A Single-Tube Screen for *Salmonella* and *Shigella*

Gary W. Procop, MD,[1] Jacqueline D. Wallace,[1] Marion J. Tuohy, MT(ASCP),[1] Margret M. LaSalvia, MT(ASCP),[1] Rachel M. Addison, MPH, MT(ASCP),[2] and L. Barth Reller, MD[2]

Key Words: Salmonella; Shigella; Screening; Gram-negative bacilli; Culture media

DOI: 10.1309/MTDBAXHDPKAL6GF5

Abstract

Salmonella *and* Shigella *species are routinely sought in stool specimens submitted for culture. It is a common practice to screen lactose-negative colonies by using triple sugar iron agar, lysine iron agar, and Christenson urea agar to determine if further identification is necessary. We designed and evaluated a novel combination of media, which are layered in a single tube, for screening isolates suspected to possibly represent* Salmonella *or* Shigella. *We tested this media combination with 106* Salmonella, *56* Shigella, *and 56 other gram-negative bacilli.*

All Salmonella *and* Shigella *isolates tested were appropriately characterized as possible* Salmonella *or* Shigella *by using an algorithm developed for use with this media combination. Similarly, 53 (95%) of 56 other gram-negative bacilli were appropriately screened as non-*Salmonella *and non-*Shigella *isolates. This unique media combination provides the most important biochemical reactions needed to screen for* Salmonella *and* Shigella *in a single-tube format, which decreases labor by two thirds (ie, 1 tube is inoculated vs 3).*

Salmonella and *Shigella* species are important causes of enteritis throughout the world; in addition, *Salmonella enterica* serotypes Typhi and Paratyphi are important causes of enteric fever in underdeveloped countries that lack adequate sewage disposal and water treatment facilities.[1-5] A wide variety of selective and differential media and identification systems have been tested during the past half century for the rapid differentiation of *Salmonella* and *Shigella* from normal enteric flora.[6-25] The urea agar is one of the most useful of these screening media, which readily differentiates the lactose-negative *Salmonella* and *Shigella* isolates, which are urease-negative, from many of the lactose-negative normal enteric flora that produce urease, such as *Proteus*, *Providencia*, and *Morganella.*[10,19,21,26] The other biochemical reactions important for the detection of *Salmonella* are lysine decarboxylation and hydrogen sulfide (H_2S) production. The fermentation of glucose without the production of gas and the absence of motility are important tests for the differentiation of *Shigella* species from other members of the Enterobacteriaceae.[26,27]

It is standard practice in many clinical microbiology laboratories to further screen lactose nonfermenting colonies that are isolated from MacConkey and Hektoen-Enteric agars, which have been directly inoculated with stool specimens.[3,5,26,27] A commonly used screening method, and the method used at the Cleveland Clinic, Cleveland, OH, uses 3 tubed/slanted agars (ie, the 3-tube set), which consists of a triple sugar iron (TSI) agar, a lysine iron (LIA) agar, and a Christensen urea agar.[3,5,27] Although useful and effective, it has been noted that some of the reactions that may occur with the TSI and LIA agars are redundant.[25] Therefore, we designed and evaluated a novel combination of media, which are layered in a single tube (the single-tube screen [STS]),

that provides the most important reactions of the 3-tube set. The STS and an algorithm that was developed to facilitate its optimal use were tested with numerous isolates of *Salmonella* and *Shigella* and other gram-negative bacilli that may be encountered in the clinical microbiology laboratory during the routine examination of bacterial isolates from stool cultures.

The STS was the forerunner of the Enteroscreen 4 (Hardy Diagnostics, Santa Maria, CA), which is now commercially available. The reactions present in the STS and the Enteroscreen 4 are the same, although there has been some slight modifications made to the formulation described herein (see https://www.hardydiagnostics.com/catalog2/hugo/EnteroScreen4.htm). In addition, the algorithm for use has also slightly been changed. Therefore, although similar, this study cannot be construed as a technical evaluation of the Enteroscreen 4, but is rather a study of the precursor of this product.

Materials and Methods

The STS system that was devised and tested consisted of 4 layers within a single tube. Three of the layers were agar media, whereas the fourth layer was sterilized petrolatum (Fisher, Hanover Park, IL) **Table 1** and **Image 1A**. The 4 layers of the STS system from the top to the bottom of the tube were as follows: (1) lysine, glucose, and peptone–containing medium with the ingredients necessary to detect H_2S production; this layer was slanted; (2) lysine, glucose, and peptone–containing media without the ingredients for H_2S production; (3) sterilized petrolatum; and (4) standard urea agar. Preliminary studies demonstrated the need for an algorithm to aid in the order in which the reactions should optimally be interpreted; such an algorithm was developed with this product **Figure 1**.

