

A selective motility medium for routine isolation of *Salmonella*

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Craigie (1931) described a technique for the selection of motile organisms from a poorly motile culture by passing them through semi-solid nutrient agar in a tube containing a narrower, open-ended tube so that, after inoculation on the agar surface in the inner tube, the motile organisms moved through twice the depth of the medium to reach the outer surface. Semi-solid agar media have been used by several workers for the isolation of salmonellas by selective motility. Jones & Handley (1945) used a semi-solid medium containing cacotheline as a selective medium after initial culture of suspected material in a broth containing hydroquinone. Ino & Graber (1955) used passage through semi-solid medium in a U tube to recover *Salmonella* from cultures contaminated with *Pseudomonas aeruginosa*. Stuart & Pivnick (1965) used a modification of Rappaport's medium in 0.6% agar to isolate salmonella from faeces. They found Craigie tubes unsuitable and devised a modified U tube in which a small-bore side arm was attached to the base of a test-tube. This technique gave about one-fifth more positive cultures than did standard methods of isolation. The average time required for the spread of salmonellas other than *Salmonella typhi* through the medium, 5 cm. deep, was 1.5 days. Harvey, Mahabir & Price (1966) and Harvey & Price (1967) used passage through semi-solid nutrient agar as a method of secondary enrichment for salmonellas after culture in selenite F broth and on selective agar media. By this method, isolations from animal feeding stuffs were more than doubled.

Harper (1968) showed that salmonellas, which spread more quickly through semi-solid nutrient agar than do other faecal organisms, can be isolated from mixed cultures or from faeces by inoculation into coiled tubes, 80 cm. long, containing semi-solid nutrient agar (1.5%). There are several disadvantages in the use of these tubes in routine work: care is required in filling the tubes to avoid introducing air bubbles; the tubes are fragile; they are space-consuming in an incubator; the passage of the organism takes 2 days.

Modifications of Shigella-Salmonella (SS) medium (Rose & Kolodny, 1942), in various depths, were tested to try to devise a medium which could be used in shorter tubes and so avoid these disadvantages. A successful medium should select the organism after passage through a much shorter length of tube; the passage of salmonella should be complete after overnight culture; the passage of other

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organisms should be retarded and salmonella should be obtained in pure culture for several days after inoculation.

MATERIALS AND METHODS

Craigie tubes

Media were prepared containing 0.2% agar (Oxoid no. 3) and distributed in Craigie tubes consisting of $\frac{3}{4} \times 6$ in. test-tubes with a central open-ended tube, of 5 mm. internal diameter, projecting about 2 cm. above the surface of the medium. The upper end of the central tube was widened so that it could be inoculated easily with little risk of contamination of the outer surface. The central tube was inoculated and, when the culture was seen to have spread to the outer surface, subcultures from this were made on to MacConkey agar plates.

Selenite F medium

Media

This was prepared as described by Leifson (1936).

Shigella-Salmonella (SS) medium

For direct plating of specimens, this was prepared as described by Rose & Kolodny (1942), except that ferric ammonium citrate was substituted for ferric citrate, the former being more easily soluble.

For use in Craigie tubes certain modifications were made as a result of extensive trials. Twenty to fifty tubes of each batch of medium prepared were inoculated with normal faeces and with faeces contaminated with *Salmonella* by adding a drop of broth culture of *S. typhimurium* to 5 ml. of emulsified faeces, or with mixtures of *Escherichia coli* and *S. typhimurium*. Faeces known to contain salmonella were also used when available. Several strains of *E. coli* and two strains of *S. typhimurium* were used in these experiments.

Modifications of SS medium

Lactose. This was omitted to avoid bubble formation in the medium. Neutral red was therefore unnecessary, and was also omitted.

Brilliant green. An increase of the brilliant green content retarded the spread of all organisms.

The presence or absence of the normal quantity of brilliant green in the medium made no difference to the speed of spread of salmonella or of *E. coli* through the medium. Brilliant green was therefore omitted.

Bile salts. The presence of bile salts in the medium retarded the spread of both *E. coli* and salmonella but this effect was greater on *E. coli*. Doubling the normal quantity of bile salts considerably retarded the spread of *E. coli* while only slightly affecting that of salmonella. The inclusion of four times the normal amount of bile salts retarded the spread of salmonella. Three commercial brands of bile salts were used in parallel: Difco, Oxoid no. 3 and B.B.L. The results using Oxoid no. 3 bile salts were more satisfactory than those using the Difco product as salmonella was more reliably isolated from overnight cultures. Difco bile salts retarded the spread of other organisms more efficiently, but also tended to retard the spread of

salmonella. B.B.L. bile salts retarded the spread of all organisms, including salmonella, to such an extent that this organism did not spread in 1 day through medium 5 cm. deep. In the final medium, therefore, 17 g./l. (twice the normal quantity) of Oxoid no. 3 bile salts was used.

