+} reaction. With the remaining 17 *Serratia marcescens* isolates, the need for further testing was indicated, since 9 gave an A_{1-}, A_{2-} reaction pattern and 8 gave a blue A_{1+} reaction. The need for further testing was indicated also for over half (11 of 20) of the *Enterobacter* strains. Seven of nine strains of *Pseudomonas aeruginosa* and the only strain of *Pseudomonas cepacia* tested gave an A_{1-}, A_{2+} combination, identical to that given by *Salmonella* isolates. A positive oxidase test, however, excluded the presence of *Salmonella* isolates in all these instances.

Table 2 shows the results for the 217 stock strains of enteric pathogens tested with API Z. All 174 *Salmonella* strains tested gave the expected reaction pattern (A_{1-}, A_{2+}), except for 5 which gave A_{1-}, A_{2-} reactions. These isolates were all group D *Salmonella* strains (three of *S. enteritidis* and two of *S. dublin*). The negativity of the A_2 reaction would not have caused any *Salmonella* strain to be missed since all isolates giving an A_{1-}, A_{2-} combination should be further tested, as recommended by the manufacturer.

All the *Shigella* and *Y. enterocolitica* strains gave A_{1-}, A_{2-} reactions, except for 8 of 19 *Shigella sonnei* strains. These eight strains gave a blue A_{1-} reaction, sometimes showing a green component. This fact should discourage considering a blue-green color change as incompatible with the presence of enteric pathogens.

DISCUSSION

Infectious enteritis is caused by a large number of microorganisms, including protozoa, bacteria, and viruses. The decision as to which bacterial pathogens to routinely search for in a given clinical microbiology laboratory depends upon many factors, including geographical location, patient population, and laboratory size (7). Screening for the presence of *Salmonella* and *Shigella* spp. is performed in virtually every laboratory concerned with the detection of enteric pathogens (7) and is generally a systematic and time-consuming operation involving various media and identification systems (5). The API Z system also screens for *Y. enterocolitica* in addition to *Salmonella* and *Shigella* spp., but it does not address other enteric pathogens or potential pathogens. Its value, therefore, would be most useful clinically in providing relevant information within 24 h regarding the absence of these specific organisms or, in the case of *Salmonella* spp., the probable presence. Depending on the systems used for identifying and reporting of these organisms in individual laboratories, the API Z system could save time and money.

The results presented in this study indicate that the API Z system can be useful for the screening of colonies suspected of being *Salmonella* or *Shigella* spp. Since only three potentially pathogenic strains were isolated during the study period, additional studies dealing with the isolation of enteric

TABLE 1. API Z reaction patterns with 197 fresh isolates from stool specimens

Organism	No. of strains	Reaction		Oxidase	Conclusions[b]
		A_1[a]	A_2		
Salmonella group B	2	−	+	−	Probable *Salmonella* spp.
Salmonella group D	1	−	+	−	Probable *Salmonella* spp.
Proteus mirabilis	60	BO			No S, S, or Y
Proteus vulgaris	18	BO			No S, S, or Y
Providencia rettgeri	7	BO			No S, S, or Y
Morganella morganii	14	BO			No S, S, or Y
	12	Y			No S, S, or Y
Serratia marcescens	13	G			No S, S, or Y
	9	−	−		Possible S, S, or Y
	8	B			Possible S, S, or Y
Serratia liquefaciens	4	G			No S, S, or Y
Enterobacter cloacae	6	G			No S, S, or Y
	2	B			Possible S, S, or Y
	2	−	−		Possible S, S, or Y
Enterobacter aerogenes	4	−	−		Possible S, S, or Y
	2	G			No S, S, or Y
Enterobacter agglomerans	3	B			Possible S, S, or Y
	1	Y			No S, S, or Y
Escherichia coli	5	−	−		Possible S, S, or Y
Citrobacter freundii	3	B			Possible S, S, or Y
	1	−	−		Possible S, S, or Y
Citrobacter diversus	2	B			Possible S, S, or Y
Klebsiella pneumoniae	2	G			No S, S, or Y
Klebsiella ozaenae	1	G			No S, S, or Y
Pseudomonas aeruginosa	7	−	+	+	No S, S, or Y
	2	Y			No S, S, or Y
Pseudomonas maltophilia	3	−	−		Possible S, S, or Y
Pseudomonas cepacia	1	−	+	+	No S, S, or Y
Acinetobacter calcoaceticus subsp. *anitratus*	2	−	−		Possible S, S, or Y

[a] BO, Brown-orange; G, green; B, blue; Y, yellow; −, no spontaneous color change.
[b] S, S, or Y, *Salmonella* spp., *Shigella* spp., or *Y. enterocolitica*.

