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Salmonella isolation with Rappaport's medium after pre-enrichment in buffered peptone water using a series of inoculum ratios

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SUMMARY

The ability of malachite green/magnesium chloride broth (Rappaport's medium) to isolate salmonellas from 25 ml quantities of sewage-polluted natural water was investigated. Samples were first pre-enriched in buffered peptone water and varying volumes of inoculum from the pre-enrichment culture were inoculated into Rappaport's broth. Inoculum ratios in the range 1:2000 to 1:10 were examined. The inoculum ratio denotes the ratio of the volume of inoculum to the volume of fluid medium into which it is introduced. Optimum results were obtained with the 1:2000 ratio, although the salmonella isolation rate was only slightly less with the 1:500 and 1:100 ratios. The 1:2000 inoculum ratio was obtained with a graduated loop holding approximately 0·005 ml of fluid. Use of a loop for inoculation has advantages in speed of performance and safety of manipulation.

INTRODUCTION

Jameson (1963) recorded the importance of the ratio of the volume of inoculum introduced into a salmonella enrichment broth to the volume of enrichment medium receiving the inoculum. The term *inoculum ratio* was used to describe this variable. The ratios used by Jameson (1961) varied between 1:25 and 1:100 in a secondary enrichment technique. Provided the inoculum to the secondary enrichment medium contained at least one viable salmonella, the greater the ratio between the volume of the medium and the volume of the inoculum the greater, normally, was the opportunity for secondary enrichment to succeed. Jameson (1963) also suggested that an isolation technique involving primary enrichment in a relatively small volume of nutrient broth followed by secondary enrichment in a relatively larger volume of enrichment medium might be advantageous. This is akin to pre-enrichment with subsequent enrichment, a technique which has proved valuable in recovery of salmonellas from naturally contaminated water supplies (Harvey & Price, 1977).

Harvey, Price & Xirouchaki (1979) contrasted selenite F, Muller–Kauffmann tetrathionate and Rappaport's media using widely differing inoculum ratios. The current study concentrates on Rappaport's enrichment alone and extends the earlier investigation in an attempt to determine an optimum inoculum ratio from buffered peptone water pre-enrichment.

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MATERIALS AND METHODS

Materials used were buffered peptone water (Edel & Kampelmacher, 1973; Anon, 1975), magnesium chloride malachite green broth (Rappaport, Konforti & Navon, 1956; Vassiliadis *et al.* 1970) and 25 ml volumes of sewage-polluted natural water. The latter provided the test inoculum for the pre-enrichment stage of the trial (Harvey & Price 1977). An earlier investigation indicated that this naturally contaminated material was of value in comparative tests on media and revealed differences which were not apparent when artificially contaminated samples were used (Harvey, Price & Crone, 1975). The water specimens were taken from the river Taff on Mondays at 09.30 h near Pontypridd and brought direct to the laboratory. Many samples contained less than 10 salmonellas/100 ml (Harvey, Price & Xirouchaki, 1979).

Each 25 ml volume of sewage-polluted water was added to 25 ml of double-strength buffered peptone water and incubated at 37 °C for 18 h. Five 10 ml quantities of Rappaport's medium were taken and the first of these was inoculated with a graduated loopful* (0·005 ml) of the buffered peptone water culture. The second tube of enrichment medium received one drop (0·02 ml) of the pre-enrichment/polluted water culture. The third, fourth and fifth tubes were inoculated in the same way with 5 drops (0·1 ml), 0·5 ml and 1·0 ml respectively. The inoculum ratios from tube 1 to tube 5 were, therefore, 1:2000, 1:500, 1:100, 1:20 and 1:10. Ten 25 ml quantities of river water were examined in this way each week so that 10 pre-enrichment cultures were prepared each Monday and 50 enrichment cultures each Tuesday for several months. The 50 inoculated enrichment broths were incubated at 37 °C for 48 h and were subcultured to brilliant green MacConkey agar (Harvey, 1956; Harvey & Price, 1974) at 24 h and 48 h. Selective agars were incubated at 37 °C for 24 h and examined for salmonellas.

RESULTS

Table 1 records salmonella isolations from each of tubes 1 to 5. There is little difference in isolation rate in tubes 1, 2 and 3 but the salmonella recovery drops sharply in tubes 4 and 5. All the positive samples revealed in tube 4 were also obtained in tube 3, and 33 additional positive samples were demonstrated in the latter tube. In this study, inoculum ratios of 1:2000 to 1:100 were necessary to ensure satisfactory functioning of Rappaport's magnesium chloride/malachite green enrichment broth.

DISCUSSION

Harvey *et al.* (1979) in a comparative study of selenite F, Muller–Kauffmann tetrathionate and Rappaport's media using two inoculum ratios (1:2000 and 1:10) recorded optimum results with Rappaport's enrichment broth with the 1:2000 inoculum. In contrast, a greater number of salmonella isolations was obtained with the 1:10 ratio than the 1:2000 using selenite F and Muller–Kauffmann tetrathionate

* Obtained from Medical Wire Equipment Co. (Bath) Ltd. Pooley, Corsham, Wiltshire.

Table 1. *Isolation of Salmonellas from Rappaport's medium using different inoculum ratios*

Tube no.	1	2	3	4	5
Volume (ml) of pre-enrichment culture added to 10 ml Rappaport's medium	0·005	0·02	0·1	0·5	1·0
Inoculum ratio	1:2000	1:500	1:100	1:20	1:10
Positive salmonella isolations	95	94	92	59	35

media. A similar trend has been noted by Collard & Unwin (1958), indicating that Jameson's (1963) hypothesis is not applicable to all enrichment broths.

In our current study, there was no significant difference in salmonella isolation in the range of inoculum ratios 1:2000–1:100 (Table 1). The purity of salmonella growth was, however, greatest with the 1:2000 ratio. Use of a loop (as in the 1:2000 ratio) has much to commend it technically for speed and convenience. Inoculation of culture media with sterile graduated capillary pipettes, *particularly if mouth pipetting is avoided for safety reasons*, slows performance in a laboratory examining many samples potentially contaminated with salmonellas. We have, therefore, adopted a graduated loop (0·005 ml) as optimum means of inoculating a 10 ml volume of Rappaport's medium from a pre-enrichment culture in buffered peptone water. It is possible that the volume of the enrichment medium could be less than 10 ml, and if it was reduced to 4 ml the inoculum ratio using a 0·005 ml loop would be 1:800. Such a volume could be contained in a small screw-capped bottle – a bijou bottle. Economy in cost and space would result. This modification in technique is under investigation.

The success of Rappaport's enrichment in demonstrating the presence of salmonellas in a loopful (0·005 ml) of an unselective fluid medium after 18 h incubation at 37 °C is in accord with calculation on the multiplication of *S. typhi* in nutrient broth (Loeffler, 1906). The relative failure of selenite F and Muller–Kauffmann tetrathionate to reveal salmonellas in 0·005 ml of buffered peptone water culture of sewage-polluted water (Harvey *et al.* 1979) suggests that Rappaport is the most sensitive of the three enrichment media for salmonella isolation. Sensitivity implies the ability of a medium to demonstrate the presence of a minimal number of salmonellas.

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