Comparison of Media for Isolation of Salmonellae and Shigellae from Fecal Specimens

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Five transport media, eight plating media, and three enrichment broth media for the isolation of salmonellae and shigellae were evaluated. Eight laboratories in widely separated regions of the United States participated in this evaluation by submitting 490 fecal specimens in the transport media provided. The results suggest that the newer transport media may not offer any advantage over the use of buffered glycerol-saline in the isolation of these enteric pathogens. Shigellae were best isolated by direct inoculation, whereas salmonellae were isolated in greater numbers after tetraionate (without Brilliant Green) enrichment with subsequent culturing on the plating medium. The use of a variety of plating media is recommended for the recovery of a larger number of these enteric pathogens.

Dependability in recovery of pathogens such as salmonellae and shigellae from fecal or nonfecal specimens is an important problem for all bacteriology laboratories. Yet, meaningful comparative data on the various types of media available are lacking. Individual enrichment and plating media have been investigated in numerous studies (16, 18, 19, 25, 27, 32–36); however, they were not studied in a directly comparable manner, and often they were studied with artificial pathogen mixtures rather than natural specimens.

In attempts to achieve optimal methods of transfer and isolation of suspected salmonellae and shigellae, five transport, three enrichment, and eight plating media have been evaluated. This paper describes the results of this evaluation.

MATERIALS AND METHODS

Processing of specimens. Eight strategically located state and hospital laboratories cooperated in this evaluation: the state health department laboratories in California, Florida, Montana, Kansas, Michigan, and Texas and the hospital laboratories at the Cincinnati Children's Hospital and Atlanta's Grady Memorial Hospital. Each laboratory was asked to send 8 to 10 enteric specimens per month until the study was terminated.

The study continued from January 1969 until June 1970, and 490 specimens were evaluated during this period. In addition, 15 stool specimens obtained from the marmoset colonies of the Ecological Investigations Program, Center for Disease Control (CDC), Phoenix, Ariz., were included in this evaluation.

Specimens were selected from the normal workload, attempting if possible to favor positive specimens. However, in the interest of freshness, they were sent before bacteriological examination was carried out.

Five transport media were used: buffered glycerol-saline (28, 36), Stuart's (30) ethylenediaminetetraacetic acid (EDTA) in glycerol-saline solution (29), Cary and Blair's (5), and Amies (1). Each laboratory was to choose at random from unlabeled, coded vials the transport medium provided (with instructions for use) for each specimen submitted. However, it should be noted that several of the participating laboratories received their specimens originally in buffered glycerol-saline. These same specimens were subsequently forwarded in this transport medium rather than subinoculated into another medium. Thus, buffered glycerol-saline was used more often for transport than the other carrier media. Isolation and subsequent identification were carried out in both the participating laboratories and in our laboratory. Each participating laboratory was considered the source of reference in determining which specimens contained salmonellae or shigellae before shipment.

Laboratory studies. To expedite the large volume of work resulting from the use of a battery of media, two groups of plating media were alternated in bimonthly cycles. Those in the first group (group 1) were Brilliant Green (BG), deoxycholate (DC), eosin-methylene blue (EMB), and xylose-lysine-deoxycholate (XLD) agars. The second group (group 2) consisted of bismuth sulfite, Hektoen enteric (HE), MacConkey's, and Salmonella-Shigella (SS) agars. All media were obtained from commercial sources and were prepared by the manufacturer's directions. The
XLD plating medium was the formulation recommended by Taylor (31).

On receipt, specimens were inoculated directly onto one of the groups of plating media and into enrichment broths. The three enrichment broths, the same for both cycles of plating media, were Hajna’s gram-negative (GN; reference 13), Selenite-F (22), and tetrahionate (26; without Brilliant Green) broths. All media were appropriately incubated at 35 to 37°C; suspicious colonies were transferred to triple sugar-iron-agar and Christensen’s urea-agar. Identification, including serotyping, was carried out by the methods of Edwards and Ewing (7).

The nomenclature used in this study is based on the taxonomic system of Ewing (9). The system of nomenclature used for the genus Salmonella is that proposed by Ewing (9) and is based on the three-species concept (3, 17).

