Rapid isolation of salmonellae from faeces

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SYNOPSIS Factors and techniques leading to the more rapid isolation of salmonellae from faeces that are negative by direct plating were examined. The aim was to increase the number of specimens on which positive reports might be given within 24 hours of receipt. Salmonellae were isolated from 279 of the specimens studied.

Brilliant green MacConkey agar was found to be the most satisfactory solid selective medium both for direct plating and as a subculture medium, giving large characteristic colonies after 24 hours' incubation. Selenite F medium, inoculated with undiluted faeces, incubated at 43°C., and subcultured after six hours' incubation on to brilliant green MacConkey agar, was the most successful rapid method of enrichment, though the results were considerably inferior to those obtained after 24 hours' incubation. Seventy-six of the positive specimens examined were negative by direct plating on three different selective media; 24 (31·6%) of these were positive on subculture from selenite F incubated for six hours at 43°C. and 21 (27·6%) when incubation was at 37°C. The use of six-hour subcultures from 43°C. selenite F as a supplement to direct plating added significantly to the number of positive results. Other techniques that were tried but found to be of no practical value were dilution of the faeces before inoculation on solid and fluid media, and replica plating from one solid medium to another after a few hours' incubation.

The rapid identification of excretors of even small numbers of salmonellae is of importance when an outbreak of food poisoning occurs in a hospital or residential institution, or when the staff of a food establishment is involved. The usual routine methods for the isolation of salmonellae from faeces consist of direct inoculation of one or more selective solid culture media, together with inoculation of one or more fluid enrichment media which, after overnight incubation at 37°C., are subcultured on to selective agars. Salmonella colonies are not found on direct culture plates of a number of specimens subsequently found to be positive, and these specimens cannot be reported on until subcultures from fluid media are examined 40 or more hours after the specimens have been received.

This paper records the results of an investigation of some techniques that might facilitate the isolation of salmonellae within 24 hours from specimens that are negative by direct plating. Three solid selective media were evaluated with regard to the rapid growth of characteristic colonies. Rapid enrichment was attempted on solid media by a replica plating technique, and with fluid media by subculture after only six hours' incubation.

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MATERIALS

The faeces specimens were received during a period of one year from patients suspected of excreting salmonellae other than *S. typhi* and *S. paratyphi*. Most of the specimens were from untreated convalescent excretors; those known to be from patients being treated with antibiotics were omitted. *S. typhimurium* was isolated from 246 (88·2%) of the 279 positive stools, *S. enteritidis*, *S. newport*, and *S. thompson* being the other serotypes found.

THE USE OF SOLID MEDIA

CHOICE OF SOLID SELECTIVE MEDIUM AND EFFECT OF DILUTING THE INOCULUM Three selective media were chosen, viz., deoxycholate-citrate agar (Leifson, 1935; Hynes, 1942), Wilson and Blair's bismuth sulphite agar (Wilson and Blair, 1927; De Loureiro, 1942), and MacConkey agar to which 1 : 25,000 brilliant green had been added. Whole plates of media were inoculated with faeces and further plates with a loopful of a 1 : 1,000 dilution of faeces in quarter-strength Ringer's solution. The plates were examined after 24 hours' incubation at 37°C. and suspicious colonies were tested by slide agglutination with diagnostic sera and by the usual biochemical tests. For comparative purposes selenite F medium (Leifson, 1936) was inoculated with faeces and subcultured after 24 hours' incubation at 37°C.

TABLE I

NUMBER OF ISOLATIONS OF SALMONELLAE WHEN SOLID AND FLUID MEDIA WERE INOCULATED WITH DILUTED AND UNDILUTED FAECES

	Direct Examination			Selenite F (24 hr.)
	BGM	DC	WB	
Undiluted faeces	189	146	94	266
1 : 1,000 faeces	135	98	56	205

BGM = brilliant green MacConkey agar; DC = deoxycholate citrate agar; WB = Wilson and Blair's medium. The selenite F medium was subcultured onto brilliant green MacConkey agar after 24 hours' incubation at 37°C. The solid media were examined after 24 hours' incubation.

