JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1983, p. 79–84
0095-1137/83/010079-06$02.00/0

Vol. 17, No. 1

Rapid Method for Detection, Identification, and Susceptibility Testing of Enteric Pathogens

CHARLES E. STAGER,[1,2] ERIC ERIKSON,[1] AND JAMES R. DAVIS[2,3]*

Ben Taub General Hospital,[1] The Methodist Hospital,[3] and Baylor College of Medicine,[2] Houston, Texas 77030*

Received 26 May 1982/Accepted 30 September 1982

Three hundred and seven colonies believed to be enteric pathogens were selected from primary plates of MacConkey, xylose desoxycholate, or salmonella-shigella agar for inoculation to lactose-sucrose broth, urea-41 motility medium, modified Andrade glucose broth with inverted Durham tube, pregrowth broth, triple sugar iron agar, lysine iron agar (LIA), and Christensen urea agar. The rapid screen consisted of interpreting the lactose-sucrose, urea-41 motility, and modified Andrade glucose broth gas reactions after 4 to 6 h at 35°C. These rapid screening biochemicals plus LIA were incubated for 24 h if further interpretation was required. Reference biochemicals (triple sugar iron, LIA, and Christensen urea agars) were interpreted at 24 h. Of 307 isolates, 49 (16%) were reported as negative for enteric pathogens after 4 to 6 h because their biochemical profiles were not compatible with those for enteric pathogens. A total of 87 (28.3%) isolates produced biochemical profiles at 4 to 6 h that were presumptive for enteric pathogens. The 87 presumptive pathogens were inoculated into the AutoMicrobic system Gram-Negative General Susceptibility Card and the AutoMicrobic system *Enterobacteriaceae*-Plus Biochemical Card (AMS-EBC+) after 4 to 6 h of growth in pregrowth broth. Of these isolates, 63 were confirmed to be enteric pathogens, of which 61 (96.8%) were correctly identified by the AMS-EBC+. One isolate was identified as *Shigella dysenteriae* by AMS-EBC+ but confirmed as *Shigella flexneri* biotype 6 by a reference laboratory. The other isolate was identified as *Arizona hinshawii* by AMS-EBC+ but was confirmed as *Salmonella enteritidis*. Of the 307 isolates, 166 (54.1%) required further interpretation of the rapid screening biochemicals plus LIA at 24 h; 5 of these were detected as enteric pathogens. The same 68 enteric pathogens were detected by both the rapid method and the reference method. The results from the general susceptibility card agreed with agar diffusion results at 99.2%. One *Salmonella enteritidis* and four *Shigella* spp. showed minor discrepancies with tetracycline. No very major or major discrepancies were observed.

Although the incidence of disease produced by the classical pathogens *Salmonella* and *Shigella* has decreased since the beginning of this century, there was a slight increase in reported isolations during the past decade. Since it has been estimated that only about 1% of all infections are reported (3) and there were more than 50,000 isolations reported in the United States alone during 1980, it is apparent that these bacteria remain a significant cause of morbidity.

The key to effective management of infectious diseases, including those produced by *Salmonella* and *Shigella*, is early diagnosis and appropriate therapeutic intervention. Therefore, laboratory techniques for isolating, identifying, and, when necessary, determining the antimicrobial susceptibility patterns of these bacteria quickly are of considerable importance. There have been some attempts to reduce the time required to identify these bacteria; in 1952, Bicknell et al. (1) reported that a preheated semisolid medium allowed them to screen stool isolates for *Salmonella* and *Shigella* in 3.5 h. In 1953, Sanders and Okabe (12) developed a lactose and sucrose "booster" broth to screen stool isolates. These investigators incubated samples for 15 to 18 h in their first report, but later reported (13) that the time could be reduced to 2 h. More recently, several investigators have reported the use of enrichment culture and coaggulutination tests to reduce the time for detection and identification of *Salmonella* and *Shigella* species (6, 10, 11). However, most clinical microbiology laboratories still use techniques for the isolation and identification of these microbes that require 3 to 4 days to obtain definitive results.

This report describes work performed in our laboratories on fresh clinical isolates that resulted in the identification and susceptibility testing of species of *Salmonella* and *Shigella* within 12 to 14 h after the bacteria were isolated. A battery of conventional media that yielded reactions within 4 to 6 h was used to screen suspicious isolates. The bacteria that produced presumptive results on the screening media were inoculated into the AutoMicrobic system *Enterobacteriaceae*-Plus Biochemical Card (AMS-EBC+) and the AutoMicrobic system Gram-Negative General Susceptibility Card (AMS-GSC; both from Vitek Systems, Inc., Hazelwood, Mo.).

