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The effect of the use of different selective media on the ability to recover salmonellae from seagull faeces

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SUMMARY

Solid media were compared for their ability to recover salmonellae from seagull faecal material after pre-enrichment in buffered peptone water and enrichment in Rappaport's broth. Of the 847 specimens examined 96 were found to be positive for salmonellae. Use of Brilliant Green agar containing sulphamandelate supplement resulted in the detection of salmonellae from each of the 96 samples found to be positive and was the most efficient medium tested. Brilliant Green agar lacking the supplement was the least effective medium, salmonellae being isolated from only 80 samples using this medium.

All of the media tested were shown to support the growth of a wide range of salmonella serotypes, although *Salmonella typhi* and *S. dublin* did not form colonies on those media which contained Brilliant Green. Hynes' modification of deoxycholate citrate agar was shown to be considerably less inhibitory to salmonellae after ageing for four days. Ageing of other media had no significant affect on their ability to support the growth of salmonellae.

INTRODUCTION

In a recent article (Fricker, Girdwood & Munro, 1983) the choice of enrichment medium was shown to affect significantly the likelihood of isolating salmonellae from seagull cloacal swabs, thus confirming the findings of others working with a variety of samples (Vassiliadis *et al.* 1977, 1978, 1979*a*, 1981; Harvey & Price, 1981; Harvey, Price & Xirouchaki, 1979). In order to develop a standard procedure for the isolation of salmonellae from seagull faecal material, it was considered necessary to investigate the effectiveness of various selective agars in recovering salmonellae after pre-enrichment in buffered peptone water and enrichment in the RB 10 form of Rappaport's broth (Rappaport, Konforti & Navon, 1956; Vassiliadis *et al.* 1976*a*).

Many selective agars have been described for the isolation of salmonellae. Although the majority of workers are agreed on the use of Rappaport's broth for the enrichment of environmental samples, there seems to be little agreement in the choice of solid media. Harvey (1956) compared bile salt–lactose medium, with bismuth sulphite and Brilliant Green MacConkey agars and preferred the latter, and this preference for Brilliant Green MacConkey agar has remained for salmonella

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isolation from environmental samples in that laboratory (Harvey & Price, 1974, 1981). Dixon (1961) also demonstrated the superiority of Brilliant Green MacConkey over deoxycholate citrate and bismuth sulphite agars and Edgar & Soar (1979) used Brilliant Green agar to good effect in the isolation of salmonellae from sewage sludge after enrichment in Muller–Kauffman tetrathionate broth. This medium has been shown to allow the growth of a wide range of salmonella serotypes (Chau & Leung, 1978), although Harvey & Price (1968) noted that some strains of *S. dublin* were inhibited by Brilliant Green. Magee & Hinton (1974), however, were unable to reproduce these findings and suggested that there was no significant difference in the recovery of *S. dublin* using Brilliant Green MacConkey or deoxycholate citrate agars. Vassiliadis and his colleagues in Greece who have carried out many studies on the use of Rappaport's broth used Brilliant Green agar supplemented with sulphadiazine, but then demonstrated the inhibitory action of this medium on some strains of salmonella (Vassiliadis *et al.* 1976*b*). Subsequently this group of workers added sodium deoxycholate to Brilliant Green agar and have used this medium effectively for the isolation of salmonellae after enrichment in RB 10 broth (Vassiliadis *et al.* 1979*b*). A further modification of BGA was described by Watson & Walker (1978), who added sodium sulphacetamide and mandelic acid (sulphamandelate supplement) and showed that this medium was effective in inhibiting the growth of competing bacteria whilst allowing the growth of a wide range of salmonella serotypes, particularly if the incubation temperature was raised to 43 °C.

Wren (1975) demonstrated the usefulness of xylose lysine deoxycholate agar in isolating salmonellae from human faeces and suggested that this medium was superior to deoxycholate citrate lactose sucrose agar when used after enrichment in selenite F broth. More recently Restaino *et al.* (1977) added novobiocin to XLD and Hektoen enteric (HE) agars and showed that this addition effectively inhibited the growth of *Proteus mirabilis* whilst allowing the growth of the eight strains of salmonellae used, thus confirming the usefulness of novobiocin-supplemented solid media as described by Shanson (1975).

The features of a good selective agar are good growth and easy recognition of the desired organism and suppression of competing organisms, particularly those which have a similar colonial appearance to the organism being sought. The aim of this study was to assess the performance of seven selective media in recovering salmonellae from seagull faecal material after enrichment in RB 10 broth.