pathogens are needed to more accurately assess the performance of this system in the clinical laboratory. However, all 174 stock *Salmonella* strains and all 40 stock *Shigella* strains tested produced reaction patterns that would have alerted the bacteriologist to the possible presence of enteric pathogens. Only three *Y. enterocolitica* strains were available for testing. All produced A_{1-}, A_{2-} reactions, indicating the necessity for further testing. Evaluation of the performance

TABLE 2. API Z reaction patterns with 217 stock strains of *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica*

Ogranism	No. of strains	Reaction		Oxidase	Conclusions
		A_1	A_2		
Salmonella group B	123	−	+	−	Probable *Salmonella* spp.
Salmonella group C_1	14	−	+	−	Probable *Salmonella* spp.
Salmonella group C_2	9	−	+	−	Probable *Salmonella* spp.
Salmonella group D	13	−	+	−	Probable *Salmonella* spp.
	5	−	−		Possible S, S, or Y[a]
Salmonella group E_1	4	−	+	−	Probable *Salmonella* spp.
Salmonella typhi	6	−	+	−	Probable *Salmonella* spp.
Shigella flexneri	21	−	−		Possible S, S, or Y
Shigella sonnei	11	−	−		Possible S, S, or Y
	8	B[b]			Possible S, S, or Y
Yersinia enterocolitica	3	−	−		Possible S, S, or Y

[a] S, S, or Y, *Salmonella* spp., *Shigella* spp., or *Y. enterocolitica*.
[b] B, Blue.

for the API Z system with this species, however, must await further investigation since only three isolates were tested.

Over 97% of the *Salmonella* strains tested produced a reaction pattern (A_1-, A_2-, oxidase negative) corresponding to correct presumptive identification. This pattern appears to be quite specific since it was not found with any stool isolate other than *Salmonella* isolates. Interestingly, the only five *Salmonella* strains that gave an A_2- reaction and could not be identified in 2 h were either *S. dublin* or *S. enteritidis*, both belonging to group D. The possibility of rapidly providing the clinician with reliable indications about the presence of *Salmonella* spp. is, perhaps, the most interesting feature of the API Z system.

It appears that this system can efficiently screen for the presence of the above-indicated enteric pathogens, since 77.3% of the clinical isolates could be excluded from further testing after 2 h. This percentage can increase significantly if one elects to consider a blue-green A_1+ reaction as excluding the presence of these enteric pathogens, as per the API Z instructions. Our experience, however, has been that distinguishing between blue and blue-green involves considerable individual variability. Some *Shigella* strains produced coloration that was called blue-green by some of us. Therefore, it is probably wiser to further test all isolates producing a color change with a clear-blue component, as per the instructions of the Rapid SST system. It is felt that producing more uniform definitions and interpretations of the color reactions contained in the package instructions of the API Z and Rapid SST systems would facilitate the exchange of data between bacteriologists from Europe and the United States.

The results presented here are essentially in agreement with those of two recent studies. Testing suspicious isolates from triple sugar iron and lysine iron agars, Heier found that the Rapid SST system detected all potential pathogens (63 *Salmoneella* and 111 *Shigella* strains) and eliminated from further testing 316 of 433 (73.0%) nonpathogens (K. A. Heier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C65, p. 247). Greene et al. found that only 1 of 57 pathogens picked from differential plates would have been missed by the Rapid SST system if this were the only screening system, whereas none of 21 *Proteeae* strains and 19 of 23 coliforms would have been tested further (L. C. Greene, P. C. Appelbaum, and J. A. Kellog, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984. C66, p. 247).

Using the API Z system is not likely to interfere with or limit the isolation of pathogens different from *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica*. Isolation and identification of these pathogens generally requires techniques quite different from the ones employed in the initial screening for *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica* (7). *Aeromonas* spp. grow well on common plating media, but isolation from these media is complicated by the fact that lactose-negative strains are not unusual (13). Thus, special media for the isolation of *Aeromonas* spp. from fecal specimens is recommended (14). However, since an oxidase reaction can be performed in cupule B of the API Z system and its contents can be subcultured onto appropriate media, this system is not incompatible, in principle, with the screening of lactose-negative colonies for the presence of *Aeromonas* spp. or other pathogens.

Various methods have been described for the rapid detection or screening of enteric pathogens, or both (1, 12). To our knowledge the API Z system is the only one to be commercially available. The system is easy to use, requires no instrumentation, has an extended shelf life, and provides valuable information within a 2-h time span. It is likely to simplify the process of screening for certain pathogens in stool specimens and to reduce the time and effort involved in this procedure.

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