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An evaluation of commercially available dehydrated Rappaport-Vassiliadis medium for the isolation of salmonellae from poultry

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SUMMARY

A total of 745 samples of chicken giblets was cultured to determine the relative efficiency of a commercially available Rappaport-Vassiliadis medium (RV-Oxoid). Experiments to determine the optimum inoculation ratio showed that 1:100 was superior to the other ratios tested. Comparison of RV-Oxoid with standard RV and RV-medium prepared using soya peptone (RV-soya) showed that after 24 h RV-soya was significantly better than RV-Oxoid ($P < 0{\cdot}05$), although there was no significant difference between standard RV and RV-Oxoid. Furthermore, when the duration of incubation was extended to 48 h there was no significant difference between the three media ($P > 0{\cdot}25$).

We conclude that RV-Oxoid is a satisfactory product for the isolation of salmonellae from poultry, providing that it is inoculated at a ratio of 1:100 and is incubated for 48 h. Its use can therefore be recommended to laboratories who wish to use a dehydrated medium.

INTRODUCTION

Since its original description in 1956 (Rappaport, Konforti & Navon, 1956) Rappaport's medium has become widely used for the isolation of salmonellae. Early studies on the use of Rappaport's medium for isolating salmonellae from human faeces were encouraging (Collard & Unwin, 1958; Hooper & Jenkins, 1965; Iveson, Kovacs & Lauric, 1964) although Sen (1964) was disappointed with its performance. In 1970 Vassiliadis and colleagues (Vassiliadis *et al.* 1970) modified the medium by reducing the concentration of malachite green slightly (R25) and in 1976 the same group of workers further modified the medium (R10) such that it became suitable for incubation at 43 °C (Vassiliadis *et al.* 1976). This medium has subsequently become known as Rappaport-Vassiliadis (RV) medium (Papadakis & Efstratiou, 1980). Since 1976 the results of many studies of the relative efficiency of RV medium have been published, and whilst some workers maintain that the R25 medium is superior (Harvey & Price, 1983), the majority of studies

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have shown RV medium to be at least as efficient as R25 (Fricker, Girdwood & Munro, 1983; Fricker, 1984*a*; Vassiliadis *et al.* 1979) and in some cases to be significantly better (Vassiliadis *et al.* 1984; Fricker & Girdwood, 1985).

Further attempts have been made to improve the RV medium and Alcaide *et al.* (1982) suggested that the addition of novobiocin to the medium (NR10) made it suitable for use as a direct enrichment broth for the examination of polluted water. Furthermore, the same group of workers suggested that direct inoculation of NR10 yielded more salmonella isolates than RV medium inoculated after pre-enrichment in buffered peptone water (Alcaide, Martinez & Garay, 1984). However, prompted by the original report on direct enrichment using NR10, Fricker (1984*b*) performed similar experiments and found that RV medium used after pre-enrichment gave significantly better recoveries than NR10 inoculated directly. The discrepancy in the results of these studies is almost certainly due to the different inoculation ratios used, since Alcaide and colleagues used a ratio of 1:10 when subculturing from pre-enrichment cultures; whereas the more widely accepted 1:100 was used by Fricker.

Although Rappaport stressed the importance of Bacto-tryptone in his medium (Rappaport, Konforti & Navon, 1956; Rappaport & Konforti, 1959) recent work has suggested that substitution of other peptones such as soya or mycological peptone may be beneficial (van Schothorst & Renaud, 1983). In comparisons of the efficiency of RV medium containing soya peptone (RV-soya) with that of the standard RV medium, we have demonstrated that RV-soya is a marginally better medium (Fricker & Girdwood, 1985; McGibbon, Quail & Fricker, 1984).

Commercially available dehydrated culture media have now found a place in most microbiological laboratories and are particularly convenient for small laboratories where facilities for media production are limited. Although there is disagreement among microbiologists as to the acceptability of some dehydrated media, convenience makes it desirable for their use to be extended, provided that the quality of microbiology does not suffer. To many small laboratories a major disadvantage of RV medium has been that a commercially dehydrated medium has not been available. Recently, however, Oxoid Limited (Basingstoke, UK) have produced such a medium, based on the RV-soya medium described by van Schothorst & Renaud (1983). We have therefore compared the commercially produced medium (RV-Oxoid) with RV and RV-soya made from individual constituents in our own laboratories.