MATERIALS AND METHODS

The solid media compared in this study were: modified Brilliant Green agar (BGA; Oxoid CM329), BGA containing (*a*) sulphamandelate supplement (BGASM) or (*b*) 2·5 g/l sodium deoxycholate (BGAD), Brilliant Green MacConkey (BGM; Harvey, 1956), xylose lysine desoxycholate agar (XLD; Oxoid CM469), XLD containing 7 mg/l sodium novobiocin (NXLD) and Hynes' modification of deoxycholate citrate agar (HDCA; Oxoid CM227). The sulphamandelate supplement was prepared by dissolving 1·0 g of sulphacetamide and 0·25 g of mandelic acid in separate 10 ml volumes of sterile distilled water and adjusting the pH to 6·9 using 40 % sodium hydroxide. The supplement was added to 1·0 l of the basal medium

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after cooling to approximately 60 °C. Then 1·0 ml of a solution containing 7 mg/ml sodium novobiocin was added to 1·0 l of XLD after cooling to approximately 60 °C. BGAD was prepared by the addition of 2·5 g of sodium deoxycholate dissolved in 50 ml of sterile distilled water to 1·0 l of the basal medium, cooled to 60 °C.

The experiments used in this study were of two types. First the ability of the seven media described above to support the growth of two strains of twenty salmonella serotypes was investigated. Following these experiments, the ability of seven solid media to recover salmonellae from seagull cloacal lavage specimens after pre-enrichment in buffered peptone water and enrichment in Rappaport's broth was investigated.

Quantitative studies

An overnight broth culture of each of the strains used was serially diluted in normal saline to produce a suspension containing approximately 500 colony-forming units (c.f.u.) per ml. Four separate 50 μl volumes of this suspension were then spotted on to each of the different media according to the methods of Miles & Misra (1938). A freshly prepared blood agar plate was included to determine the inhibitory effects of each medium. Freshly prepared plates, and plates which had been stored at room temperature in the dark for four days, were used in this experiment. All inoculated plates were incubated at 37 °C for 24 h and the mean number of colonies produced was calculated.

The serotypes used were *S. agona*, *S. anatum*, *S. bredeney*, *S. dublin*, *S. enteritidis*, *S. indiana*, *S. kedougou*, *S. mbandaka*, *S. montevideo*, *S. newport*, *S. panama*, *S. paratyphi B*, *S. rubislaw*, *S. saint-paul*, *S. senftenberg*, *S. stanley*, *S. typhi*, *S. typhimurium*. *S. virchow* and *S. virgina*. Strains were selected from those which had been submitted to the Scottish Salmonella Reference Laboratory for identification and had been stored on Dorset egg slopes at ambient temperature. Before use, they were checked for purity and their identity was confirmed by standard serological procedures.

Qualitative studies

A total of 847 seagulls were captured between March and November 1983 from various sites in Scotland by cannon netting. Cloacal lavage was performed on each of the birds by the insertion of a sterile plastic Pasteur pipette containing approximately 2 ml of buffered peptone water (BPW/Oxoid CM 508) into the bird cloaca and faecal material obtained by repeated discharging of the pipette (Fricker, 1983). The material obtained was placed directly into 20 ml volumes of BPW and transported back to the laboratory, whereupon it was incubated at 37 °C for 24 h. The pre-enrichment cultures were then subcultured to 10 ml volumes of RB10 medium at an inoculation ratio of 1:200 (Fricker, in press) and these broths incubated at 43 °C for 48 h. Each enrichment culture was subcultured on to each of seven solid media at 24 and 48 h, using a calibrated 10 μl loop, and the plates incubated at 37 °C for 24 h. Up to four presumptive salmonella colonies were examined from each plate by standard biochemical and serological techniques.

The results were compared statistically using chi-squared testing and McNemar's test for paired samples.

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Table 1. *Mean count of 20 salmonella serotypes in 50 µl of a saline suspension on eight solid media, showing the age of the media (see key)*

