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A comparison of the R25 modification of Rappaport's enrichment medium with strontium chloride B for salmonella isolation from sewage polluted natural water

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SUMMARY

The relation of salmonella isolation efficiency and the size of inoculum introduced from a buffered peptone water culture of sewage polluted water into strontium chloride B medium was investigated. Two separate studies were made, one using enrichment at 37 °C, the other at 43 °C. From these trials, two inocula suitable for efficient salmonella isolation were determined. Using this information, strontium chloride B medium was compared with modified Rappaport's broth (R25). The inoculum used with R25 was 0·005 ml, determined in an earlier study. Two incubation temperatures were employed with strontium chloride enrichment (37 and 43 °C). Rappaport's medium was incubated at 37 °C only. Elevated temperature enrichment at 43 °C improved the performance of strontium chloride B, but Rappaport's broth still gave significantly better results. This supports earlier studies on simplification of salmonella isolation and standardization of routine technique on a single enrichment medium: Rappaport broth (R25) incubated at 37 °C.

INTRODUCTION

The efficiency of four reliable salmonella enrichment media has been clearly demonstrated in the microbiological literature. These media are selenite broth (Guth, 1916; Leifson, 1936), tetrathionate broth (Muller, 1923; Kauffmann, 1930, 1935) magnesium chloride malachite green broth (Rappaport, Konforti & Navon, 1956; Rappaport & Konforti, 1959) and the fluid media based on the tolerance of salmonellas to the strontium ion (Eisenberg, 1918; Hotchkiss, 1923; Iveson & Mackay-Scollay, 1969; Iveson, 1971). Rappaport & Konforti (1959) have also recorded that the magnesium chloride in their medium could be replaced by an equimolecular quantity of strontium or barium chloride.

In a laboratory, which deals with many samples containing salmonellas, isolation techniques may develop in two different ways. Multiple techniques may be used to increase the chances of a positive isolation, or preference may be given

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to employing an optimum method thereby saving cost and laboratory time. The adjective optimum is used in a relative sense. An absolute optimum technique for all salmonella serotypes has not yet been discovered. Of recent years we have explored the second approach, but until completion of comparative tests with the different media available there was insufficient evidence to decide on the best cultural procedure.

At first, we concentrated on three of the four media: selenite F, Muller–Kauffmann tetrathionate and Rappaport's magnesium chloride malachite green broth using the R25 modification described by Vassiliadis *et al.* (1970). From studies on sewage polluted natural water and on naturally contaminated chicken giblets, Rappaport's medium was found to be the most efficient enrichment broth of the three examined (Harvey, Price & Xirouchaki, 1979; Harvey & Price, 1981).

Iveson & Mackay-Scollay (1972) in a study of sea water contaminated with abattoir effluent found strontium chloride B superior to Rappaport's broth, when samples were incubated at 43 °C. Elevated temperature incubation also improves the selectivity of other enrichment media (Harvey & Thomson, 1953). Iveson & Mackay-Scollay (1972) concluded that if a single enrichment medium and a single incubation temperature were to be used, strontium chloride B at 43 °C was the best method available for the recovery of salmonellas from swabs exposed to contaminated sea water. This statement intrigued us as, hitherto, we had adopted Rappaport's enrichment (R25) as optimal for routine salmonella isolation from environmental samples. The current study compares strontium chloride B (Iveson, 1971) with Rappaport's magnesium chloride malachite green broth as modified by Vassiliadis *et al.* (1970). The two media were examined under similar conditions to those used in previous studies (Harvey *et al.* 1979; Harvey & Price, 1980, 1981).

MATERIALS AND METHODS

The test material was sewage polluted natural water taken from the river Taff at Pontypridd. This type of sample has been shown previously to be suitable for quality control tests on media (Harvey, Price & Crone, 1975). Small inocula of pure cultures of salmonellas, in contrast, had sometimes failed to reveal differences between enrichment broths.

The media used were Rappaport's magnesium chloride malachite green both with a reduced concentration of malachite green (Vasiliadis *et al.* 1970), strontium chloride B enrichment (Iveson, 1971), buffered peptone water (Anon, 1975) and brilliant green MacConkey agar (Harvey, 1956; Harvey & Price, 1974).

