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A study of the relative efficiencies of three commercially available dehydrated Rappaport–Vassiliadis media

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SUMMARY

The relative efficiencies of three commercially available dehydrated Rappaport–Vassiliadis media have been compared with a similar medium prepared from individual constituents in our own laboratory. An inoculation ratio of 1 : 100 was found to be optimal for each of the media tested. Laboratory produced RV-medium was significantly better than the three commercial preparations after 24 h incubation. However, when the duration of incubation was extended to 48 h, there was no significant difference in the number of salmonella isolates obtained when using our own RV medium and that produced by Oxoid Ltd and Difco Ltd ($P > 0{\cdot}05$). All of these three media were, however, significantly more effective than the medium produced by Lab.M. ($P < 0{\cdot}01$).

INTRODUCTION

Procedures for the isolation of salmonellae from food and water samples have been studied intensively and whilst reference procedures have been described (Anon, 1975) many different procedures are currently in use. The International Organization for Standardization (Anon, 1975) recommends the use of tetrathionate broth for the enrichment stage although the results of many studies (reviewed by Vassiliadis, 1983) have demonstrated that Rappaport-Vassiliadis (RV) medium (Vassiliadis *et al.* 1976) is more efficient for the isolation of salmonellae from a variety of sample types. One of the major disadvantages of the RV medium has been that it has not been commercially available as a dehydrated product. Recently, however, several companies have marketed a dehydrated Rappaport medium. We have previously reported on the efficiency of one such product, produced by Oxoid Ltd, for the isolation of salmonellae from poultry samples (Fricker *et al.* 1985). This study was undertaken to determine the relative efficiencies of three commercially available dehydrated magnesium chloride-malachite green broths.

MATERIALS AND METHODS

Samples

A total of 386 samples of chicken giblets were removed from freshly thawed oven ready poultry and held at 4 °C for a maximum of 12 h prior to use. The liver was

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Table 1. *Number of salmonella isolations obtained using four enrichment media at five inoculation ratios*

Inoculation ratio	Duration	Number of positive samples			
		RV-soya	RV-Oxoid	RV-Difco	RV-Lab M
1:20	24 h	19	19	21	22
1:20	48 h	23	25	24	26
1:50	24 h	40	41	38	35
1:50	48 h	43	44	45	40
1:100	24 h	53	45	44	40
1:100	48 h	55	52	51	43
1:200	24 h	51	42	42	37
1:200	48 h	55	50	49	43
1:1000	24 h	31	32	33	29
1:1000	48 h	39	36	38	32

Table 2. *Number of samples from which salmonella were isolated with four enrichment media using an inoculation ratio of 1:100*

	RV-soya	RV-Oxoid	RV-Difco	RV-Lab M
No. of samples examined ...	386	386	386	386
Salmonella isolated after 24 h	199	174	169	150
Percentage positive for salmonella after 24 h	51·6	45·1	43·8	38·9
Salmonella isolated after 48 h	203	194	192	171
Percentage positive for salmonella after 48 h	52·6	50·3	49·7	44·3
Total salmonella isolates (24 h or 48 h)	205	200	198	186
Percentage salmonella isolated (24 h or 48 h)	53·1	51·8	51·3	48·2

selected from each packet of giblets, cut into approximately 5 mm slices and placed into 100 ml of buffered peptone water.

Media

Buffered peptone water (BPW) was prepared according to the methods of Edel & Kampelmacher (1973). Brilliant green agar containing sulphamandelate supplement (BGASM) (Watson & Walker, 1978) was prepared as described previously (McGibbon, Quail & Fricker, 1984) and used on the day of preparation (Fricker & Girdwood, 1984).

The enrichment media were obtained from Oxoid Ltd (RV.Oxoid), Difco Ltd (RV.Difco) and Lab.M (RV.Lab.M). In addition Rappaport-Vassiliadis medium containing soya peptone (RV-soya) as described by van Schothorst & Renaud (1983), was also used. The commercial media were prepared according to the manufacturer's instructions. All four enrichment media were distributed in 5 ml volumes and sterilized by autoclaving at 115 °C for 15 min.

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Table 3. *Statistical comparison (MacNemar's test for paired samples) of the number of salmonella isolates obtained after 24 h enrichment in four different RV media*

			Number of samples	χ^2	P
RV-soya	+ RV-Oxoid	+	174	25·0	< 0·001
RV-soya	+ RV-Oxoid	−	25		
RV-soya	− RV-Oxoid	+	0		
RV-soya	+ RV-Difco	+	168	28·2	< 0·001
RV-soya	+ RV-Difco	−	31		
RV-soya	− RV-Difco	+	1		
RV-soya	+ RV-Lab M	+	150	49·0	< 0·001
RV-soya	+ RV-Lab M	−	49		
RV-soya	− RV-Lab M	+	0		
RV-Oxoid	+ RV-Difco	+	166	2·4	< 0·10
RV-Oxoid	+ RV-Difco	−	8		
RV-Oxoid	− RV-Difco	+	3		
RV-Oxoid	+ RV-Lab M	+	149	22·2	< 0·001
RV-Oxoid	+ RV-Lab M	−	25		
RV-Oxoid	− RV-Lab M	+	1		
RV-Difco	+ RV-Lab M	+	148	15·7	< 0·001
RV-Difco	+ RV-Lab M	−	21		
RV-Difco	− RV-Lab M	+	2		

Procedures

The chicken livers were incubated in BPW for 24 h at 37 °C. A total of 106 samples were used to determine the optimum inoculation ratio of each of the four media. Five ratios were compared, 1 : 20, 1 : 50, 1 : 100, 1 : 200 and 1 : 1000. For this each pre-enrichment culture was inoculated into five enrichment broths of each type (250, 100, 50, 25 and 5 μl respectively). The enrichment cultures were incubated at 43 °C for 48 h, with subcultures being made to BGASM at 24 and 48 h. Solid media were incubated at 37 °C for 24 h and up to four presumptive salmonella colonies were identified by standard biochemical and serological procedures.