RESULTS

Because the study specimens arrived in the Enteric Bacteriology Unit by various means (mostly by airmail but some by surface mail or local pickup), there was some variation in the time interval between collection and plating. This ranged from 2 to 14 days, the median being 6 days. The median elapsed time for positive isolations was 5 days after collection, whereas the median elapsed time for specimens that were positive at the referring laboratory but negative on arrival was 8 days after collection. These observations suggest that elapsed time does have an influence on the isolation of enteric pathogens from transport media.

Of the 490 stool specimens examined by the contributing laboratories, 80 were positive for salmonellae and 86 were positive for shigellae. After shipment in the various transport media, 88 and 73%, respectively, were positive for the same organism (Table 1). The salmonellae appeared to survive well in all five transport media (Table 2). However, the numbers available for comparison, especially with both the EDTA and Amies transport media, are too small to be conclusive. The shigellae survived well in buffered glycerol-saline and less so in the other transport media (Table 2). Again, numbers available for comparison with the remaining transport media are too small to be conclusive.

With group 1 plating media, 50 strains of salmonellae were isolated from a total of 263 specimens, an isolation rate of 19.0% (Table 3). The rates of recovery on plates inoculated from tetrahionate enrichment broth were significantly greater than those for the other two broths (chi square, 0.01 level). Within this group, XLD had the highest rate of recovery and EMB had the lowest; however, these differences were not significant at the 0.05 level. XLD showed the highest percentage of recovery in each category of primary plating and enrichment. With group 2 plating media, 20 isolations of salmonellae were made from a total of 227 specimens, an isolation rate of 8.8%. Isolation rates for salmonellae did not differ significantly (at the 0.05 level) among the plating media of this group nor between primary plating and the various enrichments.

Table 4 shows the data for isolations of shigellae. With group 1 plating media, 28 isolations were made from a total of 263 specimens, an isolation rate of 10.6%. The higher rates of recovery observed on plates inoculated directly were significant at the 0.05 level. Within this group, DC had the highest rate of recovery and XLD had the lowest; these differences, however, were not significant at the 0.05 level. With group 2 plating

| TABLE 1. Summary of salmonellae and shigellae isolated from 490 stool specimens |
|-----------------------------------|------|------|------|------|------|
| Determination                     | Salmonellae | Shigellae |
|                                  | No. | Per cent* | No. | Per cent* |
| Known positive specimens shipped  |  80 |  16.5 |  86 |  17.5 |
| Specimens positive after receipt  |  70 |  14.3 |  63 |  12.9 |

* Per cent of total specimens.

| TABLE 2. Recovery of salmonellae and shigellae from transport media |
|------------------------|------|------|------|------|------|------|------|------|
| Media                  | Salmonellae | Shigellae |
|                        | Positive specimens shipped (no.) | Specimens positive on receipt | No. | Per cent |
|                        | Shigellae | Specimens positive | No. | Per cent |
| Buffered glycerol-saline | 29 | 24 | 83 |
| EDTA                   | 10 | 9 | 90 |
| Cary-Blair             | 21 | 18 | 86 |
| Stuart                 | 13 | 13 | 100 |
| Amies                  | 7 | 6 | 86 |

| Media                  | Salmonellae | Shigellae |
|                        | No. | Per cent |
|                        | Shigellae | Specimens positive on receipt |
|                        | No. | Per cent |
| Buffered glycerol-saline | 36 | 31 | 86 |
| EDTA                   | 14 | 8 | 57 |
| Cary-Blair             | 12 | 9 | 75 |
| Stuart                 | 11 | 6 | 55 |
| Amies                  | 13 | 9 | 69 |
media, 35 shigellae were isolated from 227 specimens (15.4% isolation rate). Primary plating resulted in significantly higher recovery rates (0.05 level) than did use of any of the enrichment broths. Moreover, primary plating on both HE and MacConkey media yielded the highest rates of recovery for shigellae.

Enteric pathogens isolated and identified to the species level included Shigella flexneri and S. sonnei; S. boydii and S. dysenteriae were not recovered. Serotypes of Salmonella enteritidis isolated were newport, murenchen, typhimurium, enteritidis, infantis, anatum, st. paul, blockey, and heidelberg. A few uncommon serotypes were also occasionally recovered. S. typhi was not isolated in this study.