	Medium														
Serotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S. agona	28	26	27	26	26	29	26	26	27	25	27	26	25	16	27
S. anatum	19	16	18	17	17	18	18	17	16	16	17	18	16	10	17
S. bredeney	32	31	29	31	28	28	30	26	31	30	29	27	28	15	28
S. dublin	21	0	0	0	0	0	0	0	0	16	17	20	19	10	21
S. enteritidis	17	18	17	19	19	18	19	18	17	18	19	17	16	9	18
S. indiana	38	37	35	37	34	35	38	37	36	36	34	36	35	21	37
S. kedougou	16	13	15	15	16	13	14	15	16	15	16	17	13	8	15
S. mbandaka	28	24	27	26	27	26	27	23	24	27	23	28	26	13	27
S. montevideo	26	26	27	23	24	25	25	25	27	22	25	23	21	14	26
S. newport	17	18	15	15	16	14	17	15	14	16	17	16	17	8	15
S. panama	19	17	16	18	17	18	20	21	16	18	16	16	18	8	17
S. paratyphi B	27	25	26	22	25	26	25	23	24	26	23	25	26	11	27
S. rubislaw	24	21	22	22	21	23	20	22	23	21	21	25	23	12	21
S. saint-paul	15	16	17	15	16	17	16	15	14	13	16	14	15	7	16
S. senftenberg	18	16	14	17	15	18	15	16	16	15	15	15	17	9	17
S. stanley	26	25	23	25	25	26	26	23	24	25	23	24	25	11	25
S. typhi	27	0	0	0	0	0	0	0	0	21	20	19	17	10	28
S. typhimurium	22	20	18	21	19	19	20	21	20	21	23	22	21	14	18
S. virchow	37	33	36	33	36	34	35	35	36	38	34	35	36	21	34
S. virginia	31	27	29	27	27	28	25	28	28	27	26	27	26	15	25

Key: (1) Blood agar (fresh). (2) Brilliant Green agar (fresh). (3) Brilliant Green agar (4 days old). (4) Brilliant Green/sulphamandelate agar (fresh). (5) Brilliant Green/sulphamandelate agar (4 days old). (6) Brilliant Green deoxycholate agar (fresh). (7) Brilliant Green deoxycholate agar (4 days old). (8) Brilliant Green MacConkey agar (fresh). (9) Brilliant Green MacConkey agar (4 days old). (10) Xylose lysine deoxycholate agar (fresh). (11) Xylose lysine deoxycholate agar (4 days old). (12) Xylose lysine deoxycholate/novobiocin agar (fresh). (13) Xylose lysine deoxycholate/novobiocin agar (4 days old). (14) Hynes' deoxycholate citrate agar (fresh). (15) Hynes' deoxycholate citrate agar (4 days old).

RESULTS

Each of the seven solid media were able to support the growth of 18 of the 20 serotypes tested. However, the four media which contained Brilliant Green were inhibitory to both *S. typhi* and *S. dublin*. Freshly prepared HDCA gave substantially lower counts than those obtained on blood agar for all of the 20 serotypes tested. When the medium had been aged for four days, however, the counts obtained increased to a level similar to that obtained on the other media used. Ageing the plates for four days had no detectable effect on the ability of the remaining media to support the growth of salmonellae. The counts obtained with each serotype on the seven solid media are shown in Table 1.

Of the 847 gulls examined, 96 (11·3%) were found to be carrying salmonellae by at least one of the procedures used. The number of salmonella isolations from each of the seven solid media, together with the number of presumptive salmonella colonies examined are shown in Table 2. Statistical analysis of the results using chi-squared testing demonstrated that there was a significant difference ($P < 0{\cdot}05$) between the number of salmonella isolations obtained with the different media.

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Table 2. *Number of salmonella isolations made on each of seven selective media showing the number of presumptive salmonella colonies examined*

	HDCA	BGA	BGAD	BGASM	BGM	XLD	NXLD
No. presumptive salmonellae	771	1934	963	741	1169	1376	1062
No. confirmed salmonellae	501	611	630	679	617	592	616
Percentage confirmed salmonellae	65·0	31·6	65·4	91·6	52·8	43·0	58·0
Samples positive for salmonella	89	80	92	96	89	85	92
Percentage positive for salmonella	10·5	9·4	10·9	11·3	10·5	10·0	10·9

HDCA = Hynes' modification of deoxycholate citrate agar; BGA = Brilliant Green agar; BGAD = Brilliant Green agar containing 2·5 g dm^{-3} sodium deoxycholate; BGASM = Brilliant Green agar containing sulphamandelate supplement; XLD = xylose lysine deoxycholate agar; NXLD = xylose lysine deoxycholate agar containing 7 mg dm^{-3} sodium novobiocin.

