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Effect of stool storage at room temperature on salmonella isolation from faeces

By R. W. S. HARVEY* and T. H. PRICE†
Regional Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff, CF4 4XW

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SUMMARY

Stools, known to have contained salmonellas were cultured in three enrichment media on arrival at the laboratory. The three media were laboratory prepared selenite F, laboratory prepared Muller–Kauffmann tetrathionate and commercially obtained Oxoid Muller–Kauffmann tetrathionate (CM343). Where faecal samples were sufficiently large, they were stored at room temperature and re-examined using the same three enrichment broths. Whether fresh or stored stools were investigated, the laboratory prepared media were significantly more efficient than the commercial medium. In a straight comparison of the two laboratory prepared media, no significant difference in efficiency was evident with fresh stool samples. With stored specimens, however, Muller–Kauffmann tetrathionate was significantly better than selenite F. This finding might be relevant in examining samples delayed in the post.

INTRODUCTION

Many years ago, we used stool samples almost exclusively for media testing (Harvey & Thomson 1953; Harvey, 1956). Such material was then readily obtainable, as major outbreaks of salmonellosis were fairly often encountered before the heat treatment of egg regulations of 1964 were introduced (McCoy, 1975). Of recent years, we have had to use sewage polluted natural water as test material for media comparisons in an attempt to rationalize methods of salmonella isolation (Harvey, Price & Crone, 1975; Harvey & Price 1977; Harvey, Price & Xirouchaki, 1979; Harvey & Price, 1980; Harvey & Price, 1982*a*). Diagnostic faecal culture is, nevertheless, an important responsibility of a PHLS laboratory and an opportunity presented during a salmonella outbreak to use faecal specimens in a comparison of salmonella recovery from selenite F broth and Muller–Kauffmann tetrathionate broth. At the time of the study, these media seemed the most appropriate for faecal examination. Material from the outbreak was submitted as faecal samples and not faecal swabs. This allowed several examinations to be made on each specimen. This was done in order to increase the numbers in the series and in the hope that stool storage at room temperature might reveal differences between the media selected. The results of the study are recorded in this paper.

* Present address: 24 South Road, Sully, Nr. Penarth, South Glamorgan.

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MATERIALS AND METHODS

The test material was human faeces. Stools known to contain salmonellas were examined on the day of arrival at the laboratory. Where suffcient of the sample had been submitted, the stool was stored at room temperature and re-examined for salmonellas on several occasions. The number of re-examinations depended on the size of the individual samples.

The media used for salmonella recovery were selenite F broth (Leifson, 1936), Muller–Kauffman tetrathionate broth (Muller, 1923; Kauffmann, 1930, 1935) and brilliant green MacConkey agar (Harvey, 1956). Two versions of tetrathionate broth were employed – the commercial medium Oxoid code No. CM343 and the formula prepared from individual ingredients (Anon, 1975). The selenite broth was sterilized by seitz filtration and not by heat and was clear and without a red deposit. Laboratory prepared media were made according to Harvey & Price (1982*b*).

Faecal inocula were prepared by introducing a throat swab into the stool sample, withdrawing it and agitating the soiled swab in 2–3 ml of peptone water. Coarse particles were allowed to settle from the suspension and three drops (approx. $3 \times 0{\cdot}02$ ml) of the supernatant were seeded into 10 ml of each enrichment medium. The media were incubated at 37 °C for 24 h and subcultured to brilliant green MacConkey agar. Selective agars were incubated at 37 °C for 24 h and examined for suspicious colonies in daylight. Salmonellas were identified by slide agglutination. Biochemical reactions were seldom required. In Cardiff, we prepare selective agars with as low a concentration of agar as is compatible with stability. This permits slide agglutination as a screening procedure with consequent time saving (Harvey & Price, 1982*b*).

RESULTS

Preliminary inspection of the data indicated that fresh and stored stools should be considered separately. This broad classification was relevant to salmonella recovery from the two laboratory prepared enrichment media. It would have been interesting to relate duration of storage to media performance, but the numbers in the series were insufficient to allow this. The results are recorded in Tables 1 and 2.

Table 1 demonstrates the significant superiority of both laboratory prepared enrichment broths over the commercial medium whether fresh or stored stools are used as inocula (MacNemar's χ^2 test for paired samples; $P < 0{\cdot}01$).

Table 2 records the results of a comparison of the two laboratory prepared enrichment broths – selenite and Muller–Kauffmann tetrathionate. Fresh and stored stools are considered separately. With fresh stools, no significant difference is evident between the media. With the stored stools, the laboratory prepared tetrathionate is significantly superior to the selenite broth (MacNemar's χ^2 test; $P < 0{\cdot}01$).

