

Isolation of Shigellae

VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue Agar with Stool Specimens

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Two enrichment broths and four plating media were compared for efficiency of detection of enteric pathogens from 1,597 stool specimens. Of 170 salmonellae isolated from the composite of all methods, direct streaking yielded but 54%, whereas enrichment in gram-negative broth found 87% and Selenite-F broth 97%. By contrast, gram-negative broth produced 100% of the 17 shigellae, Selenite-F broth but 77%, and direct streaking only 59%. Thus, enrichment methods produced almost twice the number of both pathogens as direct streaking. Comparison of the plating media revealed xylose lysine deoxycholate agar (XLD) and Hektoen enteric agar to be equal in their abilities to find both pathogens. Both were moderately better than Salmonella-Shigella agar and markedly superior to eosin methylene blue agar. XLD found 83% of salmonellae produced by the composite of four media and 90% of the shigellae. Hektoen enteric agar found 80% of both. Salmonella-Shigella agar detected 74 and 68%, respectively, and eosin methylene blue agar only 42 and 63%. The numbers of false positives accruing to each medium, however, showed Hektoen enteric and Salmonella-Shigella agars to produce more than twice as many false-positive plates as XLD. Similarly, Selenite-F broth resulted in many more false-positives for all plating media than did gram-negative broth. Consequently, the index of validity, which equates successful isolation of pathogens with total pickings, favored XLD and gram-negative broth as the media of choice, with direct streaking the poorest method by all counts.

The traditional methodology for detection of enteric pathogens invariably employs a combination of media which includes a differential plating medium, a selective plate, and an enrichment broth. The need for more than one plate has been dictated by the knowledge that if a plate is highly inhibitory to some members of the *Enterobacteriaceae*, i.e., the coliforms, there is a concomitant loss of sensitivity for the fastidious pathogens such as the shigellae. Therefore plating media embodying the extremes of sensitivity and selectivity have been chosen for the methodology. In theory, however, if one plate combined both of these qualities to the degree possessed by the two dissimilar media, a reduction in media could be effected without loss of accuracy.

Two plating media of recent origin have attempted to retain the sensitivity necessary for the less hardy pathogens and yet be selective enough to prevent overgrowth by the coliform majority. As such, these media are intermediate

in their selectivity by comparison with the traditional plating media. One of the media, xylose lysine deoxycholate (XLD; 10) agar has been compared to the conventional enteric plates in previous papers of this series (11-14) and by others (7-9). The newest such medium, Hektoen enteric (HE; 5) agar, is very similar to XLD in its inhibitor system, but resembles more closely the traditional plating media in its differential system. In this study routine stool specimens have been used to compare XLD and HE with each other as well as with the most widely used traditional plating media of both differential and selective types.

MATERIALS AND METHODS

From 4 February through 21 November 1969, a total of 1,597 stool specimens from the population of southern Louisiana was analyzed for salmonellae and shigellae. The efficacies of two enrichment broths and four plating media were compared in the course of

TABLE 1. Distribution of enteric pathogens isolated from 1,597 stool specimens

Organism	Direct				Indirect								Sub-total	Total
					GN broth				SF broth					
	XLD	HE	SS	EMB	XLD	HE	SS	EMB	XLD	HE	SS	EMB		
Salmonella	74/92 ^a (80) ^b	63 (68)	54 (59)	33 (36)	123/147 (84)	119 (81)	115 (78)	59 (40)	137/165 (83)	141 (85)	130 (79)	76 (46)	170	170
Shigella	8/10 (80)	8 (80)	9 (90)	8 (80)	17/17 (100)	14 (82)	11 (65)	10 (59)	11/13 (85)	10 (77)	7 (54)	7 (54)	17	17
Total	82/102 (80)	71 (70)	63 (62)	41 (40)	140/164 (85)	133 (81)	126 (77)	69 (42)	148/178 (83)	151 (85)	137 (77)	83 (47)	187	187
False-positives	127 (61)	281 (80)	208 (77)	112 (73)	113 (45)	264 (66)	256 (67)	78 (53)	161 (52)	443 (75)	421 (75)	146 (64)		

^a Number of positives on this medium per total number positive on all four media in this category.

^b Number in parentheses indicate percentages.

TABLE 2. Total of plates positive for pathogens after enrichment

Organism	Direct streaking	Enrichment broths	
		GN ^a	SF ^b
Salmonella	224	416	484
Shigella	33	52	35

^a Gram-negative broth.

