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NOTES

Recovery of *Salmonella* by Using Selenite Brilliant Green Sulfa Enrichment Broth

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The efficacy and sensitivity of selenite brilliant green sulfa enrichment (SBG) broth for the isolation of *Salmonella* from fecal specimens were evaluated by using both clinical and artificially infected (artificial) fecal specimens. An examination of 1,588 clinical fecal specimens found *Salmonella* in 296 specimens, including 89 cases detected by the direct-plating xylose-lysine-desoxycholate method and an additional 207 cases detected after enrichment with SBG broth. Therefore, the recovery of *Salmonella* with SBG broth is increased 3.3-fold over that by the direct-plating method alone. Furthermore, the isolation rate of *Salmonella* is higher when using SBG broth than when using gram-negative (GN) broth or GN broth supplemented with sodium selenite. To determine the sensitivity for the recovery of *Salmonella*, artificial specimens containing various amounts of *Salmonella* were prepared and analyzed. The results indicated that the sensitivity is also higher with SBG broth than with GN broth. Moreover, the optimal incubation period for SBG broth can be extended to 24 h. In conclusion, the SBG enrichment method provides a higher recovery rate of *Salmonella* from fecal specimens.

Enrichment is a critical step in enhancing the growth of certain bacterial species while inhibiting the development of unwanted microorganisms. Enrichment broths are most commonly used in clinical laboratories for the recovery of pathogens from fecal specimens. The direct-plating and enrichment methods have been combined for the best isolation of pathogens from patients with diarrhea (9). Several kinds of enrichment broth are particularly helpful in the recovery of pathogens from the stools of *Salmonella* carriers or patients with slight *Shigella* infections in whom the number of pathogens may be as low as 100/g of feces, whereas *Escherichia coli* or other enteric bacilli may reach massive concentrations, as high as 10^9/g of feces (4–8, 12, 13).

The recovery of *Salmonella* from various specimens with a variety of media and incubation conditions has been documented (1, 3, 5–7, 9–14). Recently, the efficacies of several new selective media for the isolation of *Salmonella* have been compared. These media include Hektoen enteric agar (HE), Rambach agar, SM-ID medium, xylose-lysine-tergitol 4 agar (XLT4), novobiocin-brilliant green-glycerol-lactose agar, and modified semisolid Rappaport-Vassiliadis medium. In particular, the results indicated that the sensitivity for the isolation of *Salmonella* was dramatically increased by the utilization of tetrathionate enrichment broth (2). Moreover, the effectiveness of three enrichment broths, selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium, for the recovery of *Salmonella* from contaminated food specimens has also been evaluated (7).

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Xylose-lysine-desoxycholate (XLD) agar is the most commonly used highly selective medium for the recovery of enteric pathogens from fecal specimens (15). In addition, enrichment broths are usually used to enhance the recovery of *Salmonella* species from fecal specimens. The two most frequently used enrichment broths are gram-negative (GN) broth and selenite F (SF) broth. If these broths are used, the subculture should be performed within 4 to 6 h for GN broth and within 8 to 12 h for SF broth. Selenite brilliant green sulfa (SBG) broth, originally designed for the detection of *Salmonella* in egg and food specimens, was previously used by us to perform enrichment cultures of fecal specimens. In particular, the culture period for enrichment can be extended to 24 h (12). This study was undertaken to evaluate the efficacy and sensitivity of the SBG enrichment broth for the isolation of *Salmonella* from both clinical and artificially infected (artificial) fecal specimens.

Clinical specimens. Clinical specimens were collected from patients suspected of having gastrointestinal tract infection in Yuan's General Hospital, Kaohsiung, Taiwan. Between January 1995 and December 1997, 1,588 specimens were obtained from the pediatric department. In addition, another 155 specimens were collected from the outpatient department from April to July 1997.

Bacteria and artificial specimens. To determine the sensitivity of enrichment broths, various amounts of group B and group D *Salmonella* spp. were added to stool to create the artificial specimens. All bacteria were obtained from clinical isolates and identified by biochemical and serological tests. The amount of bacteria added was adjusted depending on the bacterial turbidity by using the McFarland standard.

Enrichment broths and selective media. Four enrichment broths, GN broth, GN broth supplemented with sodium selenite (SGN broth), SF broth, and SBG broth, were used. The

TABLE 1. Isolation of *Salmonella* from 1,588 clinical stool cultures

Method or culture medium	No. of specimens	*Salmonella* spp. isolated	
		No.	%
Direct plating onto XLD agar	1,588	89	5.6
SBG broth-XLD agar	1,499	207	13.8
Total	1,588	296	18.6

isolation of *Salmonella* spp. was performed by streaking the stool specimen on plates containing the selective medium, XLD agar. The GN broth, SF broth, and XLD agar were purchased from Difco Laboratories (Detroit, Mich.). Sodium selenite (E. Merck, Darmstadt, Germany) was added to the SBG broth (Eiken Chemical Co., Tokyo, Japan).