Twenty five millilitres of river water were added to 25 ml of double strength buffered peptone water. This was incubated at 37 °C for 18 h and constituted the pre-enrichment stage of the technique. Four tubes, each containing 10 ml of strontium chloride B medium were taken and each was inoculated from the incubated pre-enrichment medium. The first of the four tubes was seeded with one loopful (0·005 ml) the second and third with one drop (0·02 ml) and five drops (0·10 ml) respectively and the fourth with 0·5 ml. A graduated loop and graduated pipettes were used for loop and drop volume measurements. The four inoculated

tubes represented the enrichment stage. They were incubated at 37 °C for 48 h and were subcultured to brilliant green MacConkey agar at 24 and 48 h. Plates were incubated at 37 °C for 24 h and were examined by shaded daylight for salmonella colonies. These were picked to moist agar slopes for further identification. One hundred and twenty samples each of 25 ml of river water were examined in this way. The whole process was repeated with 80 samples of 25 ml of river water with the pre-enrichment incubation temperature kept at 37 °C and the incubation temperature of the strontium chloride broths raised to 43 °C in accordance with the observations of Iveson & Mackay-Scollay (1972). The purpose of these separate preliminary studies was to examine the effect of varying the size of inoculum of pre-enrichment culture introduced into the enrichment media under two different conditions, i.e., to study the inter-relation of inoculum ratio (Jameson, 1963; Harvey & Price, 1980) and enrichment incubation temperature. From these investigations, two inocula appeared suitable – one drop and five drops of pre-enrichment culture to 10 ml of strontium chloride B enrichment broth. The optimum inoculum for Rappaport's medium (R25) using preliminary growth of the sewage polluted water in buffered peptone water had been determined in an earlier study. This was one loopful (0·005 ml) of pre-enrichment culture to 10 ml of Rappaport's enrichment broth (Harvey & Price 1980).

For the comparison of Rappaport's medium (R25) with strontium chloride B, the test material was, as before, 25 ml of river water. This volume was added to 25 ml of double strength buffered peptone water and the mixture was incubated at 37 °C for 18 h. Four tubes of 10 ml of strontium chloride B were taken. Two were inoculated with one drop (0·02 ml) and two with five drops (0·1 ml) of the peptone water/river water culture. The Rappaport's broth (R25) was inoculated with one graduated loopful (0·005 ml) of the same culture (Harvey and Price 1980). One tube of strontium chloride B inoculated with one drop and one tube inoculated with five drops from the pre-enrichment culture were each incubated at 37 °C. The other two tubes of strontium chloride B were incubated at 43 °C. Incubation time was 48 h in both cases and subcultures were made at 24 and 48 h to brilliant green MacConkey agars. The plates were incubated at 37 °C for 24 h and examined for salmonella colonies which were picked to moist agar slopes for further identification. The Rappaport's broth was incubated at 37 °C for 48 h and subcultured to brilliant green MacConkey agar at 24 and 48 h. Plates were incubated at 37 °C for 24 h and examined for suspicious colonies as before.

RESULTS

The results are recorded in Tables 1–3. Table 1 indicates that, in the 37 °C study, the optimum size of inoculum of pre-enrichment culture added to the strontium chloride B medium was in the region of one drop (0·02 ml). Fewer salmonella isolations were noted with inocula of 5 drops (0·1 ml) and 0·5 ml. In the 43 °C series, however, the decline in the number of isolations started with the 0·5 ml inoculum. Although the numbers are small, Table 1 suggests that a shift of the optimum

Table 1. *Strontium chloride B medium: relation between size of inoculum added to enrichment broth and number of salmonella isolations at 37 and 43 °C*

	Inoculum introduced into enrichment				Enrichment incubation temperature
	1 loopful	1 drop	5 drops	0·5 ml	
Total salmonella isolations 24 + 48 h subcultures	26	27	21	12	37 °C
	36	39	39	25	43 °C

These were separate comparisons at 37 °C and 43 °C.