The urea reaction was interpreted first in the algorithm. If the urea reaction was positive, as indicated by a color change from yellow to pink or red, the isolate was considered not to represent *Salmonella* or *Shigella* **Image 1D**. If the urea reaction was negative (ie, remained yellow), the lysine decarboxylation reaction was assessed. This was interpreted just above the hydrophobic petrolatum barrier. If the lysine decarboxylation reaction was negative, which was indicated by a yellow color **Image 1C**, the isolate was considered not to represent a *Salmonella* species. However, if the lysine decarboxylation reaction was positive, as indicated by a purple color **Image 1B**, the possibility of a *Salmonella* species remained. If the urease and the lysine decarboxylation reactions were negative, as described above, then the presence of gas production was assessed throughout the tube. If gas was present in this scenario, as determined by the presence of bubbles or cracks in the media, the isolate was considered not to be *Salmonella*, because of the negative lysine decarboxylation reaction, or *Shigella*, because of the presence of gas. If gas was absent in

Table 1
Single-Tube Screen Formulation

Ingredient	Concentration (g/L)*
A. Top agar layer, 2 mL/tube, slanted (lysine with ingredients for H_2S production)†	
1. Dextrose (BACTO), Difco Laboratories, Detroit, MI	1
2. Yeast extract, BD, Sparks, MD	3
3. Sodium chloride, J.T. Baker, Mallinckrodt Baker, Phillipsburg, NJ	5
4. Bromcresol purple, Sigma Chemical, St Louis, MO	0.02
5. L-Lysine HCl, BD	10
6. Agar (BACTO), BD	12
7. Peptone (BACTO), Difco Laboratories	5
8. Sodium thiosulfate, Mallinckrodt, Mallinckrodt Baker, Paris, KY	0.3
9. Ferric ammonium citrate, J.T. Baker, Mallinckrodt Baker‡	0.3
B. Middle agar layer, 4 mL/tube (lysine without ingredients for H_2S)†	
10. Dextrose (BACTO), Difco Laboratories	1
11. Yeast extract, BD	5
12. Sodium chloride, J.T. Baker, Mallinckrodt Baker	3
13. Bromcresol purple, Sigma Chemical	5
14. L-Lysine HCl, BD	0.02
15. Agar (BACTO), BD	10
16. Peptone (BACTO), Difco Laboratories	12
C. Hydrophobic separator, 2.0 mL/tube (sterilized petrolatum)	
D. Bottom media, 3 mL/tube (urea agar)†	
17. Urea agar base, Difco Laboratories; 10× stock	1×
18. Agar (BACTO), BD	12
19. Yeast extract, BD	1

* Unless otherwise indicated.
† Adjust each medium to pH 6.5.
‡ Ferrous sulfate is an acceptable alternative.

DOI: 10.1309/MTDBAXHDPKAL6GF5

this scenario, the isolate was considered to possibly represent a *Shigella* isolate (Image 1C).

If the lysine decarboxylation reaction was positive, the deamination reaction was considered regardless of the presence or absence of gas. The deamination reaction was interpreted at the surface aspect of the slant. A reddish or maroon color was considered positive, whereas no substantial change from the original purple was considered negative. If the deamination reaction was positive, the isolate was considered not to be a *Salmonella* isolate; *Shigella* would have already been excluded at this point by the presence of a positive lysine decarboxylation reaction. If the deamination reaction was negative in the presence of a positive lysine decarboxylation reaction, the presence of H_2S and gas was assessed. The presence of H_2S was assessed predominantly in the uppermost layer of media (Images 1B and 1D). If H_2S was produced in this scenario, with or without the presence of discernible gas, the isolate was considered a potential H_2S-producing *Salmonella* (Image 1B). If, in the presence of a positive lysine decarboxylation reaction, H_2S was not detected, but gas production was present, the isolate was considered a potential H_2S-negative *Salmonella* species.

