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# Elevated temperature incubation of enrichment media for the isolation of salmonellas from heavily contaminated materials

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## INTRODUCTION

Alteration of incubation temperature is a well-known aid to purifying cultures of bacteria, although elevated temperature techniques have been mainly used to isolate thermophilic organisms (Wilson & Miles, 1964). A study of the literature will show, however, that some authors have increased the selectivity of their media for bacteria with an optimum growth temperature of 37° C. by raising the incubation temperature (MacConkey, 1908; Wilson, 1938). The procedure is not, therefore, a new one but is profitably revived from time to time. In 1953, Harvey & Thomson described a technique using selenite F broth incubated at 43° C. for the isolation of salmonellas. This paper mentioned three temperatures: 42, 43, and 44° C. For the conditions and media in use, it was suggested that 43° C. was the optimum for incubation with the proviso that 42° C. might be safer as 43° C. possibly represented the upper end of the useful temperature range.

We have now been using 43° C. incubation of selenite F broths for 14 years, sometimes alone and sometimes in conjunction with 37° C. We feel, therefore, that we can be objective about a technique which, in common with all isolation methods, has imperfections (Burman, 1967). For much of the material examined in our laboratory, the advantages outweigh the disadvantages. Other authors have recently found elevated incubation temperatures of value in enrichment and selective culture of salmonellas (Georgala & Boothroyd, 1964; Livingstone, 1965; Spino, 1966; Burman, 1967).

## MATERIALS

The materials examined were:

(1) Gauze swabs (Moore, 1948) placed in Cardiff sewers 1953–54.
(2) Gauze swabs placed in drains from Cardiff and Barry abattoirs 1961–63.
(3) Samples of naturally polluted river water—the River Taff 1966–67.

## METHODS

The gauze swabs were covered with nutrient broth and were pressed down with a sterile rod to extrude their absorbed fluid into the broth. This liquid was decanted and divided into two parts. Each part was diluted with an equal volume of double strength selenite F broth. One of the enrichment cultures was incubated at 37° C. and the other at 43° C. The samples were, therefore, paired. The river water samples were collected in 1 l. quantities and were brought to the laboratory

within an hour. They were collected at the same sampling point each week at approximately the same time. At the laboratory they were divided into $10 \times 100$ ml. quantities and each 100 ml. was added to 100 ml. of double strength selenite F broth. Five of these enrichment cultures were incubated at 37° C. and five at 43° C. After exactly 24 hr. incubation the swab samples were subcultured to deoxycholate citrate agar, Wilson & Blair's bismuth sulphite agar and brilliant green MacConkey agar (Wilson & Blair, 1931; Harvey, 1956). The water samples, after 24 hr. incubation, were subcultured to S.S. agar (Difco or Oxoid) + 1% sucrose. The selective agars used for the swabs were incubated at 37° C and the brilliant green MacConkey plates were examined at 24 hr. The deoxycholate and Wilson & Blair plates were examined after 48 hr. incubation. The extra time was necessary for salmonella colonies to develop properly on freshly poured Wilson & Blair's agar and for optimum differentiation between proteus and salmonella colonies on deoxycholate citrate agar. The creamy brown colour sometimes shown by salmonella colonies on deoxycholate plates (Farrant, 1962) was found to be a useful distinguishing feature. The S.S. agars used for the river water were incubated at 40° C. for 24 hr. (Livingstone, 1965). A preliminary trial of this latter technique against Wilson and Blair's agar incubated at 37° C. had been promising and we wished to gain further experience of the method. Suspicious colonies were picked and examined in the usual way.

## RESULTS

The results are given in Tables 1, 2 and 3. They are self explanatory. In Tables 1 and 2, the combined results of all three plating media are recorded for the two incubation temperatures of selenite F broth.

Table 1. *Sewage samples* 1953–54

| Category | Number |
|---|---|
| Positive at 37° C. | 31 |
| Positive at 43° C. | 54 |
| Salmonellas isolated at 37° C. and not at 43° C. | 1 |
| Salmonellas isolated at 43° C. and not at 37° C. | 24 |
| Total samples | 163 |

If we give no weight to results where procedures agree $\chi^2 = 21{\cdot}16$, $P = < 0{\cdot}0001$.