MATERIALS AND METHODS

Media. Unless otherwise indicated, media were sterilized at 121°C for 15 min. The following media were prepared. (i) Lactose-sucrose broth: tryptone (Difco Laboratories, Detroit, Mich.), 20.0 g; sodium phosphate, 1.0 g; sodium chloride, 5.0 g; bromcresol purple, 0.008 g; distilled water, 900 ml. After being autoclaved, the medium was cooled to room temperature. Ten grams each of lactose and sucrose were dissolved into 100 ml of distilled water, filter sterilized. (Nalgene, 0.20 μm), and added to the tryptone base. Medium (0.5 ml) was dispensed into sterile 13 by 75-mm tubes with caps. (ii) Modified Andrade glucose broth: peptone (Difco), 10.0 g; meat extract (Difco), 3.0 g; sodium chloride, 5.0 g; glucose, 25.0 g; Andrade indicator, 10 ml; distilled water, 1,000 ml. The pH was adjusted to 7.1 to 7.2, and 1.5 ml of medium was dispensed into 13- by 100-mm tubes with caps containing an inverted Durham tube. (iii) Urea-41 motility medium: L-tryptophan (Difco), 1.0 g; peptone, 9.0 g; yeast extract (Difco), 1.0 g; potassium phosphate (monobasic), 9.5 g; agar, 4.0 g; distilled water, 1,000 ml. The medium was heated to boiling and cooled to 50 to 60°C. Urea agar base (20 g; Difco) was dissolved into the medium, and the pH was adjusted to 5.5. Medium (4 ml) was dispensed into 13- by 100-mm tubes with caps. The pH obtained after the tubes were autoclaved was 6.8. (iv) Pregrowth broth: peptone, 1.5 g; sodium chloride, 5.0 g; distilled water, 1,000 ml. After being autoclaved, the medium was cooled to room temperature. A 10% solution of glucose was filter sterilized (Nalgene, 0.20 μm), and 2.0 ml was added to the peptone base. Medium (0.5 ml) was dispensed into sterile 13- by 75-mm tubes with caps. The medium was stable for 1 month at 4°C. Triple sugar iron (TSI) agar, lysine iron agar (LIA), Christensen urea agar, MacConkey agar, salmonella-shigella agar, xylose lysine desoxycholate agar, and selenite broth were obtained from Sabco, San Antonio, Tex.

Bacteria. Fresh stool specimens were inoculated onto MacConkey and salmonella-shigella agars and into selenite broth and incubated at 35°C. Within 12 h, selenite broth was subcultured onto MacConkey, salmonella-shigella, and xylose lysine desoxycholate agars. Solid media were examined at 24 and 48 h.

Bacterial identification. Three hundred and seven colonies suggestive of enteric pathogens were inoculated into both conventional and rapid-method media. (i) Conventional method. A portion of a colony was inoculated to TSI, LIA, and Christensen urea agars and incubated at 35°C for 24 h. All bacteria that produced biochemical profiles compatible with enteric pathogens were identified by API 20E (Analytab Products, Plainview, N.Y.) and serotyped. (ii) Rapid method. Urea-41 motility medium, LIA, modified Andrade glucose broth, pregrowth broth, and lactose-sucrose broth were inoculated from the same colony used to inoculate the conventional media. The lactose-sucrose broth, pregrowth broth, and LIA required only a minimal inoculum. The urea-41 motility and modified Andrade glucose media required a heavier inoculum for characteristic reactions to take place. All media were incubated at 35°C, and urea-41 motility medium, modified Andrade glucose broth, and lactose-sucrose broth were interpreted at 4 to 6 h. The biochemical profiles most likely to represent the common enteric pathogens are shown in Table 1. The *Salmonella-Arizona-Edwardsiella* group and *Shigella* produce biochemical profiles A and B, respectively. If either biochemical profile A or B developed at 4 to 6 h, 20 μl of pregrowth broth was diluted with 1.8 ml of 0.5% sodium chloride and used as the inoculum for the AMS-GSC. The remaining volume of pregrowth broth (approximately 0.5 ml) was diluted with 1.8 ml of 0.5% sodium chloride and used as the inoculum for the AMS-EBC+ (5, 7).