If the area where the lysine decarboxylation reaction was interpreted demonstrated no color change and there was no evidence of H_2S or gas production, possibility of a non–glucose fermenter such as *Pseudomonas aeruginosa* was considered. In such cases, an oxidase reaction was performed from isolate recovered from the slant. If the test organism was oxidase positive, the isolate was considered not to be *Salmonella* or *Shigella*. If the organism was oxidase negative, the possibility of a poorly reactive *Salmonella* strain could not be excluded, so we recommended complete identification.

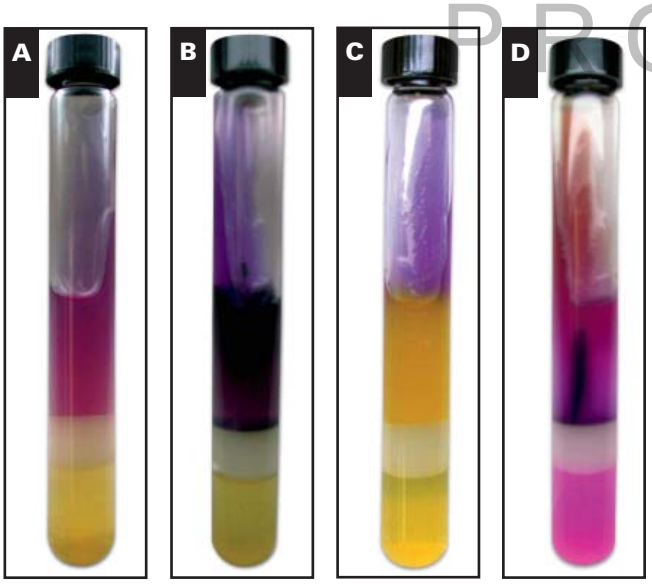

Image 1 The single-tube screen (STS). An uninoculated STS tube (**A**) shows that the sterilized petrolatum layer clearly separates the urea agar at the bottom of the tube from the lysine-containing media in the top half of the tube. The STS tube inoculated with *Salmonella* (**B**) demonstrates hydrogen sulfide production, which does not obscure the positive lysine decarboxylation reaction, and a negative urea reaction. The STS tube inoculated with *Shigella* (**C**) demonstrates glucose fermentation (no gas was produced) and negative lysine decarboxylation and urease reactions. The STS tube inoculated with *Proteus* (**D**) demonstrates a positive urea reaction, deamination of amino acids, and hydrogen sulfide production. Note that diffusion of ammonium ions from the urea agar has caused a shift in the bromocresol purple indicator in the top half of the tube. This demonstrates the need to use this system according to the associated use algorithm.

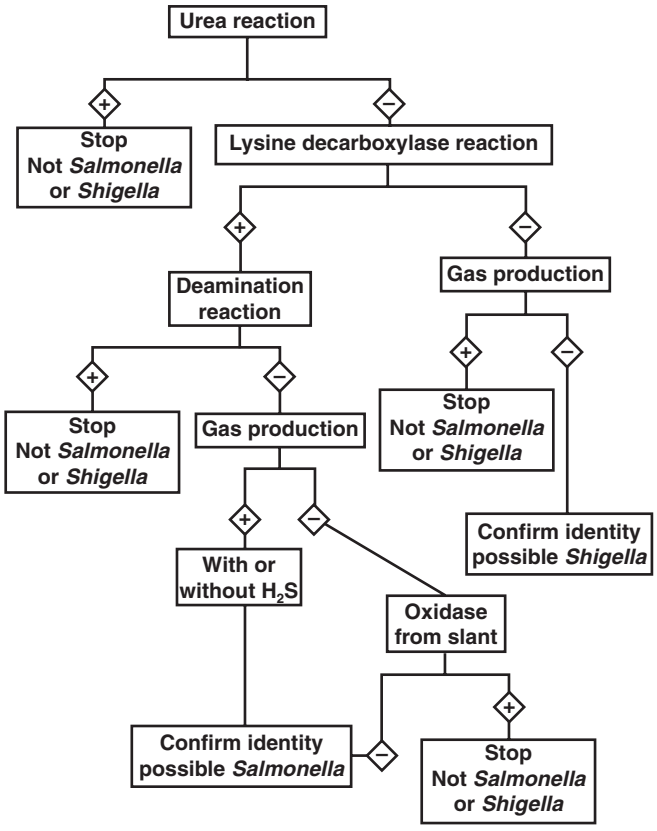

Figure 1 Algorithm for use of the single-tube screen (STS). The sterilized petrolatum layer is useful for separating these media that use different indicators. Hydrogen and ammonium ions, however, can diffuse across this barrier. Therefore, the STS should be used with this codeveloped algorithm for the optimal performance of this product.