^b Selenite F broth.

these analyses. Emulsified stools were streaked directly onto the four plates and into the enrichment broths, Selenite-F (SF; BBL) and gram-negative (GN; BBL). After 18 hr of incubation, the broths were streaked onto Salmonella-Shigella agar (SS; Difco), Levine eosin methylene blue (EMB; Difco) agar, HE (Pfizer) agar, and XLD (XL Agar Base; BBL) agar, made in accordance with the original published directions (10) for the reasons discussed in detail in another paper of this series (13, Addendum). Salmonellae and shigellae isolated were identified by biochemical and serological methods and many were submitted to the State of Louisiana Public Health Laboratories for identification of serotypes.

RESULTS

The distribution of salmonellae and shigellae on the 19,164 plates analyzed in these comparisons are shown in Table 1. No one plate or broth resulted in the detection of all of the 170 salmonellae isolated; however, the indirect method found all of them after enrichment in one or the other of the two broths. By contrast, all of the 17 shigellae were isolated on XLD agar after enrichment in GN broth. Direct streaking of stools to four plates yielded only 92 of 170 salmonellae, or 54%. Similarly, only 10 of 17 shigellae (59%)

TABLE 3. Total isolations of enteric pathogens on plating media

Organism	Plating medium ^a				Total
	XLD	HE	SS	EMB	
Salmonella	334 (83) ^b	323 (80)	299 (74)	168 (42)	404
Shigella	36 (90)	32 (80)	27 (68)	25 (63)	40
Total	370 (83)	355 (80)	326 (73)	193 (43)	444
False positives	403 (52)	988 (74)	835 (72)	336 (64)	

^a Abbreviations: XLD, xylose lysine deoxycholate; HE, Hektoen enteric; SS, Salmonella-Shigella; EMB, eosin methylene blue.

^b Figures in parentheses indicate percentages.

were found. Enrichment broths proved more effective for all pathogens, GN having found 87% of the salmonellae and 100% of shigellae, and SF produced 97 and 76%, respectively.

Selenite was slightly more effective than GN in the isolation of salmonellae, producing 16% more positive plates than GN (Table 2). Selenite resulted in 116% more salmonellae plates than direct streaking, but a negligible 6% more shigellae plates. GN produced 86% more salmonellae plates and 58% more shigellae plates than direct streaking, again attesting to the previously reported efficacy of GN in increasing shigellae isolations (11-14).

XLD agar was observed to produce more positive plates for both enteric pathogens than the other media (Table 3). For salmonellae, XLD yielded 3% more than HE, 12% more than

SS, and 99% more than EMB. XLD produced 13% more shigellae-positive plates than HE, 33% more than SS and 44% more than EMB.

The distribution of false-positive colonies picked from plating media is shown in Table 4. These organisms were identified at least to their genera and in the case of protei, speciated. In the category of lactose slow-fermenters were the slow-fermenters of the genera *Escherichia*, *Klebsiella*, and *Enterobacter*, the lactose-negative *Serratia* and *Aeromonas* species, and the nonfermentative gram-negative rods which occurred too infre-

quently to be worthy of separation for statistical purposes, such as *Alcaligenes*, *Mima*, and *Herellea*. *Citrobacter freundii* included the lactose-slow-fermenters formerly designated the Bethesda-Ballerup group. The H₂S-positive protei were predominantly *Proteus mirabilis* and the H₂S-negative were almost entirely *P. morganii*. Pseudomonads were not speciated.

In Fig. 1, a graphical presentation of the index of validity is shown. The index is formulated to equate both the successful isolations of a medium and its failures, which all too often are not con-

TABLE 4. Distribution of false positives on plating media

Organisms	Plating medium ^a			
	XLD	EMB	SS	HE
Lactose slow-fermenters ^b	103 (25.4) ^c	146 (44.5)	233 (27.0)	225 (22.8)
<i>Citrobacter freundii</i>	27 (6.7)	10 (3.0)	82 (9.5)	361 (36.5)
<i>Proteus morganii/retzgeri</i>	13 (3.2)	11 (3.4)	56 (6.5)	39 (3.9)
<i>Proteus vulgaris/mirabilis</i>	122 (30.1)	83 (25.3)	359 (41.6)	244 (24.7)
<i>Pseudomonas</i> species	140 (34.6)	78 (23.8)	134 (15.5)	120 (12.1)

^a See footnote a of Table 3.

^b See text for genera included.

^c Figures in parentheses indicate percentages.