Direct plating and subculture with XLD agar. To isolate *Salmonella* spp., all clinical specimens were inoculated onto XLD plates and into enrichment broth and then incubated overnight at 35°C in a CO_2 incubator. The subculture was performed from enrichment broth to another XLD agar plate within the optimal incubation periods of each broth, 4 to 6 h for GN broth and 8 to 12 h for SF broth (for a review, see reference 8).

Identification of bacteria. The identification media, triple sugar iron agar, Simmons citrate agar, Christensen's urea agar, sulfide-indole motility medium, ornithine decarboxylase, and oxidase reagent were purchased from Difco Laboratories. The semisolid VP medium was from Eiken Chemical Co. (Tokyo, Japan). The API 20E identification system (bioMerieux Vitek, Inc., Hazelwood, Mo.) and serological identification kit (Difco Laboratories) were used as the reference methods to identify *Salmonella* species. All media were prepared and used in accordance with the manufacturer's descriptions.

As shown in Table 1, *Salmonella* spp. were isolated in 296 clinical cultures from 1,588 specimens (18.6%). Only 89 positive cases (5.6%) were isolated by using the direct-plating method. The additional 207 positive cases (13%) were isolated by using SBG broth as the enrichment broth. This striking result indicates that the combination of direct plating with SBG enrichment increased the isolation rate 3.3-fold. Therefore, the use of SBG broth will help increase the isolation of *Salmonella* spp. from fecal specimens.

To compare the isolation rates of *Salmonella* spp. between SBG and GN enrichment broths, another 155 clinical fecal specimens were analyzed. Since the optimal enrichment periods are 4 to 6 h for GN broth and 16 to 24 h for SBG broth, we used the incubation periods of 6 h for GN broth and 16 h for SBG broth. The results shown in Table 2 indicate that the isolation rates of *Salmonella* spp. are 10.3% for SBG broth and 3.8% for GN broth. This result reveals that the isolation rate of *Salmonella* spp. is higher with SBG enrichment broth than with GN broth. To clarify the effect of sodium selenite on the isolation rate of *Salmonella* spp., sodium selenite was added to GN broth as the supplement. Ninety-four clinical specimens were used to examine the isolation rate of *Salmonella* spp. The results in Table 3 show that the isolation rate is higher with GN broth supplemented with sodium selenite than with GN broth alone. However, both GN broth and GN broth supplemented with sodium selenite have lower isolation rates than that of SBG broth.

TABLE 2. Efficacy of enrichment broths for the isolation of *Salmonella* spp.

Method or culture medium[a]	*Salmonella* spp. isolated	
	No.	%
Direct plating onto XLD agar	1	0.6
GN broth-XLD agar	6	3.8
SBG broth-XLD agar	16	10.3
Total	17	10.9

[a] A total of 155 clinical fecal specimens were analyzed for each method.

TABLE 3. Effect of GN broth supplemented with sodium selenite on the isolation rate of *Salmonella* spp.

Method or culture medium[a]	*Salmonella* spp. isolated	
	No.	%
Direct plating onto XLD agar	0	0
GN broth-XLD agar	4	4.3
GN broth plus Na_2SeO_3-XLD agar	6	6.4
SBG broth-XLD agar	11	11.7
Total	11	11.7

[a] A total of 94 clinical specimens were analyzed for each method.

To examine the optimal culture periods for various enrichment broths, artificial specimens were used. Two different culture periods, 6 and 24 h, were chosen for use in subculture. The results shown in Fig. 1 indicate that GN broth, GN broth supplemented with sodium selenite, and SF broth appear to have a better outcome with the 6-h culture period than with the 24-h culture period. In contrast, SBG broth has a better outcome with the 24-h culture period. To determine the sensitivity for the isolation of *Salmonella* spp., artificial specimens were prepared and adjusted to contain various numbers of *Salmonella* spp., with a range of 1 to 10^4 CFU. The prepared specimens were then analyzed by the direct-plating method and by using GN and SBG enrichment broths. Both group B and group D *Salmonella* spp. were included in this examination. The incubation periods were 6 h for GN broth and 16 h for SBG broth. Fifteen repeats were performed. As shown in Table 4, the prepared specimens containing 10^4 CFU of *Salmonella* spp. appeared to have an isolation rate of 100% in the direct-plating method, the GN enrichment method, and the SBG enrichment method. For specimens containing 10^3 CFU of group B *Salmonella* spp., the isolation rates were 80, 80, and 100% for the direct-plating, GN enrichment, and SBG enrichment methods, respectively. For specimens containing 10^2 CFU of group B *Salmonella* spp., the isolation rates were 20, 52, and 93% for the direct-plating, GN enrichment, and SBG enrichment methods, respectively. Furthermore, the positive isolation rates were 13, 27, and 80% for the direct-plating, GN enrichment, and SBG enrichment methods, respectively, when the prepared specimens contained 10 CFU of group B *Salmonella* spp. Thus, the sensitivity for isolation of group B *Salmonella* spp. is about 100-fold higher for SBG broth than for GN broth. The results in Table 4 also reveal that the sensitivities for isolation of group D and group B *Salmonella* spp. are similar.