MATERIALS AND METHODS

Preparation of samples

A total of 745 samples of chicken giblets was removed from freshly thawed oven-ready chickens and held at 4 °C for a maximum of 12 h before examination. The neck was removed from each packet and placed in 100 ml of buffered peptone water for culture.

Media

Buffered peptone water (BPW) was prepared according to the methods of Edel & Kampelmacher (1973), distributed in 100 ml amounts in screw-capped glass jars

and autoclaved at 121 °C for 15 min. The standard Rappaport–Vassiliadis (RV) medium was prepared as previously described (Vassiliadis *et al.* 1976) and the RV-soya medium was made by substituting soya peptone (Oxoid L44) for the tryptone in the original medium. RV-Oxoid was prepared by dissolving 30 g of the dehydrated product in 1 l of distilled water using gentle heat. All forms of Rappaport's medium were distributed in 10 ml volumes and autoclaved at 115 °C for 15 min. The pH of the medium was checked after autoclaving and was used only if in the range 4·95–5·05. All enrichment media were used within 1 week of preparation.

The plating medium used in this study was brilliant green agar containing sulphamandelate supplement (BGASM; Watson & Walker, 1978). It was prepared as previously described and used on the day of preparation (Fricker & Girdwood, 1984).

Determination of optimum inoculation ratio for commercial RV medium

To determine the optimum inoculation ratio 247 samples of chicken giblets were used. Pre-enrichment cultures were incubated for 24 h at 37 °C and inoculated into 10 ml volumes of the three RV media at ratios of 1:20, 1:50, 1:100, 1:200 and 1:1000. Enrichment cultures were then incubated at 43 °C and plated on BGASM at 24 and 48 h. Plates were incubated at 37 °C for 24 h and up to four presumptive salmonella colonies identified by standard biochemical and serological procedures.

Comparison of commercial RV medium with two forms of RV medium prepared from individual constituents in the laboratory

A total of 498 samples of chicken giblets were used to compare the efficiency of RV-Oxoid medium with that of the standard RV and RV-soya media. From the pre-enrichment cultures 0·1 ml was subcultured into 10 ml volumes of each of the three formulations of RV medium. Enrichment cultures were then incubated at 43 °C and plated on BGASM after 24 h. Samples where one or two (but not all three) of the enrichment cultures yielded salmonellae after 24 h incubation were also plated after 48 h. Presumptive salmonellae were identified as described above.

The results obtained were compared using MacNemar's test for paired samples.

RESULTS

Of the 247 samples used to determine the optimum inoculation ratios of the three media, a total of 54 were found to contain salmonellae with one or more of the inoculation ratios studied. The best system used was inoculation of RV-soya at a ratio of 1:100. The results obtained from this experiment are shown in Table 1. A ratio of 1:100 was the optimum for each of the three media studied, although the differences in the number of salmonellae isolated using 1:100 and 1:200 were small in every case. Table 2 shows the statistical comparison of the five inoculation ratios for the commercial RV medium.

After the optimum inoculation ratio had been determined for each of the three formulations of RV medium, a further 498 samples were cultured to compare the efficiencies of the three media. A total of 124 was found to contain salmonellae

Table 1. *Salmonella isolations made from 247 samples of chicken giblets cultured by pre-enrichment followed by enrichment in 10 ml of three formulations of Rappaport–Vassiliadis medium, using different inoculation ratios*

Inoculation ratio...	1:20	1:50	1:100	1:200	1:1000
Volume of pre-enrichment culture transferred (ml)	0·5	0·2	0·1	0·05	0·01
	Number of salmonella isolates				
Standard RV medium	20	41	49	49	42
RV-soya	24	44	53	51	40
RV-Oxoid	18	40	47	46	37

Table 2. *Statistical comparison (MacNemar's test for paired samples) of five inoculation ratios used for the isolation of salmonellae from 247 samples of chicken giblets*