Table 2. *Salmonella isolations with strontium chloride B and Rappaport's medium*

Medium...	Strontium chloride				Rappaport
Incubation temperature...	37 °C		43 °C		37 °C
Inoculum size...	1 drop	5 drops	1 drop	5 drops	1 loopful
Salmonella isolations 24 + 48 h subcultures	19	9	16	41	54
'Sterile' plates					
24 h subcultures	0	0	32	0	0
48 h subcultures	0	0	41	12	0

The comparisons at 37 and 43 °C were simultaneous.

inoculum to a higher value has taken place in the 43 °C study. Two inocula were selected for the final comparison – one drop and five drops (0·02 ml and 0·1 ml).

Table 2 records the comparison of the two media. It confirms Iveson & Mackay-Scollay's (1972) observation that elevated temperature incubation (43 °C) improved the performance of stontium chloride B. A maximum number of 41 salmonella isolations were made at 43 °C compared with 19 at 37 °C.

Table 2, again underlines another important point – the association of the size of inoculum taken from the pre-enrichment culture and the incubation temperature of the enrichment medium. If the one drop inoculum in the 37 °C strontium chloride series is compared with the one drop inoculum in the 43 °C series the numbers of salmonella isolations are 19 and 16 respectively. Incubation at 37 °C thus has a marginal advantage over 43 °C incubation. It is only when the *optimum* inoculum for each incubation temperature is considered that the advantage of elevated temperature is revealed.

A note was made of the number of selective agars on which no growth was evident – the so-called 'sterile' plates. These are shown in Table 2. They are recorded separately for the 24 and 48 h subcultures and the greatest number occurs with the one drop inoculum at 43 °C. Finding many plates without visible growth

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Table 3. *Significance of comparison*

Strontium chloride medium positive: Rappaport's medium positive	40
Strontium chloride medium positive: Rappaport's medium negative	1
Strontium chloride medium negative: Rappaport's medium positive	14
χ^2 (MacNemar's test)	9·6
P	< 0·01

is an indication of the inhibitory nature of the 43 °C enrichment environment. A five drop inoculum was necessary to overcome this inhibition. Inocula greater than five drops were counterindicated as the strontium chloride enrichment then became less selective in exactly the same way as Rappaport's medium (Harvey & Price, 1980).

Tables 2 and 3 record that R25 broth was the more efficient of the two media studied.

DISCUSSION

Comparison between salmonella enrichment broths lacks validity unless each is tested under conditions optimum to its functioning. This would seem to be self evident, but studies are sometimes made without regard to this truism. Some early comparisons of Rappaport's original medium with other media failed to give adequate attention to the size of inoculum introduced into the magnesium chloride malachite green broth (Sen, 1964). When examining material containing minimal numbers of salmonellas, this factor is of paramount importance (Harvey & Price, 1980). Strontium chloride B medium uses the selective inhibitory action of the strontium ion instead of the magnesium ion of Rappaport's enrichment. The potential substitution of strontium chloride for magnesium chloride was recorded by Rappaport & Konforti (1959). It seemed appropriate to examine the performance of strontium chloride B with a range of different sized inocula in the same way as we had studied Rappaport's broth earlier (Harvey & Price, 1980). The preliminary trial allowed us to investigate the efficiency of the medium under better conditions. The shift in optimum inoculum to a higher value when strontium chloride B was incubated at 43 °C instead of 37 °C is suggested by Table 1 and is even more evident in Table 2. Had the 37 °C/43 °C comparison been made with an inoculum of one drop from the pre-enrichment culture, the advantage of 43 °C incubation would not have been observed. The size of the optimum volume introduced into the enrichment broth from the buffered peptone water culture, therefore, is a function of the incubation temperature. It is significant that Vassiliadis *et al.* (1978) found it advantageous to increase the inoculum from buffered peptone water to 0·1 ml, instead of one drop from a 3 mm loop, when using a modification of Rappaport's medium (R10) developed for 43 °C incubation

(Vassiliadis *et al.* 1976). The phenomenon of 'sterile' plates with 43 °C incubation was also noted in the latter paper.

Despite the advantage of elevated temperature incubation on the performance of strontium chloride B, we were not able to confirm the statement by Iveson & Mackay-Scollay (1972) that it was a better medium for salmonella isolation than Rappaport's broth. The formula of Rappaport used by Iveson & Mackay-Scollay (1972) contained a higher concentration of malachite green than that present in the medium used by ourselves (R25). This may be relevant to the results obtained.

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