We evaluated SBG enrichment broth for the recovery of *Salmonella* from feces. With SBG broth as the enrichment, the isolation of *Salmonella* from clinical specimens was increased from 89 cases to 296 cases (Table 1). Thus, the efficacy of using SBG broth as the enrichment broth is 3.3-fold higher than that

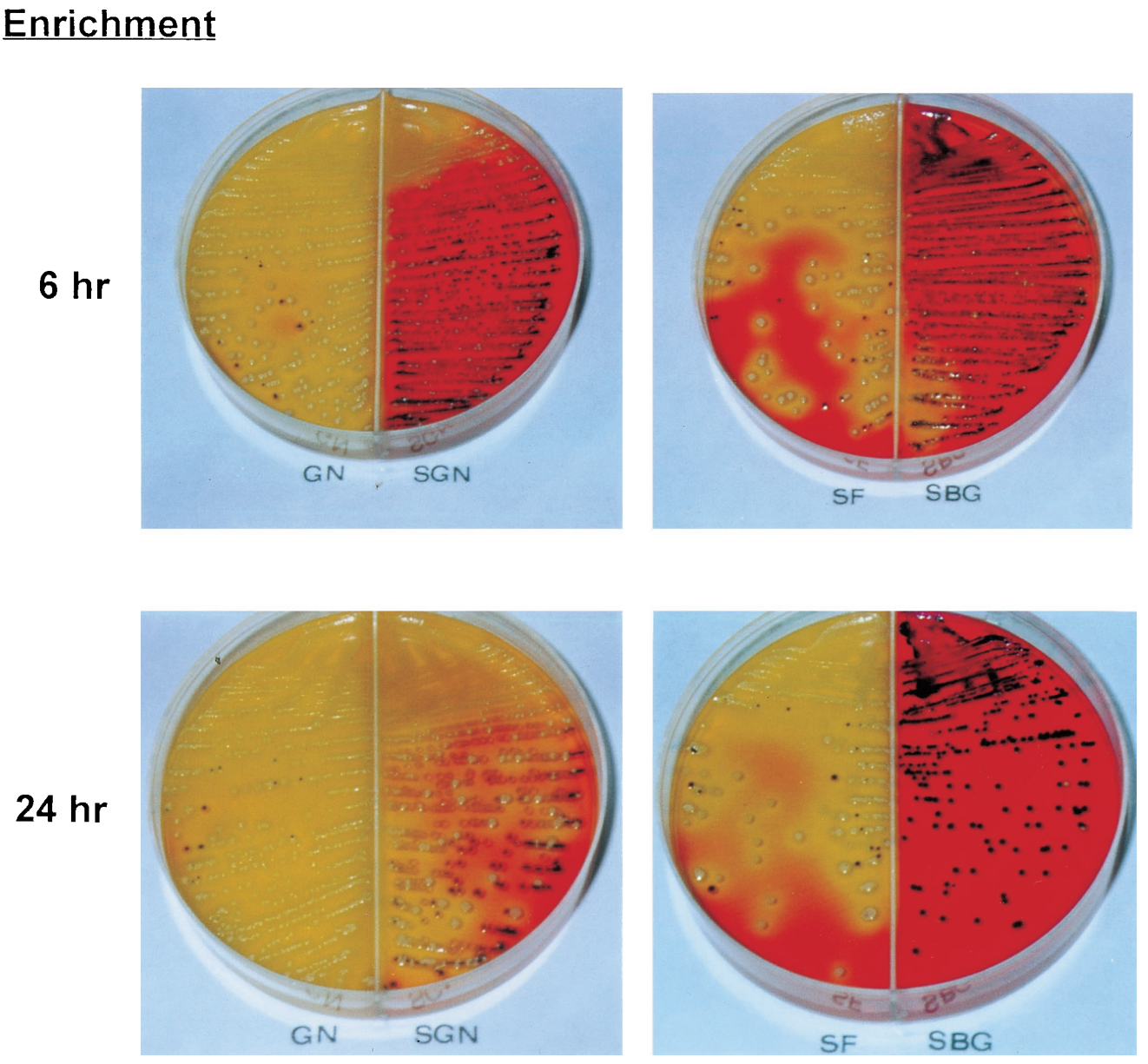

FIG. 1. Growth of *Salmonella* on XLD agar plates after enrichment. *Salmonella* spp. were incubated in GN broth, SGN broth, SF broth, and SBG broth. After either 6 or 24 h of enrichment, the cultures were inoculated onto XLD agar plates. The salmonellae appear as pink colonies with black centers. The yellow colonies are the coliforms with the characteristics of lactose-fermentative organisms.

of using the direct-plating XLD method alone. A study by Dusch and Altwegg evaluated the efficacy of enrichment using selenite broth and tetrathionate broth and found that the sensitivity for the recovery of *Salmonella* strains is higher for tetrationate broth than for selenite broth. Moreover, the sensitivity in the detection of *Salmonella* is increased by using tetrathionate at concentrations from 33 to 87% for XLT4 medium and from 34 to 79% for HE medium (2). Thus, the efficacy of using tetrathionate broth as the enrichment broth is 2.4-fold higher for the XLT4 method and 2.3-fold higher for the HE method in comparison to that of selenite broth. Therefore, we conclude that the use of SBG broth as the enrichment appears to have a higher efficacy for the recovery of *Salmonella* from fecal specimens. The sensitivities of three enrichment broths, GN broth, SGN broth, and SBG broth, in detecting *Salmonella* strains in clinical specimens were also compared. As shown in Tables 2 and 3, the results indicated that the efficacy of SBG is the highest among those of the three enrichment broths. Although the sodium selenite in SBG has the major inhibitory effect on coliforms, the method using GN broth supplemented with sodium selenite still has a lower recovery rate of *Salmonella* than the SBG broth method does. Therefore, other ingredients also contribute some effects to the recovery of *Salmonella*.