If biochemical profile A or B was not observed at 4 to 6 h, certain biochemical profiles allowed the isolate to be discarded. If the primary colony was H_2S positive and the urease or urease and lactose-sucrose reactions were positive, the isolate was discarded at 4 to 6 h. If the isolate was lactose-sucrose positive at 4 to 6 h, all media that were originally inoculated for the rapid method were incubated for a total of 24 h at 35°C. If the primary colony was H_2S negative, a two-digit code was determined. The first digit was derived by assigning a value of 1 for lactose-sucrose, 2 for urease, and 4 for motility (35°C) when the respective reactions were positive, and a value of 0 when these reactions were all negative. The values of the three reactions were added, and the sum constituted the first digit of the code. The second digit was derived by assigning a value of 1 for gas production in the modified Andrade glucose broth and a value of 0 for no gas production. Isolates yielding code numbers 21, 31, 60, 61, 70, and 71 were discarded at 4 to 6 h. If the two-digit code was 10, 20, or 30, a urea-41 motility medium was inoculated and incubated at room temperature for 18 to 20 h. Isolates that produced code number 40 were inoculated into TSI agar and incubated at 35°C for 18 to 20 h. All media that were originally inoculated for the rapid method were incubated at 35°C for a total of 24 h if the isolate was not presumptive for an enteric pathogen or discarded at 4 to 6 h as described above. A two-digit code was again determined after overnight incubation. Table 2 shows the code numbers and the additional test results that were presumptive for enteric pathogens after 24 h.

When there was a discrepancy in identification of an isolate between the conventional and the rapid methods, the isolate was sent to the Houston City Health Department Laboratory for reference serological and biochemical identification.

Antimicrobial susceptibility testing. (i) Reference susceptibility method. Agar diffusion susceptibility tests were performed from growth on the TSI agar slant according to the performance standards of the

TABLE 1. Biochemical profiles representative of the *Salmonella-Arizona-Edwardsiella* group (A) and *Shigella* (B)

Test	Biochemical profile	
	A	B
H_2S production (primary colony)	+	−
Lactose-sucrose	−	−
Urease	−	−
Motility	NI[a]	−
Gas[b]	NI	−

[a] NI, Not interpreted.
[b] Gas detected in inverted Durham tube in modified Andrade glucose broth.

National Committee for Clinical Laboratory Standards (9). (ii) AMS-GSC. The AMS-GSC consists of 30 wells containing 12 antimicrobial agents at two dilutions, one antimicrobial agent at three dilutions, one positive control broth, and two blank wells. Amikacin, ampicillin, cefamandole, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, tetracycline, tobramycin, cefoxitin, and trimethoprim-sulfamethoxazole were evaluated in this study. If biochemical profile A or B developed at 4 to 6 h, 20 μl of growth in pregrowth broth was diluted with the 1.8 ml of 0.5% sodium chloride and used as inoculum for the AMS-GSC. Generally, at 4 to 5 h an interpretive call of susceptible, intermediate, or resistant and the bracket minimal inhibitory concentration value were printed by the data terminal for each card in the reader/incubator.

RESULTS

Detection of enteric pathogens at 4 to 6 h by the rapid method. Of the 307 isolates, 87 produced biochemical profiles at 4 to 6 h that were presumptive for enteric pathogens (Table 1). Sixty-three of these isolates were confirmed by serological and biochemical results to be enteric pathogens (28 *Salmonella enteritidis* and 35 *Shigella* spp.).

AMS-EBC+ identification of enteric pathogens by the rapid method. Of 63 enteric pathogens detected by the rapid method at 4 to 6 h, 61 (96.8%) were correctly identified when inoculated into the AMS-EBC+. One isolate was identified as *Shigella dysenteriae* with a 98% probability of identification by AMS-EBC+, but was identified as *Shigella flexneri* biotype 6 by API 20E. The second isolate was identified as *Arizona hinshawii* with a 78% probability of identification by AMS-EBC+, but was identified as *Salmonella* sp. by API 20E. In both cases reference serological and biochemical tests confirmed that the API 20E results were correct.

