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Isolation of *Shigella sonnei* by fluid media

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SUMMARY

Selenite F broth prepared in the laboratory from single ingredients was found to be significantly more efficient than nutrient broth in the isolation of *Shigella sonnei* from human faeces. It was more efficient than direct plating on deoxycholate citrate agar for diagnosing Sonne dysentery in a local outbreak. The difference was significant. There was, however, no significant advantage for enrichment over direct plating when stools posted from Bristol to Cardiff were examined.

Laboratory prepared selenite F broth was preferable to the same medium purchased commercially from two different firms. The product of one firm was significantly better than that of the other.

Two methods of sterilization of laboratory prepared selenite F broth were studied. Seitz filtered selenite was less inhibitory to the growth of small numbers of *Shigella sonnei*. Sterilization by heating in free steam for periods of 15 min and over impaired the ability of selenite F broth to allow multiplication of small numbers of *Shigella sonnei*.

INTRODUCTION

Enrichment and unselective pre-enrichment fluid media are extremely efficient in salmonella isolation (Harvey & Price, 1974). Attempts at enrichment have also been made in shigella isolation with variable results (Brodie, 1942). Although *Shigella sonnei* is recorded as being capable of multiplication at temperatures above 37° C. (Braun & Weil, 1928), elevated temperature incubation techniques have not been very useful.

Two fluid media were investigated in the Cardiff Public Health Laboratory in 1952. These were MacConkey broth and citrate, rosolic acid, taurocholate broth (Brodie, 1942). The incubation temperature used was 43° C. Results of enrichment compared unfavourably with direct plating on deoxycholate citrate agar. Dartevelle & Desmet (1975), however, have recorded a partial increase in selectivity of GN broth and Hektoen agar in a study on the duration of survival of *Shigella sonnei* when media were incubated at 41° C.

Nutrient broth, incubated at 37° C, was used to recover *Shigella sonnei* from ward dust in an isolation hospital, from patches sewn onto the under-sheet of bedding of dysentery patients and from surfaces of lavatory seats. Nutrient broth has also been employed by others for shigella isolation (Hutchinson, 1956; Ewing 1972).

Armstrong (1954) and Thomas (1954) found selenite F broth of value in diagnosis of Sonne dysentery. As this is a medium of proven worth, a study of its efficiency in the isolation of *Shigella sonnei* was instigated. Certain critical factors in the preparation and choice of selenite F broths were also investigated.

MATERIALS AND METHODS

Fresh stools from victims of a Sonne dysentery outbreak, stools sent through the post from another PHLS laboratory and pure cultures of *Shigella sonnei* were used in the comparisons. The use of different inocula in quality control tests and their relevance to the results obtained has been recorded elsewhere (Harvey, Price & Crone, 1975). In some ways this paper is an extension of the same theme.

Six comparative studies were made:

(1) Selenite F broth as prepared in our media room from single ingredients was compared with ordinary nutrient broth. The selenite broth was sterilized by Seitz filtration. Fresh faeces were used in the tests. The inoculum was 0·02 ml. of a faecal suspension (0·5 g. faeces/0·5 ml. peptone water; coarse particles allowed to settle and inocula taken from the supernatant). The same inoculum from the same faecal suspension was used for each nutrient broth and selenite F broth. Volumes of each broth were 10 ml. Samples were therefore paired. The two fluid media after incubation at 37° C. for 24 h were subcultured to deoxycholate citrate agar plates. These agars were incubated at 37° C. for 24 h. and examined visually and selected colonies were tested serologically with *Shigella sonnei* antiserum. Selenite F and nutrient broths were prepared according to Harvey & Price (1974).

(2) Selenite F broth incubated at 37° C. for 24 h plated on deoxycholate citrate agar was compared with direct plating on deoxycholate citrate agar. The inoculum used was a 2 mm. loopful of solid faeces – a fairly common routine practice. DCA plates were incubated at 37° C. for 24 h. and examined for *Shigella sonnei* as before. In this study two separate series of samples were used: faeces collected in our local area and faeces posted to us from Bristol Public Health Laboratory.

(3) Laboratory-prepared selenite F broth was contrasted with commercial Oxoid selenite broth (CM 399) and B.B.L. selenite F broth (BBL 11608). Preparation of laboratory-made selenite and the inocula used were as in Comparison 1.

(4) Two laboratory-prepared selenite F broths, one sterilized by Seitz filtration the other by steaming for 30 min. were compared with each other. Inocula were as in Comparison 1.