Furthermore, artificial specimens were used in evaluating the recovery of *Salmonella* by using various enrichment broths, including GN, SGN, SF, and SBG broths. The results in Fig. 1 show that of these enrichment broths, the SBG broth has the highest efficacy for the recovery of *Salmonella* spp. Obviously, this conclusion allows us to use SBG broth as the enrichment

TABLE 4. Sensitivities for recovery of group B and D *Salmonella* spp. by the direct-plating XLD, GN enrichment broth, and SBG enrichment broth methods

No. of *Salmonella* spp. (CFU)	No. of specimens (%) with recovery[a]		
	Direct plating onto XLD agar	GN broth culture for 6 h[b]	SBG broth culture for 16 h[c]
Group B			
10^4	15 (100)	15 (100)	15 (100)
10^3	12 (80)	12 (80)	15 (100)
10^2	3 (20)	8 (53)	14 (93)
10^1	2 (13)	4 (27)	12 (80)
1	0 (0)	2 (13)	7 (47)
Group D			
10^4	15 (100)	15 (100)	15 (100)
10^3	10 (67)	11 (73)	15 (100)
10^2	3 (20)	8 (53)	12 (80)
10^1	2 (13)	3 (20)	10 (67)
1	0 (0)	1 (7)	5 (33)

[a] A total of 15 specimens were analyzed.
[b] The specimens were incubated in GN enrichment broth for 6 h and then subcultured onto XLD agar plates.
[c] The specimens were incubated in SBG enrichment broth for 16 h and then subcultured onto XLD agar plates.

broth and with an appropriate overnight culture period. This point is also demonstrated by using clinical specimens, as shown in Table 1. Therefore, these results suggest that the optimal culture period and the sensitivity of the SBG broth might contribute to the high recovery rate of *Salmonella*. With artificial specimens, the sensitivity of SBG broth for the recovery of *Salmonella* from feces was also evaluated. The results shown in Table 4 indicate that the use of SBG broth permits isolation of *Salmonella* in feces even when only a small number of bacteria are present. Thus, SBG broth enrichment allows us to isolate *Salmonella* from feces only mildly infected with *Salmonella*.

In conclusion, our results indicate that SBG broth has high sensitivity and efficacy for the recovery of *Salmonella* from feces. This finding has been proven with both clinical specimens and artificial specimens. Moreover, this result suggests that SBG broth is appropriate to be used in the recovery of *Salmonella* in clinical laboratories.

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