Depth of medium. The standard depth was 10 cm. This was reduced to 8 and 5 cm. to try to decrease the time of incubation necessary to isolate salmonella. In tubes of these lesser depths, pure cultures of *E. coli* or organisms from inoculated normal faeces reached the outer surface within 2 days. When salmonella was isolated from mixed cultures it did not usually remain in pure culture on the outer surface for more than 1 day and often *E. coli* was found within 1 day. In tubes 10 cm. deep, *E. coli* took at least 3 days to complete the passage and in mixed cultures often failed to do so in 7 days. Salmonella usually completed the passage in 1 day. In the final medium a depth of 10 cm. was used.

Storage of medium. When the medium was stored in the refrigerator for a few hours after its preparation, it appeared to be firm, but when it was inoculated on the same day, the inoculum could often be seen to sink down through the inner tube. In effect, therefore, the organisms had to spread through a shorter length of the medium to reach the outer surface and organisms other than salmonella completed the passage in a shorter time. When the tubes had been stored overnight in the refrigerator the inoculum remained on the surface of the inner tube. The medium was not therefore used until it had been stored for at least one night.

Final composition of SS medium for Craigie tubes

The final medium contained: beef extract (Lab Lemco paste) 5 g., proteose peptone (Oxoid) 5 g., sodium citrate 8.5 g., sodium thiosulphate 8.5 g., ferric ammonium citrate 1 g., bile salts (Oxoid No. 3) 17 g., agar (Oxoid No. 3) 2 g., distilled water to make 1 l. The instructions for its preparation were as follows: Dissolve all the ingredients, except agar, in about 500 ml. of distilled water. Cool and add sufficient distilled water to make 1 l. Adjust pH to 7.0. Add agar and bring to the boil, with constant stirring to prevent charring. Simmer with gas turned off for 1–2 min. Stir well. Dispense in Craigie tubes in 10 cm. depth. Insert wool plugs firmly. With the tubes in racks, cover the tops with a metal tray, to minimize wetting of the plugs. Autoclave at 10 lb. pressure for 10 min. Allow autoclave to cool slowly to prevent bubbling and soiling of the plugs. Store in the refrigerator for at least one night.

Appearance of cultures

The spread of a culture of salmonella through these tubes usually causes blackening of the medium, due to H₂S production. The edge of the culture can be seen advancing 3–4 cm. ahead of the blackening. Cultures of other organisms which produce blackening of the medium usually spread more slowly and there is often little or no space between the edge of the spreading culture and the blackened part of the medium. Most faecal organisms spread through the medium without causing blackening, or blackening only the upper part of the inner tube.

Occasionally salmonella strains fail to cause blackening in this medium although they produce H_2S in Kligler's medium (Kligler, 1918).

Some strains and serotypes do not produce H_2S .

RESULTS

This medium has been used in the routine examination of faeces and rectal specimens in this general hospital laboratory for about 3 years.

Rectal specimens were obtained by means of a hollow glass tube 12–13 cm. long, with an external diameter of about 4 mm., closed and rounded at one end, with an opening about 2 mm. in diameter, 2 cm. from the closed end. The rounded end is inserted into the rectum, withdrawn and placed in a test-tube containing about 2 ml. of nutrient broth. The specimen is sent as soon as possible to the laboratory, and the broth in the test-tube used as the inoculum for culture.

Specimens were examined by three methods. (1) Direct inoculation on SS plates; subculture of two pale colonies, if present, on Kligler's medium; subculture into urea medium if Kligler results suggest possible *Salmonella*; subculture for biochemical reactions and slide agglutinations if urea negative. (2) Inoculation into selenite-F medium; subculture on SS plate; procedure as above. (3) Inoculation of a few drops of emulsified faeces or of broth from a rectal specimen into the inner tube of the selective motility medium; subculture from the outer surface on MacConkey's medium; examination of pale colonies for biochemical reactions and slide agglutination. If the passage is not quite completed in 1 day, further overnight incubation can be avoided by withdrawing a drop from the advancing edge of the culture with a long Pasteur pipette.