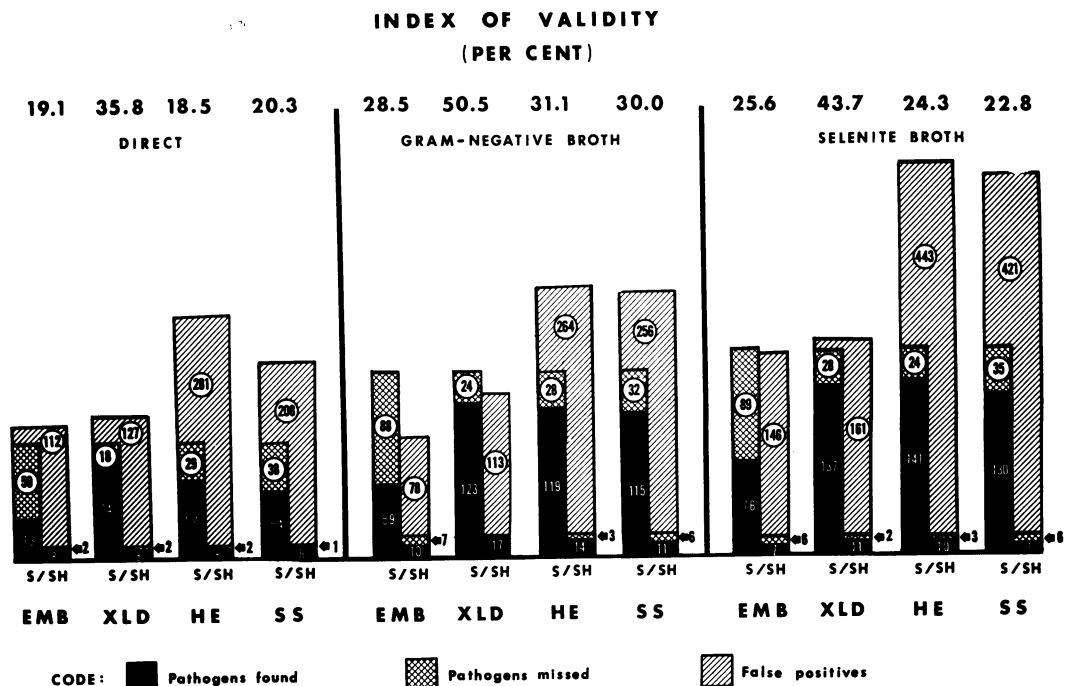


FIG. 1. Comparison of four plating media for the isolation of *Salmonella* (S) and *Shigella* (SH) from 1,597 stools. Abbreviations: EMB, eosin methylene blue; XLD, xylose lysine deoxycholate; HE, Hektoen enteric; SS, *Salmonella-Shigella*.

sidered. It is obtained by dividing the pathogens found by the sum of the pathogens, both found and missed, plus the false positives incurred. It was discussed more fully in a prior study in this series in which this concept was introduced (12). Although EMB produced the least false-positive plates (Fig. 1), it also yielded the fewest successful isolations, consequently only one-fifth of its plates were valid on direct streaking and but one-fourth after SF. The greater productivity in positive plates by XLD was accompanied by the lowest percentage of false positives. By contrast, HE, which was a very close second to XLD in successful isolations, produced the greatest number of false positives, 18% more than SS, 145% more than XLD, and 194% more than EMB. Direct streaking is shown to be the least efficient method, not only for its low productivity of positives but also for the lowest indexes of validity for all four plating media. By comparison, SF with the highest yield of salmonellae also contributed the most false positives and consequently, according to the index, proved to be less efficient than GN broth.

DISCUSSION

HE agar is the most recently formulated selective agar (5). It was reported as superior to SS and EMB (4, 6) before this study, which confirms those observations. Similarly, XLD has been found by others (1, 4, 7-9) as well as in this series (10-14) to equal the best performances of traditional plating media for salmonellae and to be superior to them for shigellae. Isenberg et al. (4) reported both media to be more efficient than the conventional plating media and too similar in efficacy to choose between them. In this study, in the detection of salmonellae and shigellae, no statistically significant difference could be found between XLD and HE agars. Both were observed to be moderately more effective than SS for both pathogens and markedly more so for salmonellae than the nonselective EMB. XLD was significantly better than EMB and SS for shigellae.

The major difference between XLD and HE lies in the occurrence of false positives, colonies indistinguishable in appearance from those of salmonellae and shigellae which, when picked, were subsequently revealed to be of no consequence. Table 3 and Fig. 1 show that 1,343 HE plates were picked to find a few less pathogens (355 positive plates) than XLD found (370) from 773 plates picked. The 988 false-positive plates from HE comprise 74%; three-fourths of the plates picked were unrewarding. By contrast, the 403 false positives on XLD were 52%, so that half were productive. Because of the number of

false positives, HE resembles SS in that respect more than XLD.

The advantage of XLD over the other selective media, SS and HE, probably lies in its more discriminating differential system. That system was discussed in detail previously (10), but in the most simple terms xylose is fermented by a great many more members of *Enterobacteriaceae* than is salicin. Thus all salicin-negative, lactose/sucrose slow-fermenters are a source of false positives on HE agar. Most of those organisms are rapid fermenters of xylose and subsequently are not false positives on XLD. SS agar, having neither salicin nor xylose as differential characters, is dependent upon its greater inhibitory system.