During a representative 9-month period of this study, plating media of both groups were evaluated from the standpoint of the number of false-positive reactions obtained. The highly selective plating media such as BG and bismuth sulfite had the fewest number of false-positive colonies, whereas the less inhibitory media such as DC, HE, MacConkey, EMB, and SS had relatively large numbers of plates with false-positive colonies.

### Table 3. Isolations of salmonellae from various enrichment and plating media

| Plating mediaa | Primary plating | Enrichment broths | Total |
|----------------|-----------------|-------------------|-------|
|                | No. | Per centb | GN | No. | Per cent | Selenite | No. | Per cent | Tetrathionate | No. | Per cent |
| Group 1        |     |           | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent |
| BG             | 22  | 44        | 23  | 46      | 25  | 50      | 40  | 80      | 110 |
| DC             | 17  | 34        | 19  | 38      | 25  | 50      | 38  | 76      | 99  |
| EMB            | 15  | 30        | 16  | 32      | 25  | 50      | 34  | 68      | 90  |
| XLD            | 25  | 50        | 34  | 68      | 32  | 64      | 43  | 86      | 134 |
| Total          | 79  | 92        | 107 | 155     |     |         |     |         |     |         |
| Group 2        |     |           | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent |
| Bismuth sulfite| 12  | 60        | 10  | 50      | 16  | 80      | 13  | 65      | 51  |
| HE             | 12  | 60        | 13  | 65      | 15  | 75      | 16  | 80      | 56  |
| MacConkey      | 9   | 45        | 11  | 55      | 10  | 50      | 14  | 70      | 44  |
| SS             | 12  | 60        | 15  | 75      | 14  | 70      | 16  | 80      | 57  |
| Total          | 45  | 49        | 55  | 59      |     |         |     |         |     |         |

a Abbreviations: BG, Brilliant Green-agar; DC, deoxycholate-agar; EMB, eosin-methylene blue-agar; XLD, xylose-lysine-deoxycholate-agar; HE, Hektoen enteric agar; SS, Salmonella-Shigella agar.
b All percentages are based on the number of known positives, 50 for group 1 and 20 for group 2.

### Table 4. Isolations of shigellae from various enrichment and plating media

| Plating mediaa | Primary plating | Enrichment broths | Total |
|----------------|-----------------|-------------------|-------|
|                | No. | Per centb | GN | No. | Per cent | Selenite | No. | Per cent | Tetrathionate | No. | Per cent |
| Group 1        |     |           | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent |
| BG             | 0   | 0        | 0   | 0      | 15  | 54      | 12  | 43      | 69  |
| DC             | 24  | 86        | 18  | 64      | 14  | 50      | 10  | 36      | 65  |
| EMB            | 22  | 79        | 19  | 68      | 12  | 43      | 14  | 50      | 64  |
| XLD            | 19  | 68        | 19  | 68      | 14  | 43      | 14  | 50      | 64  |
| Total          | 65  | 56        | 36  | 41      |     |         |     |         |     |         |
| Group 2        |     |           | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent |
| Bismuth sulfite| 0   | 0        | 0   | 0      | 23  | 66      | 9   | 26      | 78  |
| HE             | 29  | 83        | 17  | 49      | 19  | 54      | 12  | 34      | 78  |
| MacConkey      | 29  | 83        | 18  | 51      | 16  | 46      | 11  | 31      | 66  |
| SS             | 21  | 60        | 18  | 51      | 16  | 46      | 11  | 31      | 66  |
| Total          | 79  | 53        | 38  | 32      |     |         |     |         |     |         |

a For abbreviations, see footnote a, Table 3.
b All percentages are based on the number of known positives, 28 for group 1 and 35 for group 2.
DISCUSSION

Since we could not evaluate all of the many different types of media available for the transport and isolation of enteric pathogens from clinical specimens, we decided to include in this study only those now in relatively wide use in clinical bacteriology laboratories. The plating media were selected from the standpoint that the categories "differential," "selective," and "highly selective" would be adequately represented.

