

## **A comparison of the original Rappaport medium (R medium) and the Rappaport–Vassiliadis medium (RV medium) in the isolation of salmonellae from meat products**

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### SUMMARY

The Rappaport–Vassiliadis enrichment medium (RV medium) in 10 ml quantities (RV/43 °C, 10 ml) inoculated with 0·1 ml of pre-enrichment medium (P medium) was found more efficient in the isolation of salmonellae from 409 pre-enriched samples (mainly meat products), than the original Rappaport medium incubated at 43 °C (R/43 °C) and the RV medium in 5 ml quantities (RV/43 °C, 5 ml) inoculated with 0·01 ml of P medium ( $P < 0\cdot001$ , in both instances). Therefore, the inoculum from pre-enriched foods should not be less than 0·1 ml in 10 ml of RV medium.

The RV/43°, 10 ml was also better ( $P < 0\cdot01$ ) in detecting samples containing salmonellas than the original Rappaport medium incubated at 37 °C (R/37 °C, 10 ml) and the modification R25 of R medium incubated at 37 °C. The R25 modification was used in 10 ml quantities (R25/37 °C, 10 ml) inoculated with 0·1 ml of P medium and in 5 ml quantities (R25/37°, 5 ml) inoculated with 0·01 ml of P medium. The last two R25 procedures were of the same efficiency in isolating salmonellas from meat products.

### INTRODUCTION

The original Rappaport medium (R medium) (Rappaport, Konforti & Navon, 1956) as an enrichment medium for salmonellas was modified twice. In 1970 a minor modification was introduced (Vassiliadis *et al.* 1970), which was later called the R25 medium (Vassiliadis *et al.* 1976, 1977, 1978*a, b*). In 1976, two major modifications were introduced in the composition and in the use of the original R medium. The new medium was at first called R10 medium (Vassiliadis *et al.* 1976, 1977, 1978*a, b*) and later it was given the name Rappaport–Vassiliadis enrichment medium or RV medium (Papadakis & Efstratiou, 1980). It was found that, after pre-enrichment, the R25 and the RV enrichment media, were more sensitive than the original R medium (Vassiliadis *et al.* 1970, 1981*a*), as well as than the tetrathionate and selenite broths in recovering salmonellas from a variety of samples, including food products. These results were found in fifteen different studies made by our group and summarized by Vassiliadis (1983) and by Kalapothaki *et al.* (1983). Although

the R25 medium was frequently as sensitive as the RV medium the latter was preferred because it inhibits much better the competitive organisms (Vassiliadis, 1983).

The findings concerning the R25 medium were confirmed by Harvey, Price & Xirouchaki (1979), Harvey & Price (1981, 1982*a*) and Fricker, Girdwood & Munro (1983). On the other hand, Alcaide *et al.* (1982), using RV medium with the addition of novobiocin and van Schothorst & Renaud (1983), using RV medium made with soya peptone showed that the RV medium was significantly more efficient than selenite broth or Muller–Kauffmann tetrathionate broth (MK-ISO), respectively. Furthermore Fricker *et al.* (1983), reported also that RV medium was significantly more efficient than Muller–Kauffmann tetrathionate and selenite F broths, in the isolation of salmonellae from pre-enriched seagull cloacal swabs. In a recent paper Tongpim *et al.* (1984), reported also that the RV medium was significantly more sensitive in recovering salmonellae (from two sorts of meat products), than Muller–Kauffmann's tetrathionate broth. The RV medium was also found by these authors to be more specific.

However, the original R medium is still used in epidemiological studies (Jones *et al.* 1982, 1983) and even incubated at 43 °C (Williams *et al.* 1981). For this reason a comparative evaluation of R enrichment media of different formulas, inoculated with different inocula, incubated at 37 °C and at 43 °C was made. The results of this investigation are recorded in the current paper.

#### MATERIALS AND METHODS

*Samples.* From September 1982 until December 1983, 409 specimens were examined for the presence of salmonellae. Of these samples 398 were meat products and the remaining were seven specimens of animal feeds and four samples of sewage on Moore swabs. The nature of the samples examined is shown in Table 1.

The minced meat was frozen bovine meat imported from various Latin American, South African and Western European countries. The pork sausages were prepared in 26 small factories. Among the chicken carcasses examined, 66 were refrigerated and came from four different big owners of poultry farms in Greece, each of them having his own poultry processing plant, whereas the remaining 25 carcasses were imported frozen from two Western European countries. The weight of the chicken carcasses from Greece was between 1500 and 1900 g, while the weight of those imported was between 700 and 900 g. The dried powdered chicken meat was intended to be used with other ingredients, for preparing soups.