(5) A comparison was made of the dose of *Shigella sonnei* required to initiate growth in filtered and heated laboratory-prepared selenite F broths and also Oxoid and B.B.L. selenite broths. This study used 0·02 ml. inocula taken from serial ten-fold dilutions of a 24 h. broth culture of *Shigella sonnei*. The technique was, therefore, similar to that used in an earlier publication (Harvey *et al.* 1975).

(6) The final comparison investigated the effect of different sterilizing times for laboratory-prepared selenite F broth on the dose of *Shigella sonnei* required to initiate growth. The inocula used were as in Comparison 5.

Table 1. *Isolation of* Shigella sonnei *from stools cultured in nutrient broth and selenite F broth*

Selenite F broth	Nutrient broth	
+	−	96
−	+	29
Total positive samples		376

$\chi^2 = 34{\cdot}8$. $P < 0{\cdot}001$.

Table 2. *Isolation of* Shigella sonnei *from human faeces using selenite F enrichment and direct plating to deoxycholate citrate agar*

LOCAL STOOLS

Direct plating to deoxycholate citrate agar	Enrichment in selenite F subcultured to deoxycholate citrate agar	
+	−	46
−	+	102
Total positive samples		376

$\chi^2 = 20$. $P < 0{\cdot}001$.

POSTED STOOLS

Direct plating to deoxycholate citrate agar	Enrichment in selenite F subcultured to deoxycholate citrate agar	
+	−	16
−	+	28
Total positive samples		112

$\chi^2 = 2{\cdot}8$. $P < 0{\cdot}10$.

RESULTS

Table 1 records results obtained in the broth/selenite F comparison. In this series the superiority of the enrichment broth over the nutrient broth as a means of isolating *Shigella sonnei* from human faeces is significant.

Table 2 presents the results of comparisons between selenite F enrichment at 37° C. for 24 h. subcultured to deoxycholate citrate agar and direct plating to the same selective agar without an enrichment stage. Two separate series of samples were studied, the first using human faeces from a local outbreak of Sonne dysentery, the second human faeces sent through the post from another PHLS laboratory. The results of the two studies are recorded in Table 2. Enrichment in selenite F is significantly more efficient than direct plating with the local stool samples, but not with the specimens posted from Bristol.

Table 3 presents the results of the comparison between laboratory-prepared selenite F broth, Oxoid selenite F broth and B.B.L. selenite F broth as enrichment media for isolating *Shigella sonnei* from stools sent through the post from Bristol.

Laboratory-prepared selenite F broth was significantly more efficient than either B.B.L. or Oxoid selenite F. B.B.L. selenite broth was significantly better than Oxoid selenite.

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Table 3. *Comparison between laboratory-prepared selenite F broth and two commercial selenite broths for the isolation of* Shigella sonnei

BBL selenite	Laboratory-prepared selenite (filtered)	
+	+	24
+	−	0
−	+	72

$\chi^2 = 70{\cdot}0$; $P < 0{\cdot}001$.

Oxoid selenite	Laboratory-prepared selenite (filtered)	
+	+	5
+	−	0
−	+	91

$\chi^2 = 89{\cdot}0$; $P < 0{\cdot}001$.

BBL selenite	Oxoid selenite	
+	+	5
+	−	19
−	+	0

$\chi^2 = 17{\cdot}0$; $P < 0{\cdot}001$.

Total samples positive: 96.

Table 4. *Comparison of efficiency of Seitz filtered and heat-sterilized selenite F broths for isolation of* Shigella sonnei *from human faeces*

Seitz filtered selenite	Heat sterilized selenite	
+	−	29
−	+	19
Total positive samples		115

$\chi^2 = 1{\cdot}7$. $P < 0{\cdot}20$.

In our laboratory we usually sterilize selenite F broth by Seitz filtration as suggested by Hobbs & Allison (1945). This only applies to the laboratory-prepared medium. Commercial media are sterilized according to the manufacturers' instructions. Heat-sterilized selenite F as prepared in the laboratory from single ingredients was compared with the same medium sterilized by Seitz filtration. Heat sterilization consisted of steaming for 30 min. The samples of faeces were paired. The results are given in Table 4.