Transport media. It is generally agreed that in suspected bacterial enteric diseases the specimen of choice is a freshly passed stool. Rectal swabs may also be used, provided that they are used properly and adequately. Specimens that for one reason or another cannot be inoculated very soon after collection should be placed in a transport medium until they can be processed in the laboratory. Although a number of transport media have been described, they all have the same basic function: to maintain the status quo of the bacterial population in a specimen as well as to prevent overgrowth of a particular population by others that may be present. Ewing (10) recently reviewed the subject of transport media and found a large volume of comparative data, but much of it was limited in scope—too few specimens, too few media, or both.

On the basis of the total number of specimens examined (490), recovery by the participating laboratories was 17.5% for shigellae and 16.4% for salmonellae, whereas in our laboratory it was 12.9 and 14.3%, respectively. These data support previous observations (7, 24) that recovery of enteric pathogens, particularly the shigellae, is much greater when the elapsed time between patient and laboratory is kept to a minimum.

Although it is well recognized by us that the majority of stool specimens were received in buffered glycerol-saline, the data on this aspect of the evaluation are presented if, for no other reason, than to show the overall efficacy in the use of transport media for the isolation of enteric pathogens. Obviously no firm conclusions are intended to be drawn from these results. However, we do feel certain aspects regarding this data are perhaps worthy of comment.

Strains of salmonellae were recovered from the five transport media used in this study with similar degrees of consistency. The rates of recovery with three of the transport media (buffered glycerol-saline, Stuart, and EDTA) agreed well with those of Ewing, McWhorter, and Montague (11), although no S. typhi was isolated in the present study. Recovery of shigellae was poorer with all five media, although buffered glycerol-saline had the highest rate. The numbers are too limited to be conclusive, but our rate of recovery of shigellae from buffered glycerol-saline is in close agreement with results recently supported by Morris et al. (25), who noted that this transport medium was superior in the isolation of shigellae. When compared to the salmonellae, the shigellae appear to be more fastidious and thus more difficult to isolate once removed from their environment.

With the aforementioned qualifications kept in mind, the results of this evaluation would seem to suggest that salmonellae will survive well in all of the five transport media tested. However, it would appear that the newer carrier media may offer no advantage over buffered glycerol-saline in the isolation of shigellae.

Plating media. The plating media which are

| Plating mediaa | Primary plating | Enrichment broths | Total |
|----------------|-----------------|-------------------|-------|
|                | No.  | Per centb | No.  | Per cent | No.  | Per cent | No.  | Per cent |
| Group 1        |      |           |      |           |      |           |      |           |
| BG             | 16   | 10        | 11   | 7         | 11   | 7         | 8    | 5         | 46   |
| DC             | 60   | 39        | 65   | 42        | 63   | 41        | 56   | 36        | 244  |
| EMB            | 46   | 30        | 36   | 23        | 39   | 25        | 29   | 19        | 150  |
| XLD            | 19   | 12        | 22   | 14        | 17   | 11        | 27   | 17        | 85   |
| Group 2        |      |           |      |           |      |           |      |           |
| Bismuth sulfite| 21   | 14        | 7    | 10        | 8    | 5         | 49   |
| HE             | 59   | 40        | 55   | 37        | 55   | 37        | 63   | 43        | 232  |
| MacConkey      | 51   | 34        | 45   | 30        | 52   | 35        | 39   | 26        | 187  |
| SS             | 57   | 39        | 71   | 48        | 60   | 41        | 69   | 47        | 257  |
| Total          | 329  | 315       | 307  | 299       |      |           |      |           |

a For abbreviations, see footnote a, Table 3.

b Percentages are based on a total of 155 specimens in group 1 and 148 specimens in group 2.
classified as "differential" or "slightly selective" are EMB (14) and MacConkey's (23). These media and Endo's (8) and Leifson's deoxycholate (21) originally were designed for the isolation of S. typhi with emphasis on detecting subclinical cases and carriers. Not until the development of DC and SS agars as plating media was there any awareness of the isolation of shigellae. The "selective" or "highly selective" media evaluated were bismuth sulfite-agar (38), BG agar (20), SS agar, and DC agar (15, 21). Both DC and SS agars generally support growth of shigellae as well as salmonellae and suppress the growth of coliform bacteria. Although highly selective, bismuth sulfite-agar is an especially effective medium for the isolation of S. typhi and members of the genus Arizona, particularly when incubated at 37°C for 48 hr. The other highly selective medium evaluated in this study, BG, is useful in the isolation of salmonellae other than S. typhi.