		χ^2	P
1:100 +ve 1:20 +ve	18	20·0	< 0·001
1:100 +ve 1:20 −ve	20		
1:100 −ve 1:20 +ve	0		
1:100 +ve 1:50 +ve	30	5·6	< 0·025
1:100 +ve 1:50 −ve	8		
1:100 −ve 1:50 +ve	1		
1:100 +ve 1:200 +ve	45	0·7	> 0·5
1:100 +ve 1:200 −ve	2		
1:100 −ve 1:200 +ve	1		
1:100 +ve 1:1000 +ve	36	8·4	< 0·005
1:100 +ve 1:1000 −ve	11		
1:100 −ve 1:1000 +ve	1		

Table 3. *Number of samples from which salmonellae were isolated using three formulations of Rappaport–Vassiliadis medium after inoculation at a ratio of 1:100 and incubation at 43 °C for 24 h*

Medium	No. examined	No. positive at 24 h	Positive %
Standard RV	498	95	19·1
RV-soya	498	104	20·9
RV-Oxoid	498	92	18·5

after 24 h incubation with at least one of the enrichment media although none of the media allowed the isolation of salmonellae from all samples. RV-soya was shown to be the most efficient medium, 104 samples being positive with this medium. The number of salmonella isolations made after 24 h using the three media is shown in Table 3, and the statistical comparison of these results is shown in Table 4. The difference between standard RV medium and RV-soya after 24 h incubation is not statistically significant ($P < 0·05$) although RV-soya did facilitate the isolation of more salmonellae than did standard RV medium. Whilst RV-soya was shown to be significantly better than RV-Oxoid ($P < 0·05$), the

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Table 4. *Statistical comparison of the efficiency of standard RV, RV-soya and RV-Oxoid in recovering salmonellae from chicken giblets after 24 h incubation*

			χ^2	P
RV-soya +ve	RV +ve	83		
RV-soya +ve	RV −ve	21	2·5	> 0·05
RV-soya −ve	RV +ve	12		
RV-soya +ve	Oxoid RV +ve	82		
RV-soya +ve	Oxoid RV −ve	22	4·5	< 0·05
RV-soya −ve	Oxoid RV +ve	10		
RV +ve	Oxoid RV +ve	76		
RV +ve	Oxoid RV −ve	19	0·3	> 0·5
RV −ve	Oxoid RV +ve	16		

Table 5. *Salmonella isolations at 24 and 48 h from 50 samples which were positive using at least one but not all three formulations of Rappaport–Vassiliadis medium after 24 h incubation*

	Standard RV	RV-soya	RV-Oxoid
No. of samples plated at 24 and 48 h	50	50	50
No. positive after 24 h	22	31	19
Positive after 24 h (%)	44	62	38
No. positive after 48 h	35	38	35
Positive after 48 h (%)	70	76	70

Table. 6. *Statistical comparison of the efficiency of three formulations of Rappaport–Vassiliadis medium in isolating salmonellae from chicken giblets after 48 h incubation*

	No. of samples	χ^2	P
RV-soya +ve Standard RV +ve	26		
RV-soya +ve Standard RV −ve	12	0·5	> 0·25
RV-soya −ve Standard RV +ve	9		
RV-soya +ve RV-Oxoid +ve	29		
RV-soya +ve RV-Oxoid −ve	9	0·7	> 0·25
RV-soya −ve RV-Oxoid +ve	6		
Standard RV +ve RV-Oxoid +ve	25		
Standard RV +ve RV-Oxoid −ve	10	0·05	> 0·75
Standard RV −ve RV-Oxoid +ve	10		

difference between standard RV medium and RV-Oxoid was not significant ($P < 0·5$).

Fifty samples showed differences in the media after 24 h incubation, and of these, 49 were positive by at least one procedure after 48 h incubation. RV-soya was the most efficient medium after 48 h incubation, detecting salmonellae in 38 of the 49 samples which were found to be positive after this time. Table 5 shows the number of samples found to contain salmonellae after 48 h incubation and Table 6 shows the statistical comparison of the results. Clearly the difference in the relative efficiencies of RV-soya and RV-Oxoid is reduced if the media are plated at 48 h ($P > 0·25$).