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Table 1. *Salmonella isolation from laboratory prepared enrichment media and commercial enrichment medium (CM343)*

Medium and result	Fresh stools	Stored stools
Laboratory prepared tetrathionate positive, CM343 positive	5	52
Laboratory prepared tetrathionate positive, CM343 negative	28	52
Laboratory prepared tetrathionate negative, CM343 positive	0	0
Both media negative	0	2
Laboratory prepared selenite positive CM343 positive	5	35
Laboratory prepared selenite positive CM343 negative	26	51
Laboratory prepared selenite negative CM343 positive	0	0
Both media negative	2	20

N.B. Total tests performed in each comparison are not identical

Table 2. *Salmonella isolation from laboratory prepared media*

Medium and result	Fresh stools	Stored stools
Selenite positive, tetrathionate positive	114	84
Selenite positive, tetrathionate negative	2	1
Selenite negative, tetrathionate positive	4	19
Both media negative	4	1

DISCUSSION

Muller–Kauffmann tetrathionate came into current prominence as a result of a series of international studies in Europe (Edel & Kampelmacher, 1969). In these trials Oxoid tetrathionate broth base (Code No. CM29) and Oxoid desiccated ox bile (Code No. L50) were used to prepare the medium. Later, the desiccated ox bile was incorporated in the base and this combination was offered under the code name Oxoid CM343.

In 1975, we collaborated with the Newcastle Public Health Laboratory in examining both CM29 and CM343 for their ability to recover salmonellas (Media D and E, Harvey, Price & Crone, 1975). Laboratory prepared media were used as controls (Media F and G). Using CM29, we prepared a medium which permitted multiplication of small numbers of salmonellas but with CM343 the resulting enrichment broth was inhibitory and small numbers of salmonellas usually failed to grow. The temperature of incubation (37 or 43 °C) did not alter the inhibitory nature of the medium in comparison with other tetrathionate broths. The two commercial media were also examined for their ability to recover salmonellas from

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25 ml of sewage polluted natural water. A laboratory prepared Muller–Kauffmann tetrathionate was used as control. Out of 92 tests, the control medium cultured salmonellas from 43 samples. The CM29 medium recovered salmonellas from two samples and the CM343 from none and three samples respectively, depending on the incubation temperature used.

Both CM343 and laboratory prepared Muller–Kauffmann tetrathionate have been shown to inhibit salmonella multiplication by other microbiologists (Vassiliadis *et al.* 1974; van Schothorst *et al.* 1977). This inhibition could be counteracted by addition of 5% w/v of human faeces to CM343 or by using a pre-enrichment stage in buffered peptone water with the laboratory prepared medium. We have examined CM343 after pre-enrichment of material in buffered peptone water. Sewage polluted natural water samples were used in this study. Even with pre-enrichment, the commercial medium was again significantly inferior to the laboratory prepared medium (Harvey, Price & Xirouchaki, 1979).

The results recorded here with CM343 directly inoculated with faeces (Table 1) are in keeping with those of other workers (Roberts *et al.* 1975) and we question whether this medium is suitable for use in an international standard for salmonella isolation (Anon, 1975). From past experience, stools of patients suffering from salmonellosis contain large numbers of salmonellas (Thomson, 1955). The test described here is, therefore, not performed at a critical level.

Other questions are raised relevant to quality control. Are commercially obtainable bacteriological media more reliable than the laboratory prepared products? If so, we need evidence for this (Stokes, 1978). There are records that this is not always the case (Taylor & Schelhart, 1965). The variability of commercial brilliant green agars has also been noted (Read & Reyes, 1968). Our own experience of commercial selenite, tetrathionate and Rappaport's enrichment broths demonstrated the significant superiority of laboratory prepared media (Harvey, Price & Xirouchaki, 1979). Questions such as 'Can bacteriologists agree?' (Anon, 1970) are unlikely to be answered satisfactorily until media variability is more deeply studied by the consumer.

Table 2 records an unexpected result. We have always considered selenite F as the medium of choice for faecal culture. It is the optimum medium available in the UK for the isolation of *Salmonella typhi* and *S. dublin* (Harvey & Price, 1975). We have not had the opportunity of testing strontium selenite broth (Iveson & Mackay-Scollay, 1969). Selenite F is also of value in the isolation of *Shigella sonnei* from stools (Price, 1976). From Table 2, however, it is possible that laboratory prepared Muller–Kauffmann tetrathionate might be useful in the examination of faecal samples arriving at the laboratory after postal delays.

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