Table 3. *Salmonella serotypes isolated from* 96 *of* 847 *gulls showing the solid media on which they were isolated*

Serotype	HDCA	BGA	BGAD	BGASM	BGM	XLD	NXLD
S. agona	5	4	5	5	5	3	4
S. bredeney	11	9	11	11	10	9	11
S. indiana	4	4	4	4	4	3	4
S. infantis	7	6	8	8	8	7	7
S. montevideo	2	1	2	3	2	2	2
S. newport	5	5	5	5	5	5	5
S. stanley	3	3	3	3	2	2	3
S. typhimurium	24	22	25	27	26	25	27
S. virchow	28	26	29	30	27	29	29
Total	89	80	92	96	89	85	92

HDCA = Hynes' modification of deoxycholate citrate agar; BGA = Brilliant Green agar; BGAD = Brilliant Green agar containing 2·5 g dm^{-3} sodium deoxycholate; BGASM = Brilliant Green agar containing sulphamandelate supplement; BGM = Brilliant Green MacConkey agar; XLD = Xylose lysine deoxycholate agar; NXLD = Xylose lysine deoxycholate agar containing 7 mg dm^{-3} sodium novobiocin.

Brilliant Green agar containing sulphamandelate supplement gave the highest number of salmonella isolations (96), with the same medium lacking the antibiotic supplement yielding the lowest recovery (80). Comparison of the results obtained with BGASM and each of the other solid media showed that BGASM was significantly more effective ($P < 0{\cdot}05$) in recovering salmonellae than were HDCA, BGA, BGM and XLD. The difference between the number of salmonella isolations made on BGASM and BGAD or NXLD was not statistically significant ($P < 0{\cdot}25 > 0{\cdot}10$).

Table 3 shows the salmonella serotypes isolated during the study and the media from which they were recovered. Nine serotypes were isolated, with *S. virchow* and *S. typhimurium* together accounting for 57 of the 96 strains isolated.

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DISCUSSION

The effectiveness of different selective media in recovering salmonellae has received relatively little attention, whilst enrichment and pre-enrichment techniques have been extensively researched. This study has demonstrated that there are marked differences in the recovery of salmonellae from seagull faecal material depending on the solid medium employed, and it is likely that these differences would apply to salmonella isolation from other environmental samples.

Quantitative studies showed that the seven selective media compared were all able to support the growth of a wide range of salmonella serotypes, with little difference in the amount of growth obtained. Media containing Brilliant Green were however inhibitory to *S. dublin* and *S. typhi*. Whilst this may constitute a problem in the examination of some environmental specimens for salmonellae, these particular serotypes have not been isolated from seagulls in Scotland despite the employment of culture techniques which would facilitate their isolation (Fricker, Girdwood & Munro, 1983). In addition, since Rappaport's broth is inhibitory to *S. dublin* and *S. typhi* (Rappaport, Konforti & Navon, 1956; Harvey & Price, 1975), any system which employs this medium is unlikely to detect these particular serotypes.

An interesting finding was that freshly prepared HDCA had a considerable inhibitory effect on all strains of salmonella tested, whereas plates which had been stored for four days allowed good growth of salmonellae. This is similar to the effect described by Cook (1952) when studying the ability of two forms of bismuth sulphite to recover *S. typhi* and *S. typhimurium*.

A significant difference in the ability to recover salmonellae was seen between some of the media compared. Brilliant Green agar containing sulphamandelate supplement gave the highest number of salmonella isolates whilst the same medium lacking the antibiotic supplement gave the lowest. In addition the number of colonies which had the appearance of a salmonellae and were therefore further identified varied widely between different media. The number of presumptive salmonella colonies which were selected for further identification was considerably lower for BGASM, BGAD and HDCA than for the other media tested, and consequently the percentage of colonies selected which were confirmed as salmonellae was high for these media. Use of BGASM, therefore, results in a reduction in the technical time required for screening samples and also dramatically reduces the cost of examination of specimens. The reduction in growth of competing bacteria on BGASM, BGAD and HDCA also allows small numbers of salmonella colonies to be identified which may have been overgrown on some of the other media used.

On the basis of this and a previous study (Fricker, Girdwood & Munro, 1983) we would recommend the following protocol for the examination of seagull faecal material for salmonellae: pre-enrichment in buffered peptone water (37 °C for 24 h), enrichment in the RB10 formulation of Rappaport's broth (43 °C for 48 h, inoculated at a ratio of 1:200) and plating onto Brilliant Green agar containing sulphamandelate supplement and 4-day-old plates of Hynes' modification of deoxycholate citrate agar at 24 h and 48 h. The inclusion of HDCA as a plating medium is justified since occasional strains of salmonellae may be encountered

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which are usually sensitive to certain antibiotics used in selective media or to Brilliant Green dye.

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