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Table 7. *Statistical analysis (using MacNemar's test for paired samples) of the number of salmonella isolations made from 50 samples plated at 24 and 48 h for three formulations of Rappaport–Vassiliadis medium*

	No. of samples	χ^2	P
RV-soya			
24 h +ve 48 h +ve	30		
24 h +ve 48 h −ve	1	5·6	< 0·025
24 h −ve 48 h +ve	8		
Standard RV			
24 h +ve 48 h +ve	21		
24 h +ve 48 h −ve	1	5·6	< 0·025
24 h −ve 48 h +ve	8		
RV-Oxoid			
24 h +ve 48 h +ve	18		
24 h +ve 48 h −ve	1	14·3	< 0·001
24 h −ve 48 h +ve	17		

Table 7 shows the statistical comparison of plating the three enrichment media at 24 and 48 h. It can be seen that the increase in salmonella isolations obtained by plating the enrichment media at 48 h is significant ($P < 0·025$).

DISCUSSION

Commercially prepared dehydrated media are used extensively in microbiology laboratories and are particularly useful in small laboratories where facilities are limited. Whilst it has been suggested that commercially prepared products are often not as efficient as those produced from individual constituents (Harvey, Price & Xirouchaki, 1979) their convenience makes their use desirable. However, before any dehydrated product is used for routine work its efficiency should be compared with the same medium prepared from individual constituents. Recently, Rappaport–Vassiliadis medium has become available as a dehydrated product and it is essential that controlled trials are carried out to determine the relative efficiencies of these media against that of laboratory-prepared RV medium. This study has compared the RV medium prepared by Oxoid Ltd with RV and RV-soya produced in our own laboratories from individual constituents.

The importance of inoculation ratios in the isolation of salmonellae has been demonstrated by many workers (Jameson, 1961; 1963; Harvey & Price, 1980; Fricker, 1984*a*) and a ratio of 1:100 has been recommended for RV medium (Vassiliadis, 1983). In this study, an inoculation ratio of 1:100 was shown to be superior to 1:20, 1:50, 1:200 and 1:1000 for isolating salmonellae from chicken giblets after pre-enrichment in buffered peptone water, for all three formulations of Rappaport's medium used. For RV-Oxoid, 1:100 was significantly better than 1:20 ($P < 0·001$), 1:50 ($P < 0·025$) and 1:1000 ($P < 0·005$) although there was no significant difference between 1:100 and 1:200 ($P > 0·5$).

Subculture of the three enrichment media after 24 h yielded salmonellae from a total of 124 samples. RV-soya was the most efficient medium, detecting 104 positive samples. A significant difference was demonstrated between the number

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of positive samples obtained with RV-soya and RV-Oxoid ($P < 0{\cdot}05$) although the differences between RV-soya and standard RV ($P > 0{\cdot}05$) and standard RV and RV-Oxoid ($P > 0{\cdot}5$) were not significant. However, when the enrichment cultures from the 50 samples which showed differences after 24 h incubation were plated after 48 h, the difference between all the media were reduced to non-significant levels ($P > 0{\cdot}25$). It has been suggested that incubation of RV medium can be limited to 24 h (Vassiliadis, 1983) and other workers have confirmed this when using RV-soya (van Schothorst & Renaud, 1983). In this study, however, a significant difference in the number of salmonella isolates obtained after 24 and 48 h was demonstrated for all three formulations of Rappaport's medium and we therefore suggest that incubation of enrichment cultures for the isolation of salmonellae from poultry samples should be extended to 48 h.

In this comparison of RV-Oxoid and standard RV and RV-soya prepared in our own laboratory, the commercial medium recovered salmonellae from almost as many samples as did the two media produced in the laboratory. If the duration of incubation is extended to 48 h, the differences between the three media are not significant and we are therefore able to recommend RV-Oxoid for the isolation of salmonellae from poultry products provided that the duration of incubation is extended to 48 h. It is likely that the performance of this medium will be similar when used for other samples although this must be tested by experiment. Further studies are required to determine batch variation and to compare the efficiencies of other commercially prepared RV media.

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