DOI: 10.1309/MTDBAXHDPKAL6GF5

In this manner, we tested the STS with 218 gram-negative bacilli. These consisted of 106 isolates of *Salmonella* (23 *Salmonella typhi* strains and 83 nontyphi *Salmonella* strains, with representatives strains from 19 serotypes), 56 *Shigella* isolates (with representatives from each of the 4 species), and 56 other gram-negative bacteria, representing commonly encountered lactose-negative Enterobacteriaceae and *P aeruginosa* ▮**Table 2**▮. The bacteria had been identified by traditional biochemical testing and/or the Vitek GNI card used with the Vitek legacy system (bioMerieux, St Louis, MO). Traditional testing with the TSI, LIA and Christensen urea agars was performed on isolates for which the screening result with the STS was inconsistent with the organism identification. The identifications of the *Salmonella* species, *Shigella* species, *Escherichia coli* O157:H7, and *Yersinia enterocolitica* isolates were confirmed by the public health laboratories in the states in which the organisms were initially recovered.

▮**Table 2**▮
Bacterial Isolates Tested With the Single-Tube Screen

	No. of Isolates
Salmonella isolates (n = 106)	
Salmonella enterica serotype Typhi	23
Salmonella enterica serotype Paratyphi*	83
Salmonella enteritidis	23
Salmonella heidelberg	5
Salmonella havana	1
Salmonella Saintpaul	1
Salmonella ealing	1
Salmonella typhimurium	26
Salmonella choleraesuis	5
Salmonella senfrenberg	1
Salmonella poona	1
Salmonella oslo	1
Salmonella berta	1
Salmonella java	2
Salmonella agona	1
Salmonella alachua	1
Salmonella newport	2
Salmonella infantis	8
Salmonella javiana	1
Salmonella anatum	1
Salmonella braenderup	1
Shigella isolates (n = 56)	
Shigella sonnei	27
Shigella flexneri	21
Shigella boydii	6
Shigella dysenteriae	2
Other gram-negative bacilli (n = 56)	
Citrobacter species	12
Hafnia alvea	2
Yersinia enterocolitica	6
Morganella species	11
Escherichia coli	1
Providencia species	4
Pseudomonas aeruginosa	2
Enterobacter species	2
Proteus species	15
Klebsiella pneumoniae	1

* The names following the genus name are the serotypes of *S enteritidis*.

The bacterial isolates were recovered from frozen stock on sheep's blood agar before testing. One additional passage of each isolate, was performed and growth from the fresh transfer was used to inoculate the STS system. The tubes containing the STS media were inoculated with 1 of the test isolates each. Inoculation was performed with a sterile wooden stick; the tubed media was stabbed once to the bottom of the tube, and the slant was streaked. The tubes were incubated for 18 to 24 hours at 35°C with air.

Results

All 106 isolates of *Salmonella* and 56 isolates of *Shigella* were appropriately screened in as possible *Salmonella* and possible *Shigella* by the STS system. Of the 56 non-*Salmonella* and non-*Shigella* bacterial isolates, 53 (95%) were appropriately screened out by the STS using the associated algorithm. The results of the TSI, LIA, and urea agars that were performed on the discrepant isolates demonstrated that these were uniformly weak producers of urease. The 3 isolates inappropriately characterized as possible *Salmonella* or *Shigella* by the STS were 1 isolate each of *Y enterocolitica*, a *Citrobacter* species, and an *Enterobacter* species. We hypothesize that the abundant fermentation of glucose in the upper layers of media resulted in sufficient hydrogen ion production to afford a slight penetration of the petrolatum layer and neutralization of the weak urea reaction. Therefore, the detection of urea in the STS is not as sensitive as detection using Christensen urea agar alone.

Discussion

The STS system is a novel combination of the media, some of which have been modified, that have proven effective through years of use in clinical microbiology for the differentiation of *Salmonella* and *Shigella* isolates from other members of Enterobacteriaceae and other gram-negative bacilli. The 2 lysine-containing media in the upper half of the STS system are identical in composition, except for the ingredients necessary for H_2S production, which are present only in the uppermost layer. These were constructed so that when H_2S production occurred, it would not obscure the area in which the lysine decarboxylation reaction was interpreted, which may occur with the LIA medium. These media contain glucose for fermentation and gas production. In addition, peptones are present that afford the aerobic amino acid deamination reaction that occurs on the slant with certain nonenteric pathogens, such as *Proteus* species.