The results of the examination of 279 positive specimens are presented in Table I. Brilliant green MacConkey agar was the most successful of the three media. In most instances the colonies on the brilliant green MacConkey agar were more than twice the diameter of the corresponding colonies of the pathogens on the other two media. The medium was prepared by the addition of brilliant green in a concentration of 1 : 25,000 to ordinary MacConkey agar including neutral red. The amount of brilliant green required may vary with the particular brand or batch of dye and sodium taurocholate, but is readily determined by testing plates containing concentrations of dye in the range 1 : 20,000 to 1 : 35,000. Judex JTS brilliant green¹ has been found to be very satisfactory. Specimens found positive by direct plating on one or more media after inoculation with undiluted faeces totalled 203; inoculation of diluted faeces resulted in 150 isolations. Dilution of the specimens had no value in aiding the rapid isolation of salmonellae by direct plating on any of the three media.

REPLICA PLATING AND ENRICHMENT ON SOLID MEDIA The technique used was that of Lederberg and Lederberg (1952). A plate of medium was inoculated with faeces and incubated for two to five hours at 37°C. Bacteria from the micro-colonies that resulted were then transferred to a velvet surface by pressing the inoculated surface of the agar on to the velvet. The velvet was secured to one end of a cylindrical wooden block of slightly smaller diameter than a Petri dish. Replicate inoculations from the velvet surface on to other selective and non-selective media were then made by pressing on further plates. The replicate plates were incubated at 37°C. for 18 hours and then examined as described in the previous section.

Various combinations of the three selective media previously described and MacConkey agar were used for primary and replicate platings, but none of the combinations was of value in the enrichment of small numbers of salmonellae present in specimens of faeces. It was sometimes difficult to recover salmonellae by these methods from faecal specimens that were found to be positive by direct plating.

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THE USE OF EARLY SUBCULTURE FROM FLUID MEDIA

Tubes of selenite F medium (Leifson, 1936) and nutrient broth were inoculated with approximately 0.1 g. faeces or 1 ml. of a 1 : 1,000 dilution of faeces. The tubes were incubated in a water-bath at either 37°C. or 43°C. and subcultured on to brilliant green MacConkey agar plates after six hours' incubation. The plates were incubated for 18 hours at 37°C., then examined as already described. For comparison, the faeces samples were also inoculated directly and in a 1 : 1,000 dilution on to the three selective agars previously used, and the selenite F incubated at 37°C. was subcultured after 24 hours' incubation. Salmonellae were isolated from 279 of the specimens. The results of most interest were those of the 76 positive specimens that were negative on the three selective media inoculated with undiluted faeces; they are presented in Table II. Selenite F inoculated with undiluted faeces and incubated at 43°C. gave the greatest number of positive results of the rapid methods; 24 isolations were made,

TABLE II

RESULTS OF EXAMINATION BY VARIOUS ENRICHMENT PROCEDURES OF 76 POSITIVE FAECAL SPECIMENS FOUND NEGATIVE FOR SALMONELLAE BY PLATING AN UNDILUTED INOCULUM ON THREE SELECTIVE AGARS

	Six-hour Subculture			24-hour Subculture
	Selenite 37°C.	Selenite 43°C.	Broth 43°C.	Selenite 37°C.
Undiluted faeces	21	24	19	68
1 : 1,000 faeces	7	6	—	32

All subcultures were on to brilliant green MacConkey agar.

which represented 31.6% of the specimens that were negative by direct plating but from which salmonellae were finally isolated. These, however, were far below the number (68) isolated from selenite cultures incubated for 24 hours at 37°C. Incubation of selenite for six hours at 37°C. gave 21 positives (27.6%) and nutrient broth at 43°C. yielded 19 (25.0%). Dilution of the faeces before inoculation of selenite was not helpful at either temperature of incubation.