*Pre-enrichment of the samples.* The specimens were brought in the laboratory early on Mondays. They were pre-enriched in buffered peptone water (Edel & Kampelmacher, 1973; Anonymous, 1975) (P medium) at 37 °C for 18–22 h. To each of 25 g of bovine minced meat, of finely cut pork sausages, of dried powdered chicken meat, and of animal feeds, 225 ml of P medium were added and mixed with the sample before incubation. To each Moore swab in big jars 500 ml of P medium were added and the jars were incubated as described above. The chicken carcasses were washed in sterile plastic bags with 500 ml of P medium, the carcasses were removed and the wash was incubated at 37 °C for 18–22 h.

*Rappaport's enrichment media.* The preparation of the original R medium is

described by Rappaport *et al.* (1956). The R25 modification was introduced by Vassiliadis *et al.* (1970) and was named in 1976 R25 medium (Vassiliadis *et al.* 1976). Finally the RV medium was introduced by Vassiliadis *et al.* (1976). Its preparation is described in detail by Vassiliadis *et al.* (1976, 1977, 1981*a, b*) and by Vassiliadis (1983). The R medium and the R25 medium are intended to be incubated at 37 °C, while the RV medium *must* be incubated at 43 °C.

*Procedures with R media.* Two test tubes, each containing 10 ml of the original Rappaport medium, were inoculated with 0.1 ml of P medium. One tube was incubated at 37 °C (R/37 °C, 10 ml) and the other at 43 °C (R/43 °C, 10 ml). Two more tubes, one containing 10 ml of R25 medium (R25/37 °C, 10 ml) and the other 5 ml of the same medium (R25/37 °C, 5 ml) were seeded, the first with 0.1 ml of P medium and the second with one loopful (approx. 0.01 ml) of P medium. Both these tubes were incubated at 37 °C. Finally one tube containing 10 ml of RV medium was inoculated with 0.1 ml of P medium and another tube containing 5 ml of RV medium was seeded with a loopful (approx. 0.01 ml) of P medium. Both tubes containing the RV medium were incubated at 43 °C, (RV/43 °C, 10 ml and RV/43 °C, 5 ml, respectively). All the six tubes of R media were incubated for 48 h. From all the tubes after 24 and 48 h incubation, subcultures were made on plates of brilliant green deoxycholate agar (BGDA-Oxoid) (Vassiliadis *et al.* 1979*c*). The plates were incubated at 37 °C for 24 h. From the plates showing suspicious growth, usually two colonies were cultured on Kligler iron agar, and were further examined by appropriate biochemical and serological tests.

The statistical evaluation of the results was made using MacNemar's test for paired samples.

## RESULTS

The nature of the 409 samples examined and the number of positive specimens found in each category are summarized in Table 1. The number of positive samples and the number of serotypes and strains isolated with the six different enrichment procedures are shown in Table 2. The performance of each of the enrichment methods employed for the examination of the specimens naturally contaminated with salmonellae is recorded in Table 3. The data in terms of statistical significance using MacNemar's test for paired samples are presented in Table 4. The results obtained with RV broth, in 10 ml quantity, incubated at 43 °C and inoculated with 0.1 ml of pre-enrichment (RV/43 °C, 10 ml) and with 0.01 ml in 5 ml of RV medium (RV/43 °C, 5 ml) are compared in Table 4. It can be seen that the RV/43 °C, 10 ml is better than the RV/43 °C, 5 ml, the difference being highly significant. This observation shows that 10 ml of RV medium should be inoculated with not less than 0.1 ml of pre-enrichment medium. A similar difference is observed in Table 4 when the RV/43 °C, 10 ml is compared with the original Rappaport medium (R medium) incubated at 43 °C (R/43 °C, 10 ml). The RV/43 °C, 10 ml method is also superior to both the original Rappaport medium incubated at 37 °C (R/37 °C, 10 ml) and to the R25 medium incubated at 37 °C (R25/37 °C, 10 ml or R25/37 °C, 5 ml), the difference being significant (Table 4). The isolations using the two procedures with R25 media (R25/37 °C, 10 ml and R25/37 °C, 5 ml) are almost the same. (Tables 2 and 3.) This observation confirms the data reported by Harvey *et al.* (1979) and Harvey & Price (1980, 1981, 1982*b*). These authors have shown

Table 1. *Recovery of salmonella from 409 samples*

Nature of the sample	No. examined	No. positive	Percent positive
Bovine minced meat	225	19	8.4
Chicken carcasses	91	69	75.8
Pork sausages	63	6	9.5
Dried powdered chicken meat	19	12	63.2
Animal feed and sewage	11	5	45.5
Total	409	111	27.1

Table 2. *Isolation of salmonellae with six different procedures using modified Rappaport's media*

	Enrichment procedures					
	R/37 (10 ml)	R/43 (10 ml)	R25/37 (10 ml)	R25/37 (5 ml)	RV/43 (10 ml)	RV/43 (5 ml)
No. of positive samples	94	84	95	94	106	94
Percent of positive	23.0	20.5	23.2	23.0	25.9	23.0
No. of serotypes isolated	22	22	21	24	27	22
No. of strains isolated	98	89	103	101	124	103

Total samples examined: 409

Total positive samples by at least one enrichment procedure: 111 (27.1%).