Unlike the TSI, we did not include sucrose or lactose in the STS. Lactose- and/or sucrose-fermenting strains

DOI: 10.1309/MTDBAXHDPKAL6GF5

of *Salmonella* have been reported that cause serious disease.[1,10,15,21,22] For example, *Salmonella paratyphi* A is known to produce an acid-over-acid reaction in the TSI tube; it is thereby mistaken for non-*Salmonella* members of Enterobacteriaceae. The amount of glucose present in the STS is greater than that in the LIA but similar to the amount found in the TSI. This concentration of glucose was chosen to facilitate the assessment of gas production. The presence of the slanted uppermost medium allows recovery of the organism for additional testing, such as oxidase testing. The bottommost layer that consists of standard urea agar readily identifies strong urease producers, thereby distinguishing them from *Salmonella* and *Shigella*.

The sterilized petrolatum layer separates the urea medium, with its potentially basic reaction, from the lysine-containing media in which glucose fermentation and acid production occur; these different media also use different pH indicators. Although effective, this barrier is an incomplete hydrophobic layer. The vigorous fermentation of glucose could produce sufficient acid to neutralize a weak urea reaction, which was seen with a few isolates in this study. Similarly, the vigorous production of a urease could produce sufficient ammonium ions to shift the pH in the lysine-containing media to produce a false-positive lysine decarboxylation reaction. Fortunately, this latter contingency is considered in the algorithm (ie, the lysine decarboxylation reaction is not important if a positive urease reaction is present). The false-positive lysine decarboxylation reaction due to strong urease production does not result additional testing to exclude the possibility of a *Salmonella* isolate because the urease reaction is the first reaction considered in the algorithm and isolates with positive urease reactions are excluded as non-*Salmonella* and non-*Shigella* isolates.

The detection of all 162 *Salmonella* and *Shigella* isolates tested demonstrates the high sensitivity (100%) of the STS system for appropriately characterizing these genera. High sensitivity is a desired characteristic in a screening test, whereas high specificity is desired for a confirmatory test. Of 56 isolates of non-*Salmonella* and non-*Shigella* gram-negative bacilli tested, 3 were erroneously screened as potential pathogens, yielding a specificity of 95%. Organisms that are currently inappropriately screened as potential *Salmonella* and *Shigella* isolates with the 3-tube set would also be expected to be similarly screened with the STS.

The STS seems promising as a single-tube alternative to the 3-tube set (TSI, LIA, and Christensen urea) for the screening of lactose-negative stool isolates that may represent *Salmonella* or *Shigella*, but it must be used with the associated algorithm. Given the false-negative urease reaction for an isolate of *Y enterocolitica*, we believe the STS should not be used to screen for the possibility of this enteric pathogen. Finally, a modified version of the STS is available as the Enteroscreen 4. Although the reactions are essentially the same as those described herein, the formulation of the media in the commercially available product is slightly different (see https://www.hardydiagnostics.com/catalog2/hugo/EnteroScreen4.htm), and the algorithm for use is slightly changed. Therefore, although similar, this study cannot be construed as a technical evaluation of the Enteroscreen 4, but rather is a study of the forerunner to this product.

From the [1]Section of Clinical Microbiology, Cleveland Clinic Foundation, Cleveland, OH; and [2]Department of Clinical Microbiology, Duke University Medical Center, Durham, NC.

Address reprint requests to Dr Procop: Clinical Pathology/L11, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195.