The difference in efficiency between filtered and heated selenite F broth is not significant using a suspension of faeces as inoculum, nevertheless selenite broth sterilized by filtration had a slight advantage over the identical medium sterilized by heating. In an earlier paper, it was shown that comparative studies on enrichment broths gave different results with different inocula (Harvey *et al.* 1975). Sensitivity, as measured by the number of organisms required to initiate growth (the growth initiating dose), was a separate property of an enrichment broth from *selectivity*. The latter is the ability of an enrichment medium to separate the desired organism from unwanted competing bacteria. It was decided to examine the sensitivity of filtered and heated selenite F broths by means of small inocula of

Table 5. *Number of* Shigella sonnei *necessary to initiate growth in different selenite F broths (modal value of range)*

	Culture dilution								
	10^0	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
No. of organisms initiating growth	No growth	2×10^6	2×10^5	2×10^4	2×10^3	2×10^2	2×10	2	Selenite medium
No. of tests in	—	—	—	—	1	2	8	9	Filtered
which growth was	—	—	—	—	1	10	7	2	Heated
initiated by an	—	—	—	2	5	7	4	2	BBL
inoculum	13	5	2	0	0	0	0	0	Oxoid

Filtered and heated selenite broths were prepared in the laboratory from basic ingredients and differed only in their method of sterilization.

Table 6. *Comparison of growth-initiating inocula expressed as highest tenfold dilution of broth culture of* Shigella sonnei *producing positive growth in Seitz filtered and heated selenite F broths*

Growth-initiating inoculum smaller in filtered selenite than in heated selenite	19
Growth-initiating inoculum smaller in heated than in filtered selenite	0

$\chi^2 = 17. \quad P < 0{\cdot}001.$

pure cultures of *Shigella sonnei*. The results are recorded in Table 5, which presents in frequency distribution form the growth initiating doses in 20 tests.

It will be noted that the modal inoculum necessary to produce growth in Oxoid selenite F differs considerably from those found in the other versions of selenite F broth. The Oxoid medium is very inhibitory to the growth of *Shigella sonnei*.

A study of the sensitivity of filtered and heat-sterilized selenite F broths was made in an alternative manner. Once again, serial tenfold dilutions of a 24 h. broth culture of *Shigella sonnei* were used as inocula. The results are given in Table 6. Each tube of selenite producing growth after incubation at 37° C. for 24 h. was plated on MacConkey agar and deoxycholate citrate agar. Selective agars were incubated at 37° C. for 24 h. and examined for *Shigella sonnei*.

Table 6 demonstrates that when small numbers of a pure culture of *Shigella sonnei* were used as test inocula, a significant difference in efficiency could be shown between filtered and heat-sterilized selenite F prepared from basic ingredients. This significant difference was not apparent when inocula of naturally infected faeces were employed (Table 4).

Information was finally sought on the effect of progressive times of heat sterilization on selenite F. Eight different periods of heating were studied. The method of heating was exposure to free steam. Each batch of selenite F heated for a particular period was subdivided into seven portions of 10 ml. Each quantity was contained in a 28 ml. screw-capped container. There were, therefore, $8 \times 7 = 56$ containers.

Table 7. *Effect of different heating periods on growth initiation in selenite F broth by* Shigella sonnei

	Culture dilution						
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
No. of organisms initiating growth ...	2×10^{6}	2×10^{5}	2×10^{4}	2×10^{3}	2×10^{2}	2×10	2
Steaming period (min.)							
0	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
15	+	+	+	+	−	−	−
30	+	+	+	−	−	−	−
60	+	+	+	−	−	−	−
120	+	+	+	−	−	−	−
180	+	+	+	−	−	−	−
240	+	+	+	−	−	−	−

+ = growth. − = no growth.

Each container of a batch of medium heated for a single time period was inoculated with 0·02 ml. of a serial tenfold dilution of a broth culture of *Shigella sonnei* incubated at 37° C. for 24 h. The first of a row of containers was inoculated with 0·02 ml. of undiluted culture, the last of the row with the same volume of a 10^{-6} dilution of the same broth culture. The containers were incubated at 37° C. for 24 h. and each was then plated on a MacConkey and a deoxycholate citrate agar plate. Plates were incubated at 37° C. for 24 h. and examined for colonies of *Shigella sonnei*. The peptone water culture used to prepare the tenfold dilutions contained 10^{8} *Shigella sonnei*/ml. Results are given in Table 7. Columns represent the numbers of organisms inoculated and rows the different times of steaming of the selenite F broths.