It is an incontrovertible fact that the picking of suitable colonies is dependent upon the skill and training of the technologist and his familiarity with the medium. Since, for example, all four plating media tested utilize carbohydrate fermentations as differential criteria, a nonfermentative organism such as *Pseudomonas* will appear similar to the salmonellae and shigellae on all of the media. Then, whether a well-trained technologist is able to discern aberrant colony characteristics which will distinguish the *Pseudomonas* colony from the *Shigella* does not accrue to the credit of the medium, but only to the skill of the user. In this study, as in others of this series, the skills of the technologists have been deliberately minimized to remain as objective as possible in the picking of colonies from all plates, so that long familiarity with some media might not weigh as heavily in their favor, and to put the onus solely on the formulae being tested. For instance, certain false positives would be known or suspected by their odor (*Proteus*, *Pseudomonas*, *Citrobacter*) on the media, or consistency, or size of black centers in the colonies, etc. It was required that these markers be ignored insofar as they were the result of acquired skills with the media and not formula characteristics.

When there is a legitimate biochemical differential marker incorporated in the medium, the type and number of false positives picked will be proscribed by that marker. Thus, whether sucrose-fermenting *Serratia* will appear identical to lactose-fermenting *Escherichia* on EMB, which contains both lactose and sucrose, or will mimic lactose-negative salmonellae and shigellae on Levine EMB agar, containing only lactose, is determined by the inclusion or exclusion of sucrose, irrespective of technologist skills.

Just as the differential system is a factor, so is the degree of accommodation to the inhibitor system of a given medium a factor in the determination of the false positive. The combination

of differential and selective elements in this study may best be illustrated in the occurrence of *Citrobacter* as a false positive (Table 4). *Citrobacter* is not greatly inhibited by EMB, HE, or XLD agars, but SS agar is very inhibitory to them; consequently, they are among the least frequent false positives on SS (9.5%). On EMB, *Citrobacter* simply appears to be overgrown by *Escherichia/Enterobacter* so that they are not evident unless their numbers are very large. XLD is not very inhibitory to *Citrobacter*, but the differential system causes them to produce yellow colonies and mimic the coliforms because even the lactose slow-fermenters are xylose-positive and lysine-negative (6.7%). HE agar, however, has neither the growth inhibitors nor the differential system to distinguish *Citrobacter* from *Salmonella* as both may be lactose-, sucrose-, and salicin-negative and H₂S-positive; thus *Citrobacter* becomes the most frequent false positive (36.5%) on HE.

Each of the four media predisposes a different organism as its most numerous false positive. Just as HE failed to inhibit or differentiate *Citrobacter*, SS failed to do either to *P. mirabilis* (41.6%). EMB found the lactose slow-fermenters to be the greatest source of false positives (44.5%), since it lacks selectivity. XLD, dependent upon xylose fermentation to screen the *Enterobacteriaceae*, is ineffectual against nonfermenters such as pseudomonads (34.6%). Numerically, *P. mirabilis* was seen to be the most commonly occurring false positive on the four media, yet it should be noted that it was not greatly apparent on the direct plates. After enrichment broths, however, protei and pseudomonads, both of which grew luxuriantly in GN and SF, predominated as false positives. *P. morganii* and *P. rettgeri* colonies simply fit the description of shigellae on all four plates, and it is fortunate that their occurrence is not more common.

The existence of the false positive indicates a more serious shortcoming of the methodology for detection of salmonellae and shigellae than just picking an analytical artifact. Hentges has demonstrated and amply documented the deleterious effects of normal intestinal flora on the concomitant growth of shigellae (2, 3), which is borne out in the experience of others who have noted the rapid disappearance of shigellae from mixed cultures (4) or feces (8). The profuse growth of protei and pseudomonads in enrichment broths must certainly be instrumental in decreasing the efficacy of media purporting to facilitate growth and enhance the detection of shigellae. It is apparent that, whereas the total

isolations of salmonellae and shigellae are significantly increased by enrichment broths, there is a similar increase in the occurrence of false positives (Fig. 1). It appears that the greatest need for definitive improvement in stool methodology lies in the formulation of a more selective enrichment broth which will still not inhibit the most fastidious salmonellae and shigellae strains.

The index of validity (Fig. 1) is the ratio of the actual isolation of stool pathogens to the apparent isolation of them. The index, expressed as a percentage of successful positive isolations, thus indicates the degree of confidence which may be placed upon the performance of media compared in these trials. From the practical viewpoint of the clinical laboratory, the value of the index is greatest when, as occurs here, two media seem to be equal in ability but one requires twice as many pickings to find the same number of pathogens as the other, which enables one to obtain comparative efficiencies of those media. Since each false positive involves inoculation of additional identification media, and thus a delay in the final report to the physician, such comparisons are of more than just academic value.

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