Total serotypes isolated by at least one enrichment procedure: 28.

Total strains isolated by at least one enrichment procedure: 145.

\* R/37 °C, original Rappaport medium, incubation at 37 °C for 48 h; R/43 °C, incubation at 43 °C for 48 h; R25/37 °C, medium containing 25 ml of malachite green solution in 1125 ml of final medium, incubation at 37 °C for 48 h; RV/43 °C, medium containing 10 ml of malachite green solution in 1110 ml of final medium, incubation at 43 °C for 48 h. All tubes containing 10 ml of Rappaport medium were inoculated with 0.1 ml of pre-enrichment medium, while all tubes containing 5 ml of Rappaport medium were inoculated with a loopful of pre-enrichment medium (approx. 0.01 ml).

Table 3. *Recovery of salmonella from 111 contaminated samples with the six different enrichment methods used*

	Enrichment procedures* (°C)					
	R/37 (10 ml)	R/43 (10 ml)	R25/37 (10 ml)	R25/37 (5 ml)	RV/43 (10 ml)	RV/43 (5 ml)
No. of positive samples†	94	84	95	94	106	94
Percentage isolation efficiency	84.7	75.7	85.6	84.7	95.5	84.7

\* See footnote on Table 2.

† All 111 specimens were positive by at least one method (100%).

that using the R25 medium with an inoculum ratio (from the pre-enrichment) of 1:2000 optimum results were obtained, although the salmonella isolation rate was almost the same within the 1:500 and 1:100 ratios. In Table 5 the specificities of the six methods used are compared.

During this trial a total of 28 serotypes and 145 different strains of salmonella were isolated.

Table 4. Statistical comparison of six procedures of enrichment

(The results compared are RV/43 °C, 10 ml; RV/43 °C, 5 ml; R/37 °C; R/43 °C; R25/37 °C, 10 ml; and R25/37 °C, 5 ml)\*

		X <sup>2</sup>	P
RV/43°/10 ml + ve, RV/43°/5 ml + ve	94	12.0	< 0.001
RV/43°/10 ml + ve, RV/43°/5 ml - ve	12		
RV/43°/10 ml - ve, RV/43°/5 ml + ve	0		
RV/43°/10 ml + ve, R/43°/10 ml + ve	82	18.6	< 0.001
RV/43°/10 ml + ve, R/43°/10 ml - ve	24		
RV/43°/10 ml - ve, R/43°/10 ml + ve	2		
RV/43°/10 ml + ve, R/37°/10 ml + ve	93	10.3	< 0.01
RV/43°/10 ml + ve, R/37°/10 ml - ve	13		
RV/43°/10 ml - ve, R/37°/10 ml + ve	1		
RV/43°/10 ml + ve, R25/37°/10 ml + ve	94	9.3	< 0.01
RV/43°/10 ml + ve, R25/37°/10 ml - ve	12		
RV/43°/10 ml - ve, R25/37°/10 ml + ve	1		
RV/43°/10 ml + ve, R25/37°/5 ml + ve	93	10.3	< 0.01
RV/43°/10 ml + ve, R25/37°/5 ml - ve	13		
RV/43°/10 ml - ve, R25/37°/5 ml + ve	1		

\* For symbols, see footnote on Table 2.

Table 5. Specificities of the six enrichment methods for salmonella isolation

	Enrichment procedures* (°C)					
	R/37 (10 ml)	R/43 (10 ml)	R25/37 (10 ml)	R25/37 (5 ml)	RV/43 (10 ml)	RV/43 (5 ml)
No. of suspicious plates	247	149	234	233	179	151
No. of colonies examined	498	303	477	472	360	309
Percentage of salmonella colonies	37.8	60.1	38.8	39.8	64.7	67.0
Percentage of 'false positive' colonies	62.2	39.9	61.2	60.2	35.3	33.0

\* See footnote on Table 2.