References

1. Bern C, Martines J, de Zoysa I, et al. The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bull World Health Organ.* 1992;70:705-714.
2. Moeller V. Distribution of amino acid decarboxylases in *Enterobacteriaceae*. *Acta Pathol Microbiol Scand.* 1954;35:259-277.
3. Procop GW. Gastrointestinal infections. *Infect Dis Clin North Am.* 2001;15:1073-1108.
4. Procop GW, Cockerill FR III. Enteritis caused by *Escherichia coli* & *Shigella* & *Salmonella* species. In: Wilson WR, Sande MA, eds. *Current Diagnosis & Treatment in Infectious Diseases.* New York, NY: McGraw-Hill; 2001:548-566.
5. Reller LB, Mirrett S. Motility-indole-lysine medium for presumptive identification of enteric pathogens of Enterobacteriaceae. *J Clin Microbiol.* 1975;2:247-252.
6. Andrews WH. Evaluation of a selective medium for hydrogen sulfide production by *Salmonella*. *J Assoc Off Anal Chem.* 1975;58:579-580.
7. Bhar P, Myers RM. Lysine iron agar medium in routine diagnostic enteric bacteriology. *Indian J Med Res.* 1969;57:995-998.
8. Blazevic DJ. Improved motility-indole medium. *Appl Microbiol.* 1968;16:668.
9. Bonev SI, Zakhariev Z, Gentchev P. Comparative study of media for determination of lysine decarboxylase activity. *Appl Microbiol.* 1974;27:464-468.
10. Christensen WB. Urea decomposition as a means of differentiating *Proteus* and *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. *J Bacteriol.* 1946;52:461-466.
11. Cox JM. Lysine-mannitol-glycerol agar, a medium for the isolation of *Salmonella* spp, including *S typhi* and atypical strains. *Appl Environ Microbiol.* 1993;59:2602-2606.
12. Ederer GM, Clark M. Motility-indole-ornithine medium. *Appl Microbiol.* 1970;20:849-850.
13. Ederer GM, Lund ME, Blazevic DJ, et al. Motility-indole-lysine-sulfide medium. *J Clin Microbiol.* 1975;2:266-267.
14. Gooch WM III. Evaluation of a multitest system for rapid identification of *Salmonella* and *Shigella*. *Am J Clin Pathol.* 1980;73:570-573.
15. Hinrichs J, Gauthier A, Keating M, et al. Evaluation of a new screening system for enteric pathogens. *Diagn Microbiol Infect Dis.* 1985;3:397-401.

DOI: 10.1309/MTDBAXHDPKAL6GF5

16. Igarashi H, Ota K, Zenyoji H. A new differential medium for enteric pathogens, lysine-indole-motility (LIM) medium [in Japanese]. *Nippon Saikingaku Zasshi.* 1969;24:338-344.
17. Kohbata S, Takahashi M, Yabuuchi E. Lactose-fermenting, multiple drug-resistant *Salmonella typhi* strains isolated from a patient with postoperative typhoid fever. *J Clin Microbiol.* 1983;18:920-925.
18. Miller SI, Hohmann EL, Pegues DA. *Salmonella* (including *Salmonella typhi*). In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases.* 4th ed. New York, NY: Churchill Livingstone; 1995:2013-2028.
19. Smith PB, Rhoden DL, Tomfohrde KM, et al. R-B enteric differential system for identification of Enterobacteriaceae. *Appl Microbiol.* 1971;21:1036-1039.
20. Stuart CA, Van Stratus E, Rustigan R. Further studies on urease production by *Proteus* and related organisms. *J Bacteriol.* 1944;49:437-444.
21. Sulkin SE, Willett JC. A triple sugar–ferrous sulphate medium for use in identification of enteric organisms. *J Lab Clin Med.* 1940;25:649-653.
22. Traub WH, Raymond EA. Modified motility-indole-ornithine-H2S medium (MIOH) for the routine identification of rapid lactose-fermenting Enterobacteriaceae. *Zentralbl Bakteriol [Orig].* 1970;215:401-403.
23. Villasante PA, Agulla A, Merino FJ, et al. Rapid automated method for screening of enteric pathogens from stool specimens. *J Clin Microbiol.* 1987;25:584-585.
24. Yong DC, Thompson JS, Prytula A. Rapid microbiochemical method for presumptive identification of gastroenteritis-associated members of the family Enterobacteriaceae. *J Clin Microbiol.* 1985;21:914-918.
25. Rosa Fraile M, Vega Aleman D, Fernandez Gutierrez C. Evaluation of urea-motility-indole medium for recognition and differentiation of *Salmonella* and *Shigella* species in stool cultures. *J Clin Microbiol.* 1980;12:310-313.
26. Farmer JJ III. Enterobacteriaceae: introduction and identification. In: Murray PR, Baron EJ, Pfaller MA, et al, eds. *Manual of Clinical Microbiology.* 7th ed. Washington, DC: ASM Press; 1999:442-458.
27. Bopp CA, Brenner FW, Wells JG, et al. *Escherichia, Shigella,* and *Salmonella.* In: Murray PR, Baron EJ, Pfaller MA, et al, eds. *Manual of Clinical Microbiology.* 7th ed. Washington, DC: ASM Press; 1999:459-474:459-474.

DOI: 10.1309/MTDBAXHDPKAL6GF5