The results of the 279 positive specimens were analysed to establish the value of six-hour subculture from selenite F incubated at 43°C. as a supplement to direct plating on to three selective media. Triple direct plating gave 203 positive results 24 hours after receipt of the specimens, whereas the additional use of 43°C. six-hour selenite enrichment increased the number found positive at this time to 227; this increase is significant by the χ^2 test ($\chi^2 = 5.79$; $P < 0.02$). A more significant increase was demonstrated when the short enrichment was used as a supplement to direct plating on to a single medium. One hundred and eighty-nine specimens of faeces were positive at 24 hours by the most successful single plating medium (brilliant green MacConkey agar) and this figure was increased to 223 by the additional use of six-hour subculture from selenite F at 43°C. ($\chi^2 = 10.69$; $P < 0.01$).

DISCUSSION

The value of brilliant green MacConkey agar for the isolation of salmonellae was demonstrated by Harvey (1956). The present investigation confirms this finding and indicates the rapidity with which characteristic salmonella colonies develop on the medium; for this reason it is of particular value for six-hour subcultures from fluid media which are, of necessity, made late in the day. The possibility that use could be made of the differing action of various selective agars led to the experiments making use of the replica plating technique of Lederberg and Lederberg (1952). Possibly the inhibitory action of the selective media allowed too few bacteria to be transferred to the replica plates for the method to be of value.

There are few reports on the value of early subculture from enrichment media. Boecker and Silberstein (1932), Boecker (1935), Waldhecker (1935), and Fry (quoted by Hobbs and Allison, 1945) found early subculture from tetrathionate media of value. H. W. Smith (1952) could not recover salmonellae before the fifteenth hour of incubation from selenite and tetrathionate broths that had been inoculated with artificially-infected faeces. H. G. Smith (1959), however, working with pure cultures, considered the selective action of selenite broth to occur quite early in the growth period. Graphs presented by Leifson (1936) indicated that typhoid bacilli outnumbered coli more quickly in selenite F when a 1:1,000 dilution of faeces was inoculated than when undiluted faeces were used. Diluted faeces have been used with success for inoculating selective agars with specimens from chronic enteric carriers (Thomson, 1954), but had no advantage over undiluted faeces when used with either solid or fluid media in the present study. The report by Harvey and Thomson (1953) that incubation of selenite broth at 43°C. facilitated the isolation of *S. paratyphi B* from faeces suggested the use of this temperature for rapid enrichment in selenite. As selenite F does not support the growth of salmonellae as well as nutrient broth (Banwart and Ayres, 1953), and Thomson (1955) noted that nutrient broth was of some value for the isolation of *S. typhimurium* from faeces, a trial of nutrient broth incubated at 43°C. for six hours was also included.

Duplication of media inoculated with specimens will always increase the number of isolations of pathogens, but the degree of increase will vary with the detail of the method used. In the present investi-

gation six-hour subcultures from selenite F inoculated with undiluted faeces and incubated at 43°C. gave the greatest increase in isolations of salmonellae within 24 hours. Incubation at 37°C. gave slightly fewer positive results than at 43°C. but some laboratories might find the former temperature more convenient. Most of the specimens examined were from convalescent cases because of the difficulty of obtaining a sufficient number of scanty positives from contacts, but there was no indication that these gave different results from specimens from contacts. The impression was gained during the serial examinations of faeces from convalescent cases that, after direct plating had become negative, six-hour enrichment continued to be positive for a few days until finally the pathogens could be found on 24-hour subculture plates only. It is not known whether similar results would have been obtained with specimens containing other salmonella serotypes, because the degree of enrichment occurring in selenite broth varies from species to species (H. W. Smith, 1952; Rappaport, Konforti, and Navon, 1956). *S. typhimurium*, which predominated in this study, has been the salmonella found most frequently in faeces in Great Britain for many years, and the other species isolated were among the six isolated most frequently from human excretors in 1958 (Report of Public Health Laboratory Service, 1959). There are certain circumstances when the speedy detection of persons excreting small numbers of salmonellae is important; the more successful techniques described in this paper might then prove of value when used as a supplement to direct plating.

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