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Isolation of Shigellae

V. Comparison of Enrichment Broths with Stools

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Many enteric media are more efficient for the detection of salmonellae than of shigellae. Comparisons of three enrichment broths and three plating media were made during analysis of 1,405 stool specimens to choose a combination of media which would enhance detection of shigellae as well. Gram-Negative (GN), Selenite, and Silliker's Broths were streaked to E M B, *Salmonella-Shigella* (SS), and xylose lysine deoxycholate (XLD) Agars. The enrichment broths produced a twofold increase in isolations of both salmonellae and shigellae over direct streaking. All three broths performed equally well for *Salmonella* detection, but GN and Silliker's produced twice as many *Shigella* isolates as did Selenite. Comparison of the plating media showed that XLD was markedly more efficient than either E M B or SS Agar for the recovery of both genera. SS Agar was superior to E M B for isolation of salmonellae after enrichment, whereas E M B was better for isolation of shigellae by direct streaking. Both E M B and SS were more effective when used after GN and Silliker's than after Selenite. GN Broth and XLD Agar were the most efficient combination of media. During these analyses, 158 salmonellae and 49 shigellae isolates were obtained.

The current prominence of the salmonellae as national and international hazards to the health of man and domestic animals has, in part, overshadowed the importance of the shigellae as causative agents of diarrheal disease. Historically, the worldwide importance of typhoid fever dominated the enteric scene during the early years of microbiology from the 1880's through the 1930's. With the decline of typhoid fever, particularly in the United States, the increase of salmonellosis, other than typhoid fever, has gradually preempted center stage. As the recognizable serotypes of salmonellae classifiable with the Kauffmann-White schema exceeded 1,000 and the incidence of disease attributable to them continues to rise, the scientific interest in the isolation and identification of salmonellae and the search for a means of control or eradication of them are imminently justifiable. However, the threat of the shigellae also exists, and in the absence of media that will readily disclose their presence in patients, the population at large, food handlers, and even in some foods, the threat may well be likened unto the iceberg which discloses only the smallest portion of its bulk and conceals the menace of its true proportions.

In a previous report (5), the sensitivities of four enrichment broths were compared for shigellae specifically. One of these, Tetrathionate Broth Base, was totally unsuitable for the isolation of shigellae and was not considered further. However, Selenite-F Broth, although found to be inimical to the growth of *Shigella flexneri* by Leifson (2), was included in the comparisons to follow because it is probably the most familiar and widely used of the traditional enteric enrichment broths in clinical microbiology.

The first enrichment broth designed (1) to meet the needs for propagation of shigellae as well as salmonellae was Gram-Negative Broth (GN). The most recent such broth was a formula proposed by Silliker et al. (3). Having previously demonstrated the abilities of these broths to support the growth of pure cultures of shigellae adequately, comparisons of their abilities to implement isolation of enteric pathogens from stool cultures were made, and the results are reported in this paper.

MATERIALS AND METHODS

During the period of 10 January to 31 August 1966, clinical specimens collected from the population of southern Louisiana were used in this study. Three plating media were inoculated by swab and streaked by loop for isolation. Since three enrichment media were being compared, each stool specimen was used to inoculate three swabs with approximately the same amount and kind of inoculum to be added to each of

the enrichment broths. When rectal swabs were ordered, triplicate swabs were requested on the patient. In the rare cases in which only two such were obtained, Selenite and Silliker's Broths received them, and GN Broth was decanted directly into the carrier tube and incubated. The enrichment broths compared were GN Broth (Difco), Selenite-F Broth (Difco), and Silliker's broth made according to his formula. The plating media were Eosin Methylene Blue Agar (E M B, Difco), *Salmonella-Shigella* Agar (SS, Difco), and xylose lysine deoxycholate agar (XLD) made according to the previously published instructions (4). Biochemical and serological identification of enteric pathogens was performed as previously described (6), with frequent confirmation of serotypes by the State of Louisiana Public Health Laboratories at Lafayette and New Orleans.

RESULTS AND DISCUSSION

The results given in Table 1 represent 1,405 stool specimens, inoculated in replicate into three different enrichment broths and streaked to three plating media for a total of 16,860 plates observed for the occurrence of enteric pathogens.

Only one-half the number of salmonellae and shigellae were detected by direct streaking, as compared to enrichment broths. While this pattern has been long established for recovery of salmonellae, the most effective isolation of shigellae has usually been achieved by direct streaking. The excellent performance of Selenite and Tetrathionate Broths with the salmonellae for which they were formulated has only recently been challenged by the newer media, such as GN and Silliker's; in this series of analyses, the Selenite proved to be a poor third to both the newer media not only for isolation of shigellae, which could have been predicted (2, 5, 7), but also for a relatively poor performance with salmonellae. It was observed that if it had not been for XLD, the Selenite series would have found only 49 and 73 of the 139 salmonellae isolates on E M B and SS, respectively (Table 1). By contrast, E M B and SS detected 76 and 115 of 143 salmonellae from GN broth and 76 and 107 of 141 from Silliker's. Thus, Selenite found only 64% and 67% of the salmonellae detected on the traditional E M B and SS media, as compared to GN and Silliker's. It is probable that only the ability of XLD to detect small populations of salmonellae caused the Selenite to even approximate the number of successful detections produced by the other broths.

The total number of positive plates observed in each category again indicates the shortcomings of Selenite with regard to salmonellae and also its lack of suitability for shigellae to a far greater degree (Table 2).

The total of 38 shigella-positive plates represents only 32% of the GN, 37% of Silliker's, and 67% of the direct streaking isolates.

The newer enrichment broths have brought the same degree of efficacy to shigellae detection that previously was obtained only for salmonellae (Tables 1 and 2). Selenite, while roughly comparable to those broths in recovery of salmonellae, produced only one-half their shigellae and only the same number as by direct streaking, even with the aid of XLD agar.