DISCUSSION

In this paper the original Rappaport medium (Rappaport *et al.* 1956) and five procedures involving modifications of this medium were compared with respect to their ability to recover salmonellas from meat products. In a previous study, using feces of normal pigs, it was shown that the RV and R25 modifications of the R medium were more efficient in the detection of positive samples than the original R medium (Vassiliadis *et al.* 1981a). In this same study, as well as in a previous investigation, using decimal dilutions of cultures of salmonella in RV and in R25 media, it was shown that the RV medium was slightly better than the R25 medium (Vassiliadis *et al.* 1976). However, in three investigations the two media, R25 and RV, were found of equal efficiency in the detection of salmonellas from minced meat, from sewage and from pork sausages (Vassiliadis *et al.* 1978a, b, 1979b), while in three other trials on meat products and feces of pigs the RV medium was slightly

better than the R25 medium. However, in the latter trials, the difference was not statistically significant (Vassiliadis *et al.* 1977, 1979*a*; Xirouchaki *et al.* 1982). In all these studies the RV medium was much more specific than the R25 medium; it inhibited much better the growth of the competitive organisms, especially of the bacteria which are lactose and sucrose negative on BGDA. In spite of the slight superiority of the RV medium in isolating salmonellae and its great specificity, Xirouchaki *et al.* (1982) concluded that the R25 (R25/37 °C) medium may be used as an alternative to the more efficient RV medium (RV/43 °C) when the only available incubation temperature is 37 °C. However, in the current study the RV medium is again significantly more efficient than the R25 medium ( $P < 0.01$ ) in the isolation of salmonellae (Tables 3 and 4).

In a recent paper Harvey & Price (1983) have found in Cardiff, that using sewage polluted natural water as test material, the R25 medium was significantly more efficient in the recovery of salmonellas than the RV medium ( $P < 0.01$ ) although the latter medium inhibited better the competitive organisms. These authors insist that their results in salmonella recovery from sewage polluted water must not be projected without further experiment to cover other material. They also add that test samples can have a profound influence on outcome. This view is supported by the fact that, in an earlier study, from feces of normal pigs (Vassiliadis *et al.* 1981*a*) and in the present study dealing with meat products, the RV medium was significantly better than the R25 medium in detecting positive samples contaminated with salmonella (Table 4). Moreover, Harvey & Price (1983) have pointed out that other microbiologists have drawn attention to failure to obtain satisfactory salmonella recovery at 43 °C from river water and sewage effluent samples (Burman, 1967; McCoy, 1962) and, thus, their results may reflect this peculiarity. Furthermore, in a subsequent study in Cardiff, made between July and October 1983, on artificially contaminated minced meat (European Economic Community trial) the RV medium was marginally better than R25, although there was no significant difference between the two media (Harvey, 1983, personal communication).

In this study 87.9% of the local chicken carcasses examined were contaminated with salmonellae. This percentage is high. However, since we started using the technique of washing the whole chicken carcass with buffered peptone water the percentages found positive were as high or higher, reaching 100% (Mavromatti *et al.* 1981). Of the imported chicken carcasses, 44% were contaminated with salmonellae. However, the imported carcasses were about half the size of the chickens grown in Greece.

Table 3 shows that with at least one of the six enrichment methods used, 111 specimens were found positive (100%), whereas none of the six methods alone was able to isolate all the salmonellas that were recovered by at least one procedure. The closest results were obtained with RV medium in 10 ml quantities, seeded with 0.1 ml of pre-enrichment medium and incubated at 43 °C (RV/43 °C, 10 ml), which resulted in the recovery of 106 positive samples (95.5%). Next in efficiency were the methods using R25 medium inoculated with 0.1 ml or with 0.01 ml of pre-enrichment and incubated at 37 °C (R25/37 °C, 10 ml and R25/37 °C, 5 ml) which resulted in the detection of 85.6 and 84.7% respectively, of all positive samples. The same efficiency (84.7%) was shown by the methods involving enrichment in 5 ml of RV/medium incubated at 43 °C and inoculated with 0.01 ml

of pre-enrichment (RV/43 °C, 5 ml), and the original R medium incubated at 37 °C (R/37 °C, 10 ml). Finally, the least efficient method was the one involving the original R medium incubated at 43 °C (R/43 °C, 10 ml) which permitted the detection of only 75.7% of the positive samples.

In the current study, the growth of competing organisms lactose and sucrose negative was recorded (Table 5). A greater inhibition of these organisms was observed with the RV/43 °C medium than with the other R media. With the RV/43 °C, 10 ml medium, 35.3% of the suspicious colonies examined were false positive colonies, while with the two R25 media these figures were 60.2 and 61.2% respectively. However, in other studies the inhibition of these organisms with the RV medium, was much more spectacular as the false positive colonies observed in previous work varied from 3.5 (Vassiliadis *et al.* 1979*b*) to 15.3% (Vassiliadis *et al.* 1979*a*).

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