Isolates discarded at 4 to 6 h with the rapid method. Of the 307 isolates, 49 (16.0%) were discarded at 4 to 6 h. Of these, 25 were H_2S-positive primary colonies that were urease posi-

TABLE 2. Two-digit codes and additional tests presumptive for enteric pathogens

Two-digit code	Test[a]			Presumptive identification
	TSI agar	LIA	Motility (RT) medium	
01	ND	K/K or /N, H_2S+	ND	*Salmonella* (rare),[b] *Arizona* (rare)
10	ND	R/A	NI	*Providencia stuartii*
10	ND	K/A	+	*Yersinia enterocolitica*
11	ND	K/K or /N, H_2S+	(NI)	*Salmonella* (rare), *Arizona* (rare)
20	ND	K/A	+	*Yersinia pseudotuberculosis*
30	ND	K/A	+	*Yersinia enterocolitica*
40	K/A, H_2S+[c]	K/K or /N, H_2S+	ND	*Salmonella typhi*, *Salmonella* (rare), *Arizona* (rare), *Edwardsiella* (rare)
40	K/A, H_2S+	K/K or /N	ND	*Salmonella* (rare)
40	K/A	K/K or /N, H_2S+	ND	*Salmonella typhi* (rare)
40	K/A	K/K or /N	ND	*Salmonella typhi* (rare)
41	(K/a)	K/A	ND	*Salmonella paratyphi* A
41	(K/a)	K/K or /N, H_2S+	ND	*Salmonella* (rare), *Arizona* (rare)
41	(K/a, H_2S+)	K/K or /N, H_2S+	ND	*Salmonella*, *Arizona*, *Edwardsiella*
50	(K/a)	R/A	(NI)	*Providencia stuartii*
51	(A/a, H_2S+)	K/K or /N, H_2S+	(NI)	*Salmonella* (rare), *Arizona* (rare)

[a] Key: R, Red, oxidative deamination of lysine; K/, alkaline slant; A/, acid slant; /K, alkaline butt; /N, neutral butt; /A, acid butt; /a, acid and gas in butt; H_2S+, hydrogen sulfide production. Abbreviations: RT, Room temperature; ND, not done; NI, not interpreted. Parentheses indicate the test may or may not have been done.
[b] Indicates rare biotype.
[c] A very small area of black precipitate near the point of inoculation would be characteristic of *Salmonella typhi*.

tive (23 isolates) or urease and lactose-sucrose positive (2 isolates) at 4 to 6 h, and 24 were H_2S-negative primary colonies that yielded code numbers 21 (2 isolates), 60 (11 isolates), 61 (10 isolates), and 71 (1 isolate) at 4 to 6 h.

Isolates requiring additional biochemical tests with the rapid method. At 4 to 6 h, 166 H_2S-negative primary colonies yielded a code number consistent with that of an enteric pathogen when the rapid method was used. Of these isolates, 21 required additional biochemical tests at 4 to 6 h to assure that an enteric pathogen was not missed (Table 3). None of the 21 isolates was presumptive for an enteric pathogen when the biochemicals were interpreted at 24 h (Table 2).

Detection of enteric pathogens at 24 h by the rapid method. A total of 23 isolates produced code numbers at 24 h that required additional evaluation (Table 4). Twenty-two of these isolates produced code 41 at 24 h, and eighteen of these produced K/A reactions on LIA (refer to footnote *a*, Table 2). These isolates required additional biochemical and serological tests to exclude *Salmonella enteritidis* bioserotype paratyphi A. *Salmonella enteritidis* bioserotype paratyphi A was not identified, nor were any other enteric pathogens. The other four isolates with code 41 produced either K/K H_2S-positive colonies (two isolates) or K/N H_2S-positive colonies (two isolates) on LIA. Additional biochemical and serological tests identified the isolates as *Salmonella enteritidis*. One isolate yielded code 40 at 18 to 24 h and was K/N H_2S positive on LIA; although the inverted Durham tube did not demonstrate gas production, the isolate was confirmed by serological and additional biochemical tests as *Salmonella enteritidis*.

Detection of enteric pathogens at 24 h by the conventional method. The same 68 enteric pathogens detected by the rapid method were detected by the conventional method. In addition to the 68 enteric pathogens, 44 isolates produced biochemical profiles presumptive for an enteric pathogen by the conventional method. Additional biochemical and serological evaluation of these 44 isolates showed that they were not enteric pathogens.

AMS-GSC susceptibility testing of enteric pathogens by the rapid method. Fifty-five of the enteric pathogens (26 *Salmonella enteritidis* and 29 *Shigella* spp.) were tested for antimicrobial susceptibility by AMS-GSC and agar diffusion. The results from AMS-GSC agreed with agar diffusion results at 99.2%. One *Salmonella enteritidis* strain and four *Shigella* spp. had minor discrepancies with tetracycline. No very major or major discrepancies were observed.

