Technical methods

Isolation of salmonella using a standardised inoculum and a rotary plating technique

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Enrichment media for salmonella isolation require a standardised inoculum (Leifson, 1936; Rappaport *et al.*, 1956). If the optimum inoculum is not added to the enrichment medium, subculture results in an overgrowth of normal flora or insufficient growth of salmonellae. Furthermore, large particles of faeces suppress the action of sodium biselenite and in turn allow growth of normal flora. Plating of faeces directly on to culture media for salmonella and shigella isolation results in poor colony separation. It is the aim of this paper to outline a simple method for the routine culture of faeces which will overcome these problems.

Material and methods

Faeces samples from known salmonella and shigella cases, from asymptomatic excretors, and from negative patients were used. Using sterile wooden applicators, a portion of faeces about the size of a large pea was taken and a suspension was made in 4 ml of $\frac{1}{4}$ strength sterile Ringer's solution; this was shaken on a vortex mixer for 15-20 seconds, to give approximately a 25% suspension of faeces. The suspension was left for 2-3 minutes to allow any large particles to settle, and the supernatant fluid was used as the inoculum.

The optimum inoculum for solid media (DCA: Oxoid) (Hynes, 1942) was determined by trial and error, and inocula for the enrichment media were arrived at by adding increasing volumes of suspensions of faeces to enrichment media, examining the resultant subcultures. The enrichment media used in this laboratory, Selenite F (Oxoid), and Rappaport broth (Rappaport *et al.*, 1956), were inoculated with increasing volumes (1-30 drops) of faecal suspension from a Pasteur pipette calibrated to deliver approximately 30 drops per ml.

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Fig. 1 DCA plate being spread by rotary plater and nichrome wire triangle.

The DCA plates were spread using a rotary plater (Denley Instruments) modified to revolve at 150 rpm, and a nichrome wire triangle ($2 \times 2 \times 3$ cm) was fixed to a standard loop holder (Fig. 1).

The fluid was allowed to soak into the medium, and the inoculum was then pushed with the wire triangle slowly from the centre to the periphery of the plate, taking 4-5 seconds to reach the edge. The DCA plates and Rappaport and Selenite F broths were incubated at 37°C for 18 hours. After incubation the plates were examined in the usual way (Edwards and Ewing, 1972).

Enrichment media were subcultured by taking one loopful (5 mm internal diameter) of each broth on to the centre of a DCA plate. The fluid was allowed to soak into the medium and the plate was spread as before, but this time the spreader was pushed from the centre to the periphery and back again to the centre of the plate, taking in total 4-5 seconds. The plates were incubated for 18 hours at 37°C.

Results

It was found, using a Pasteur pipette to inoculate all media with the faecal suspension, that the following inocula were most suitable:

Fig. 2 DCA plates spread by (left) conventional technique and (right) rotary plating technique.

- (a) 1 drop of faecal suspension to the centre of a DCA plate;
- (b) 2-5 drops of suspension to 10 ml of Rappaport broth;
- (c) 15-25 drops of suspension to 10 ml of Selenite F.

DCA plates spread as above gave better colony separation than conventional techniques (Fig. 2). This has proved to be of particular advantage with direct plating when low numbers of shigella are present.

The inoculum for Selenite F need not necessarily be counted; with experience a volume from a Pasteur pipette may be added. Selenite F subcultures resulted in either no growth, or a heavy growth of non lactose fermenters, including salmonellae (Table 1). Rappaport broth subcultures resulted in either no growth or a moderately heavy, pure growth of salmonellae other than *Salmonella typhi* (Rappaport *et al.*, 1956) (Table 2).

It will be seen from Tables 1 and 2 that the optimal inoculum is one that will allow growth of small numbers of salmonella and suppress normal flora or allow only minimal growth of normal flora. This resulted in an improved yield and purity of salmonellae from the enrichment media.

Various media, such as bismuth sulphite, xylose lysine desoxycholate agar, brilliant green agar, and brilliant green MacConkey, have been tried, using this plating technique, but have proved

inferior to DCA because they are less inhibitory and gave very poor colony separation.

Discussion

The use of a suspension of faeces is better than unemulsified faeces as the inoculum for salmonella enrichment media (Leifson, 1936; Rappaport *et al.*, 1956). The suspension of faeces serves three purposes: firstly, a roughly standard inoculum can be added to the enrichment media; secondly, suppression of the selective properties of the broth is avoided; and, thirdly, it can be used for the primary inoculum. The suspension has the added advantage that it may be used for direct microscopy for the presence of WBCs and RBCs.

The variation in spreading technique between direct and subculture plates was determined by trial and error; the return of the spreader to the centre of the subculture plates increased colonial separation, but on direct plating this method resulted in decreased separation.

Finally, the time taken to spread the plates is much shorter by this than by conventional techniques, and is much simpler. It has been found that the completely inexperienced can be taught the technique in a matter of minutes and, with very little practice, can produce excellent, reproducible results.

Table 1 *Selenite F*

Inoculum in drops from Pasteur pipette	1	2	3	5	10	15	20	25	30
Growth of salmonella from positive faeces	+	+	+	+	++	+++	+++	+++	+++
Growth of <i>E. coli</i> , proteus, etc, from negative faeces	-	-	-	±	+	+	+	+	++

15-25 drops of faecal supernatant were found to be the best inoculum giving good recovery of small numbers of salmonella with good suppression of normal flora.

± very light growth
+ light growth
++ moderate growth
+++ heavy growth

Table 2 *Rappaport*

Inoculum in drops from Pasteur pipette	1	2	3	5	10	15	20	25	30
Growth of salmonella* from positive faeces	++	++	++	++	+++	+++	+++	+++	+++
Growth of <i>E. coli</i> , proteus, etc, from negative faeces	-	-	-	+	++	++	+++	+++	+++

2-5 drops of faecal suspension were found to be the best inoculum giving good recovery of small numbers of salmonella (other than *Salmonella typhi*) with good suppression of normal flora.

See footnote to Table 1.

*Other than *Salmonella typhi*.

References

- Edwards, P. R., and Ewing, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd edition, pp. 7-20. Burgess, Minneapolis.
- Hynes, M. (1942). Isolation of intestinal pathogens by selective media. *Journal of Pathology and Bacteriology*, **54**, 193-207.
- Leifson, E. (1936). New Selenite enrichment media for the isolation of typhoid and paratyphoid (salmonella) bacilli. *American Journal of Hygiene*, **24**, 423-432.
- Rappaport, F., Konforti, N., and Navon, B. (1956). A new enrichment medium for certain salmonellae. *Journal of Clinical Pathology*, **9**, 261-266.
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