The role of the plating media in summary form is given in Table 3.

The superiority of XLD over SS and E M B is obvious. For salmonellae, SS is more effective than E M B, producing 46% more isolations. However, E M B detected 37% more shigellae than did SS plates. XLD produced 45% more salmonellae positives than did SS and 33% more shigellae than E M B. It may be observed that if the traditionally employed method of direct streaking onto E M B and SS Agars and enrichment with Selenite Broth had been employed in these analyses, one would have detected at most 73 (45%) of the 158 salmonellae isolates (Sele-

TABLE 1. *Isolation of enteric pathogens from 1,405 replicate stool specimens*

Organism	Direct			Indirect									Subtotal	Total
				GN Broth			Selenite-F Broth			Silliker's broth				
	EMB	XLD	SS	EMB	XLD	SS	EMB	XLD	SS	EMB	XLD	SS		
Salmonella.....	34	78[a]/80	48	76	141/143	115	49	138/139	73	76	140/141	107	158	158
Per cent.....	42.5	97.5	60.0	53.2	98.6	80.4	35.3	99.3	52.5	53.9	99.3	75.9		
Shigella.....	21	24/24	12	41	46/47	31	8	23/24	7	33	44/47	25	49	49
Per cent.....	87.5	100.0	50.0	87.2	97.9	65.9	33.3	95.8	29.2	70.2	93.6	53.2		
Total, per cent..	52.9	98.1	57.7	61.6	98.4	76.8	34.9	98.8	49.1	57.9	97.9	70.2	100	100

[a] Number positive on this medium per total number positive by all three media in this category.

nite and SS) and 21 (43%) of the 49 shigellae (E M B, direct streaking). In contrast, XLD direct streaking alone proved superior by finding 78 salmonellae and 24 shigellae.

The distribution of the shigellae isolates is presented in Table 4. The 49 shigellae isolated represented strains from 45 different patients. There were 33 (73.3%) strains of *S. boydii*, 10 (22.2%) of *S. sonnei*, and 1 each of *S. dysenteriae* and *S. flexneri*. As in the preceding report (6), the high rate of isolation of *S. boydii* strains is unexplained because it varies greatly from both regional and national rates of occurrence for this group.

TABLE 2. *Performance of enrichment broths in the isolation of enteric pathogens*

Organism	Direct	Indirect		
		GN broth	Selenite broth	Silliker's broth
Salmonella	160[a]	332	260	323
Shigella	57	118	38	102
Total	217	450	298	425

[a] Total number of plates showing pathogens.

TABLE 3. *Performance of plating media in the isolation of enteric pathogens*

Organism	EMB	XLD	SS	Total
Salmonella	235[a]	497	343	503[b]
Per cent	(47)	(99)	(68)	—
Shigella	103	137	75	142
Per cent	(73)	(96)	(53)	—
Total	338	634	418	645
Per cent	(52)	(98)	(65)	—

[a] Aggregate of plates from direct and all three enrichment broth platings.

[b] Maximal isolations per specimen from all media used.

ADDENDUM

Subsequent to the study reported here, the widespread use of XLD agar from commercially prepared dehydrated media has occurred. In addition, XLD poured plates are now offered by many different manufacturers of media for use by clinical and food laboratories. With greater numbers of varieties of XLD now available, I have received an increased number of communications indicating that not all of these products are performing up to expectations.

All of the data reported in this series of publications to date and those in press have been obtained on XLD agar made from the components (4) or from commercially prepared XL Agar Base (BBL, Difco) to which the heat-labile solutions were added after autoclaving and cooling the base. Under these conditions, there are minimal opportunities for the abuse of this medium, and I am quite willing to stake my reputation on the performance of the finished plating medium.

By contrast, the dehydrated complete XLD agar, in which all of the ingredients are included for convenience in a heat and pour formulation, appears quite easily damaged by overheating and thus subject to considerable abuse. Most of the unfavorable comments received have been caused by untoward results with these media, such as the swarming of *Proteus* (indicative of the destruction of most of the deoxycholate inhibitor system) or failure to blacken by well-isolated *Salmonella* colonies (suggesting oxidation of the thiosulfate to stable sulfates). Under these circumstances, I cannot recommend the use of these media.

Various methods of making finished plates of XLD agar are being used by the different companies in this field. I am not knowledgeable of their manufacturing policies. In only one case have I been able to suggest to a neophyte in this field (who was just about to sell plates which he had made from the dehydrated complete XLD medium to several hospitals) that he destroy them and make new media from XL

TABLE 4. *Distribution of Shigella isolates*

Organism	Direct			Indirect									Total
				GN			Selenite			Silliker's			
	EMB	XLD	SS	EMB	XLD	SS	EMB	XLD	SS	EMB	XLD	SS	
S. dysenteriae	1	1[a]/1	0	1	1	1	1	1	0	1	1	1	1
S. flexneri	1	1/1	1	1	1	1	1	1	0	1	1	1	1
S. boydii	16	17/17	9	33	35	26	6	17	5	25	33	20	36
S. sonnei	3	5/5	2	6	9	3	0	4	2	6	9	3	11
Total	21	24/24	12	41	46/47	31	8	23/24	7	33	44/47	25	49

[a] Number positive on this medium per total number positive by all three media in this category.

Agar Base with the appropriate additions. Since it is not apparent from the appearance of the finished plate that it is or is not lacking in the selectivity necessary for the best performance of the medium as originally formulated, I can place no confidence in the purchased media when the method of manufacture is unknown.

In essence then, the published method for making XLD, amended to permit the use of XL Agar Base, made as directed and with the additions noted (4) constitutes the only valid and reliable form of XLD agar that I recommend.

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