TABLE 3. Isolates that required additional biochemical tests at 4 to 6 h by the rapid method

No. of isolates (n = 21)	Two-digit code	Biochemical test required
4	10	RT[a] motility
6	20	RT motility
3	30	RT motility
8	40	TSI

[a] RT, Room temperature.

TABLE 4. Biochemical profiles presumptive for enteric pathogens at 24 h by the rapid method and final identification

No. of isolates (n = 23)	Two-digit code	LIA[a]	Final identification
18	41	K/A	Nonpathogen
2	41	K/K, H_2S+	*Salmonella enteritidis*
2	41	K/N, H_2S+	*Salmonella enteritidis*
1	40	K/N, H_2S+	*Salmonella enteritidis*

[a] See Table 2, footnote *a*.

DISCUSSION

Of 307 isolates, 136 (44.3%) produced biochemical profiles that allowed final interpretation to be made at 4 to 6 h with the rapid method. A total of 87 isolates produced biochemical profile A or B (Table 1) and consequently were inoculated into the AMS-GSC and AMS-EBC+; 49 isolates produced biochemical profiles that allowed them to be discarded at 4 to 6 h by the rapid method. Therefore, nearly half of the isolates were either in the process of final identification and susceptibility testing or were discarded within 4 to 6 h after the primary colony was picked. The generation of code numbers to determine which isolates required further processing greatly simplified interpretation and made it easy for weekend and rotating personnel to use this approach.

The rapid method was efficient; 63 of 87 (72.4%) isolates that produced biochemical profile A or B (Table 1) at 4 to 6 h were confirmed as enteric pathogens. If a laboratory rarely recovered enteric pathogens it would not realize this level of efficiency, since most isolates yielding biochemical profile A or B would not be enteric pathogens. This method would be best for laboratories that recover numerous enteric pathogens. However, laboratories that recover few enteric pathogens but desire a fast turnaround time for better patient care might consider using this approach.

We observed in this study that approximately 10% of the colonies suggestive of enteric pathogens on isolation media were too small to provide sufficient inoculum for all of the biochemical tests and therefore had to be screened by conventional methods. *Yersinia enterocolitica*

and *Yersinia pseudotuberculosis* are known to produce small colonies, and therefore the scheme reported here may not be suitable for their identification. However, 13 isolates in this study did produce a two-digit code at 4 to 6 h that indicated a *Yersinia* species (Table 3), and a room-temperature motility medium was inoculated. When the biochemical tests were interpreted at 24 h, no *Yersinia* organisms were found. Strains of *Y. enterocolitica* are not always motile at room temperature and nonmotile at 35°C (4). However, most strains of *Y. enterocolitica* do produce typical motility patterns (2, 8) and, assuming that enough inoculum was available, would be properly identified by the rapid method. An additional eight isolates required that TSI agar be inoculated at 4 to 6 h (Table 3) to ensure that an enteric pathogen was not missed. Both urea-41 motility medium and TSI agar can be inoculated easily and quickly and have been well accepted into the workflow of the enteric station in our laboratories.

Only 23 of 307 (8.8%) isolates required additional tests at 24 h when the rapid method was used. The conventional method required additional tests for 48 of 307 (14.3%) isolates.

The rapid method detected 100% and correctly identified 96.8% of *Salmonella* and *Shigella* species when compared with a commonly used conventional approach. Two isolates were not correctly identified by the rapid method. A *Shigella flexneri* biotype 6 isolate that was mannitol negative was misidentified as *Shigella dysenteriae* with a 98% probability of identification by AMS-EBC+. The AMS-EBC+ computerized data base cannot differentiate *Shigella dysenteriae* from *Shigella flexneri* biotype 6 strains that are mannitol negative (5). Therefore, although this isolate was not correctly identified to species by the rapid method, misidentification was not caused by using organisms grown in pregrowth broth at 4 to 6 h as inoculum for the AMS-EBC+. A *Salmonella enteritidis* strain was misidentified as *A. hinshawii* with a 78% probability of identification by AMS-EBC+ on the basis of a false-positive malonate reaction. Because of the low percent probability of identification, this latter isolate would have had additional biochemical tests performed in our laboratory.

The antimicrobial susceptibility results from the rapid method demonstrated an outstanding correlation of 99.2% with agar diffusion tests. The majority of the isolates tested were sensitive to all of the antimicrobial agents tested and did not stress the susceptibility test methods. However, since many *Salmonella* and *Shigella* species are sensitive to the commonly tested antimicrobial agents, in the routine setting a high correlation might be expected.