Included in this study were two plating media, developed relatively recently, which show particular promise in the isolation of enteric pathogens. Both of these media are selective, although HE (18, 19) has been reported to be more highly selective (16) than XLD (31). In our studies, however, XLD was more selective. Several recent studies (16, 18, 19, 25, 27, 32-35) have indicated the superiority of these two plating media over the so-called traditional plating media in the isolation of enteric pathogens, especially the shigellae.

Enrichment media. Through the years, numerous methods have been described for the isolation of enteric pathogens from feces and rectal swabs (4, 12, 22, 26). Most of the enrichment media are highly selective, and their values are based on ability to support the growth of enteric pathogens as well as to inhibit or suppress other intestinal organisms.

Salmonellae were isolated most frequently after enrichment with tetrathionate. Certain combinations with Selenite-F and GN broths compared favorably with tetrathionate in the isolation of salmonellae: GN broth with SS agar and Selenite-F broth with bismuth sulfite, HE, or SS agar. Shigellae were isolated most frequently in both plating media groups by primary plating of fecal specimens. This observation agrees with some reports by others (7, 24, 25) but is at variance with reports (6, 32, 34, 35) of greater isolation of shigellae by the use of GN broth than by direct plating. It is difficult to reconcile these differences except that perhaps the use of the transport media in this evaluation stimulated better growth of these organisms on primary plating. Furthermore, the size of the inoculum on the plating medium in the respective studies may have been a contributing factor.

With the group 1 plating media, GN broth showed the highest number of isolations, whereas with the group 2 plating media Selenite-F broth showed the greatest number of recoveries of shigellae. No doubt several variables account for this apparent discrepancy between the two groups, not the least of which would be the differences among the plating media themselves. Moreover, the possibility exists that the group 2 isolation rate of shigellae is weighted in favor of the number of S. sonnei recovered as compared to group 1. Although Selenite-F was designed only for enrichment of salmonellae, including S. typhi, from feces (22), a number of commercial sources have modified it slightly and some shigellae may be recovered after plating from it. Indeed, some investigators (2, 37) have reported that Selenite-F broth is useful in the isolation of S. sonnei.

The results of this evaluation indicate that, in the examination of a stool specimen suspected of harboring either salmonellae or shigellae, it would be desirable to plate the specimen directly on DC, EMB, HE, or MacConkey agar and also to inoculate the same specimen in tetrathionate broth for enrichment before plating on XLD or some other agar. Also desirable perhaps would be the use of more than one plating medium at each of these two steps. However, one is still faced with the possibility of missing approximately 5% of the potential isolations of shigellae by primary plating alone and approximately 10% of the potential isolations of salmonellae by using tetrathionate enrichment alone. By combining the data from both groups of plating media, one can calculate the number of isolations for both enteric pathogens that would have been missed if tetrathionate enrichment or the direct plating method had been used alone. If the former, 7 of a total of 70 isolations of salmonellae would have been missed. Similarly, if direct plating had been used alone, 4 of 63 isolations of shigellae would have been missed. An increase in these isolation rates could be expected by the respective use of GN and Selenite-F enrichment.

General comment. The data suggest several compromises that can be made in selecting media. For example, direct plating on DC, EMB, HE, or MacConkey followed by tetrathionate enrichment would limit the varieties of plating media needed and yet permit reasonable isolation of enteric pathogens. However, all four of these plating media produce a large number of false-positive colonies. The use of XLD plating medium, both for primary plating and after enrichment in tetrathionate, decreases the number of false-positive reactions. Moreover, this plating medium plus enrichment in GN broth provided the best combination for the isolation of both salmonellae and shigellae in this evaluation (overall recovery rate, 68%). However, some
shigellae ferment xylose (7) and thus may be missed.

Although enteric pathogens usually can be isolated from any one of the aforementioned plating media, it is not advisable to rely on only one plate of a single medium. The recommendation is to use at least one plate of each of the slightly selective and the highly selective media.

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