The remaining 280 samples were processed in the manner described above, except that an inoculation ratio of 1 : 100 was used for all samples. The results obtained were analysed by MacNemar's test for paired samples.

RESULTS

The results of the study of inoculation ratios showed that 1 : 100 and 1 : 200 were superior to the other ratios tested ($P < 0{\cdot}05$) although there was no significant difference between 1 : 100 and 1 : 200 ($P > 0{\cdot}5$) for any of the media tested. An inoculation ratio of 1 : 100 resulted in a slightly higher salmonella isolation rate than 1 : 200 with RV-Oxoid and RV-Difco whilst the two ratios were equally effective for RV-soya and RV-Lab M. Table 1 shows the results obtained with different inoculation ratios for each of the four media.

Comparison of the four enrichment media showed that RV-soya (produced from individual constituents in our own laboratory) was the most efficient medium (205 salmonella-positive samples) and RV-Lab M the least efficient (186 positive samples). RV-soya was significantly more efficient than all three media after 24 h

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Table 4. *Statistical comparison (MacNemar's test for paired samples) of the total number of salmonella isolates obtained using four enrichment media plated out at 24 h and 48 h*

			Number of samples	χ^2	P
RV-soya	+ RV-Oxoid	+	199		
RV-soya	+ RV-Oxoid	−	6	2·3	> 0·10
RV-soya	− RV-Oxoid	+	1		
RV-soya	+ RV-Difco	+	194		
RV-soya	+ RV-Difco	−	11	3·3	> 0·05
RV-soya	− RV-Difco	+	4		
RV-soya	+ RV-Lab M	+	185		
RV-soya	+ RV-Lab M	−	20	17·2	< 0·001
RV-soya	− RV-Lab M	+	1		
RV-Oxoid	+ RV-Difco	+	195		
RV-Oxoid	+ RV-Difco	−	5	0·625	> 0·25
RV-Oxoid	− RV-Difco	+	3		
RV-Oxoid	+ RV-Lab M	+	183		
RV-Oxoid	+ RV-Lab M	−	17	9·85	< 0·05
RV-Oxoid	− RV-Lab M	+	3		
RV-Difco	+ RV-Lab M	+	182		
RV-Difco	+ RV-Lab M	−	16	7·25	< 0·01
RV-Difco	− RV-Lab M	+	4		

($P < 0·05$). After 48 h however, there was no significant difference between RV-soya and either RV-Oxoid or RV-Difco, although RV-Lab M was still significantly less efficient ($P < 0·05$). The number of samples from which salmonella was isolated at 24 h and 48 h with all four media is shown in Table 2. Table 3 shows the statistical comparison of salmonella isolations after 24 h incubation and Table 4 shows the comparison of the total salmonella isolations made by plating at 24 h and 48 h.

DISCUSSION

The use of commercially available dehydrated culture media has become widespread in most microbiology laboratories. Whilst it may be argued that production of large batches of media by commercial companies results in a more standardized product, some workers have questioned the efficiency of such media when used to isolate salmonellas from environemental samples (Harvey, Price & Crone, 1975). In a recently reported evaluation of commercially available Rappaport–Vassiliadis medium (RV-Oxoid), Fricker *et al.* (1985) demonstrated that if the duration of incubation of enrichment cultures was extended to 48 h the performance of RV-Oxoid was similar to RV-medium prepared from individual constituents in our own laboratories. The data presented here confirms our previous findings with RV-Oxoid and also demonstrates that RV-Difco is of a similar quality. However, the product marketed by Lab M appears to be somewhat less efficient.

The importance of correct inoculation ratios has again been demonstrated, confirming our previous findings that ratios of 1 : 100 and 1 : 200 are superior to 1 : 20 or 1 : 1000 (Fricker, 1984*a*, *b*; Fricker & Girdwood, 1985).

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Despite repeated reports of the superior efficiency of RV-medium over selenite and tetrathionate broths (Fricker, Girdwood & Munro, 1983; Fricker 1984*a*; Vassiliadis, 1983), some workers continue to use the latter two media in preference to RV-medium (e.g. Juven *et al.* 1984; Wray & Callow, 1985). Perhaps the most likely explanation of this is that until recently RV-medium has not been available as a dehydrated product. Now that this situation has been remedied it is important that we know the relative efficiencies of the products marketed by different companies and the conditions under which they function best. On the basis of this study we would recommend that if commercial RV-medium is to be used, the products marketed by Oxoid Ltd and Difco Ltd are of similar efficiency and that, provided an inoculation ratio of 1 : 100 is used and the enrichment cultures incubated for 48 h, then the results are similar to those obtained using RV-medium prepared from individual constituents.

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