Pregrowth broth is a minimal nutrient medium that was developed in our laboratories which allows good but limited growth of *Enterobacteriaceae* because the nutrients are exhausted within 4 h of incubation at 35°C. A 20-μl amount of this growth diluted with 1.8 ml of 0.5% sodium chloride yielded excellent results when used as inoculum for the AMS-GSC. When organisms grown in 0.5 ml of pregrowth broth were diluted with 1.8 ml of 0.5% sodium chloride, the inoculum for the AMS-EBC+ was consistently equivalent to an 0.5 McFarland standard. Even though this is one half the recommended inoculum for the AMS-EBC+, 61 of 63 enteric pathogens were properly identified.

AMS-GSC results were completed 4 to 5 h after inoculation of the card, whereas AMS-EBC+ results were completed at 8 h. Therefore, both susceptibility tests and biochemical identification of enteric pathogens were completed 12 to 14 h after the primary colony was picked. An equally important advantage of this method is the obvious cost effectiveness of screening isolates before inoculating them onto a costly identification system. Only 87 of 307 (28.3%) colonies that were suggestive of enteric pathogens yielded biochemical profiles at 4 to 6 h that required their inoculation onto an identification system.

In conclusion, this report describes a rapid method for detecting enteric pathogens. Presumptive pathogens detected on the screen were inoculated into the AMS-GSC and AMS-EBC+, with results being final 12 to 14 h after the primary colony was picked. Rare biotypes of enteric pathogens that were screen negative were detected by further interpretation of biochemicals at 24 h.

ACKNOWLEDGMENT

We thank J. Vernon Knight and Robert P. Williams for critical review of the manuscript and Janice Edwards for secretarial assistance.

LITERATURE CITED

1. **Bicknell, A. K., F. Butt, and L. Mattman.** 1952. The rapid identification of enteric organisms by microtechnique. Am. J. Public Health **42**:437–439.
2. **Bottone, E. J.** 1977. *Yersinia enterocolitica*: a panoramic view of a characteristic microorganism. Crit. Rev. Microbiol. **5**:211–241.
3. **Centers for Disease Control.** 1980. Annual summary 1979. Morbid. Mortal. Weekly Rep. **28**:73–74.
4. **D'Amato, R. F., and K. M. Tomfohrde.** 1981. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J. Clin. Microbiol. **14**:347–348.
5. **Davis, J. R., C. E. Stager, R. D. Wende, and S. M. H. Qadri.** 1981. Clinical laboratory evaluation of the AutoMicrobic system *Enterobacteriaceae* biochemical card. J. Clin. Microbiol. **14**:370–375.
6. **Edwards, E. A., and R. L. Hilderbrand.** 1976. Method for identifying *Salmonella* and *Shigella* directly from the

primary isolation plate by coagglutination of protein A-containing staphylococci sensitized with specific antibody. J. Clin. Microbiol. 3:339–343.
7. **Isenberg, H. D., T. L. Gavan, P. B. Smith, A. Sonnenwirth, W. Taylor, W. J. Martin, D. Rhoden, and A. Balows.** 1980. Collaborative investigation of the AutoMicrobic system *Enterobacteriaceae* biochemical card. J. Clin. Microbiol. **11**:694–702.
8. **Martin, W. J., and J. A. Washington II.** 1980. *Enterobacteriaceae*, p. 195–219. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
9. **NCCLS Subcommittee on Antimicrobial Susceptibility Testing.** 1979. Performance standards for antimicrobial disc susceptibility tests. Approved standard no. MS-A2. The National Committee for Clinical Laboratory Standards, Chicago.
10. **Rockhill, R. C., L. W. Rumans, M. Lesmana, and D. T. Dennis.** 1980. Detection of *Salmonella typhi* D, Vi, and d antigens by slide coagglutination in urine from patients with typhoid fever. J. Clin. Microbiol. **11**:213–216.
11. **Sanborn, W. R., M. Lesmana, and E. A. Edwards.** 1980. Enrichment culture coagglutination test for rapid, low-cost diagnosis of salmonellosis. J. Clin. Microbiol. **12**:151–155.
12. **Sanders, A. C., and K. Okabe.** 1953. A method for screening nonpathogens in feces. U.S. Armed Forces Med. J. **4**:1053–1055.
13. **Sanders, A. C., and K. Okabe.** 1954. A rapid screening method for the isolation of enteric pathogens. Public Health Lab. **12**:12–14.