A heating period longer than 5 min. destroyed the ability of selenite F broth to allow multiplication of small numbers of *Shigella sonnei*. This study was repeated once with identical results. Put in a different way, 2000 cells of *Shigella sonnei* were required to initiate growth in selenite steamed for 15 min. and 20,000 cells were necessary to produce growth in selenite F steamed for 30 min. and over.

DISCUSSION

The question has been put in a leading article 'Can bacteriologists agree?' (Lancet, 1970). In a previous paper it was demonstrated that media given identical names – selenite or tetrathionate broths – behaved very differently according to whether they were laboratory-made or purchased from commercial sources. The test organisms were salmonellas in this study (Harvey *et al.* 1975). That investigation has been extended in the current paper and one reason why bacteriologists disagree is apparent. They call by the same name media which are *not* identical in their behaviour. The commercial media chosen here were not selected by chance. Oxoid selenite (Medium L) had shown itself to be less efficient than B.B.L. selenite

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(Medium H) in the isolation of salmonellas (Harvey *et al.* 1975). The current study shows that order of relative efficiency to be the same with *Shigella sonnei*.

Crone (1948) has recorded that there is a limit to the amount of faeces which may be used to inoculate a selective agar, as faecal detritus may interfere with colony formation. Enrichment broths are more tolerant of heavy inoculation with faeces and this gives them an immediate advantage over direct plating independent of the selectivity of the fluid medium. The same reasoning may be valid in shigella diagnosis. It was necessary, therefore, to compare unselective and presumed selective broths with paired samples of faeces to determine whether selenite F broth had any advantage over nutrient broth for isolation of *Shigella sonnei*. Both fluid media have their advocates (Armstrong, 1954; Thomas, 1954; Ewing, 1972). In the series of faecal samples studied, selenite F broth had a significant advantage over nutrient broth and could therefore be recommended for routine practice.

In evaluating a fluid enrichment medium it is usual to assess its efficiency against direct plating on a selective agar. In this investigation, two types of sample were investigated: fresh stools from a local outbreak and specimens of faeces posted from Bristol Public Health Laboratory. Only in the series of stools collected locally was enrichment significantly better than direct plating. It is possible that selenite enrichment may be less effective with stools passed several days previously and therefore the samples examined should be as fresh as possible.

Commercial media are commonly used in many microbiological laboratories. It was, therfore, advisable to examine the performance of two commercial selenite F broths compared with laboratory prepared selenite F. The reasons for the choice of the two commercial media have been given. In our hands, selenite F broth prepared from basic ingredients was significantly more efficient than either of the commercial media. Others have made similar observations with selective agars (Taylor & Schelhart, 1968). In the present study, B.B.L. selenite F broth was more efficient than Oxoid selenite F broth. This could explain the failure of Vassiliadis, Pateraki & Politi (1966) to obtain satisfactory enrichment of *Shigella sonnei* with Oxoid selenite broth. Only one batch of each commercial medium was examined.

In Cardiff, we sterilize selenite F broth by Seitz filtration, although Hobbs & Allison (1945) stated that either steaming for not longer than 30 min. or filtration were suitable sterilizing methods. Ewing (1972) indicates that sterilization is unnecessary if the medium is to be used immediately. This is probably ideal. Table 4 records the relative efficiency of filtered and heated selenite broths in the culture of *Shigella sonnei* from human faeces. No significant difference was found. Using a pure culture technique, however, demonstrates that heated selenite broth is more inhibitory to the growth of small numbers of *Shigella sonnei* than Seitz-filtered selenite and when results are recorded, as in Table 6, the difference is significant. It should not surprise us that faecal samples give different results from pure cultures, as addition of faecal matter is known to affect the performance of enrichment media (Silliker, Deibel & Fagan, 1964; Vassiliadis, Pateraki, Papadikis & Trichopoulos, 1974).

When the effect of different heat-sterilization times on the ability of selenite broth to grow small numbers of *Shigella sonnei* is investigated, a period of heating

by free steam for 15 min. and over is found to impair this property. Gillespie & Gibbons (1975) have commented on dangers of overheating media in laboratory autoclaves. Lesser degrees of heat sterilization may also prevent growth of small numbers of certain common pathogenic bacteria if faecal matter is not present.

Although selenite F broth prepared as described here was better than nutrient broth for isolation of *Shigella sonnei* from faeces, we should always wish to use an unselective fluid medium to recover this organism from environmental samples.

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