Salmonella, other than *S. typhi*, was isolated from 300 specimens which had been fully examined by all these three methods. The specimens were obtained from 117 patients, five of whom had double infections with two salmonella serotypes. The specimens were mostly faeces and rectal specimens, but also included three pus swabs and two urines. The 122 organisms isolated from 117 patients included twenty-four serotypes. Fifty-five (45%) were *S. typhimurium*. Other serotypes isolated from more than two patients were: *S. adelaide* (6), *S. anatum* (3), *S. birkenhead* (5), *S. bovismorbificans* (7), *S. chester* (10), *S. derby* (3), *S. give* (3), *S. muenchen* (5) *S. newington* (3), *S. potsdam* (4), *S. saintpaul* (3).

The isolations of salmonellas by the three methods used are shown in Table 1. Table 2 shows the number of successful isolations by each method. While salmonellas were isolated from nearly 90% of the specimens by means of the motility medium, the other methods were successful with less than half the specimens and the combined use of these two methods isolated the organism from less than two-thirds of the specimens. By use of the selective motility medium, salmonellas were isolated from 123 specimens from which it was not isolated by either of the other methods. However, salmonellas were isolated from thirty-one specimens by one or both of the other methods when the motility tube method failed.

Isolation of *Salmonella* from faeces by passage through long tubes of semi-solid nutrient agar is not reliable when the patient has received antibiotic treatment

(Harper, 1968). This limitation does not apply to the selective motility medium. The thirty-one specimens from which salmonellas were not isolated in this medium were not all from treated patients. Some were first specimens from untreated patients. Others were from patients whose later specimens yielded salmonellas from this medium. At least half of the positive specimens were from patients who had received antibiotic treatment. One patient continued to excrete *Salmonella* for 4 months after its first isolation and had several courses of antibiotic treatment during this time. Of twenty-eight positive specimens from this patient, only one failed to yield the organism from the Craigie tube.

Table 1. *Isolation of Salmonella by three methods*

(The figures in parentheses are percentages of all positive specimens.)

| SS agar | Selenite F broth | Craigie tube | No. of specimens | Combined use of SS agar and selenite broth | Craigie tube | No. of specimens |
|---------|------------------|--------------|------------------|--|--------------|------------------|
| — | — | + | 123 | — | + | 123 (41·0) |
| + | + | + | 67 | + | + | 146 (48·7) |
| + | — | + | 52 | | | |
| — | + | + | 27 | | | |
| — | + | — | 20 | + | — | 31 (10·3) |
| + | — | — | 6 | | | |
| + | + | — | 5 | | | |
| Total | | | 300 | Total | | 300 |

Table 2. *Efficiency of three methods of isolation*

(Total positive specimens, 300. The figures in parentheses are percentages of all positive specimens.)

| | Positive | Negative |
|--|------------|----------|
| SS agar | 130 (43·3) | 170 |
| Selenite F broth | 119 (39·7) | 181 |
| Combined use of SS agar and selenite F broth | 177 (59·0) | 123 |
| Craigie tube | 269 (89·7) | 31 |

S. typhi differs from other motile salmonellas in that cultures of this organism spread much more slowly through semi-solid nutrient agar (Harper, 1968). Spread through the selective medium is also slow and the organism cannot be isolated by this method from mixed cultures of *S. typhi* and *E. coli*. During the time this medium was in routine use, *S. typhi* was isolated nineteen times from a carrier and once each from two other patients. In no case was it isolated from the motility medium.

DISCUSSION

Motile salmonellas other than *S. typhi* can be isolated from mixed cultures inoculated into Craigie tubes containing a modified Shigella-Salmonella medium in 0·2% agar.

In routine use, in this laboratory, this medium has proved to be a valuable addition to the methods used in examination of faeces for the presence of *Salmonella*.

The method is simple and involves few manipulations. Pure cultures of salmonella are usually obtained by subculture from the Craigie tubes on MacConkey agar plates after incubation for 1 day. Pure cultures can usually still be obtained after incubation for several days. This fact is useful, as results still can be obtained if the tubes are left in the incubator for an extra day owing to pressure of other work over week-ends and holidays. In the absence of salmonellas, other faecal organisms do not usually spread through the medium to the outer surface within 3 days.

The medium has been successfully used in the examination of specimens from individual patients thought to be suffering from gastro-intestinal infections.

It seems that it would also be useful as a method of screening large numbers of specimens of faeces or other material for the presence of salmonellas.

SUMMARY

A modified Shigella-Salmonella medium in semi-solid agar was shown to inhibit the spread of most faecal organisms through a Craigie tube, while selectively allowing the passage of *Salmonella*.

A medium for routine use is described.

Over a period of 3 years, in this laboratory, 90% of specimens from which salmonellas were isolated gave positive results with this medium. Over 40% of these specimens would have been reported as negative if the method had not been in use. However, 10% would have been missed if this method alone had been used. It should therefore be used in conjunction with other culture methods, of which direct plating on SS agar was the most successful in this series.

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