Table 2. *Abattoir samples* 1961–63

| Category | Number |
|---|---|
| Positive at 37° C. | 46 |
| Positive at 43° C. | 72 |
| Salmonellas isolated at 37° C. but not at 43° C. | 7 |
| Salmonellas isolated at 43° C. but not at 37° C. | 33 |
| Total samples | 501 |

If we give no weight to results where procedures agree $\chi^2 = 16{\cdot}9$, $P = < 0{\cdot}0001$.

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Table 3. *River Taff water samples* 1966–67

| | Temp. of incubation | |
|---|---|---|
| | 37° C. | 43° C. |
| Salmonellas isolated from | 147 | 174 |
| Salmonellas not isolated from | 73 | 46 |
| Total | 220 | 220 |

These data cannot be arranged as in Tables 1 and 2. The specimens were derived from separate litre samples. Each litre was shaken vigorously and divided into ten equal parts five of which were incubated at 37° C. and five at 43° C.

## DISCUSSION

We have shown, for three different materials, that incubation of selenite F broth at 43° C. had advantages over incubation at 37° C. The specimens examined were all heavily contaminated with organisms other than salmonellas. A few faecal coli counts on the River Taff were over 18,000 per 100 ml. Each material was sampled over a period of at least 12 months. All seasons were, therefore, represented. The total observations covered 1953–67.

Lack of success with 43° C. incubation, in some laboratories, is probably explained on the basis of differences in sample or technique. Specimens that yield salmonellas easily at 37° C. are unlikely to yield more by the use of 43° C. Samples containing minimal numbers of salmonellas, or organisms in need of resuscitation, may be better examined at 37° C. (Burman, 1967). In the isolation of salmonellas from heavily contaminated materials, we constantly tread a technical tight-rope between sensitivity and selectivity. Others also have recognized this difficulty (Jameson, 1962).

Media preparation is also relevant to the use of 43° C. incubation. In our laboratory selenite F broth is sterilized by Seitz filtration (Hobbs & Allison, 1945). Heat is never used and the medium is crystal clear without a trace of red deposit. It is relatively non-toxic in that it usually allows multiplication and enrichment of *Shigella sonnei*. Other enrichment broths are, in our experience, more toxic than selenite F. If, for example, the formula of Rolfe's 'B' tetrathionate (Rolfe, 1946) is expressed in terms of molar tetrathionate (0·39 M) it will be noted that this medium is somewhat inhibitory (Knox, Gell & Pollock, 1943). A 'balanced' tetrathionate broth may easily be titrated to function very well at 43° C. and we have used such a medium at this temperature for the isolation of salmonellas from river water. Multiplication of media is, however, inconvenient for routine practice. The enrichment medium of Rappaport, Konforti & Navon (1956) can be adjusted to 43° C. incubation, but the results are not very satisfactory. We have not a great deal of experience with this medium, but in our hands it is toxic to *S. dublin*, *S. pullorum* and *S. typhi*. It therefore distorts the serotype pattern. This phenomenon is being currently investigated using selenite F broth as control.

If we can alter the formulae of enrichment media to function at 43° C., we can, just as easily, alter the incubation temperature to allow the use of more toxic enrichment broths. Spino (1966) for example, used a temperature of 41·5° C. with

selenite brillant green broth and Kauffmann's tetrathionate broth. Adjustment of temperature may well be more convenient for laboratories using commercial media.

Plating media are also important in elevated temperature enrichment. The best results are obtained with brilliant green MacConkey agar. This medium suppresses the growth of *Proteus* species, does not react with selenite F broth with the production of an area of no growth at the site of heaviest inoculation and requires only 18–24 hr. incubation. It is particularly useful if multiple subculture from selenite F is used (Harvey & Phillips, 1955). Other authors have also found brilliant green agars useful (Georgala & Boothroyd, 1964; Spino, 1966). Deoxycholate citrate agar and S.S. agar are valuable if *S. dublin* is being searched for as some strains of this organism are inhibited by brilliant green MacConkey. Wilson and Blair's agar is not so well suited to 43° C. incubated selenite. This is, perhaps, because in Cardiff we do not ripen our plates in the refrigerator.

## SUMMARY

In three separate series, samples were examined for salmonellas by culture in selenite F broths incubated at 37 and 43° C. The samples used were:

(1) Gauze swabs placed in sewage.

(2) Gauze swabs placed in drains in abattoirs.

(3) Sewage-polluted river water.

In each series the higher temperature gave better results.

The modification of tetrathionate broth for incubation at 43° C. and the adjustment of the incubation temperature to suit more inhibitory enrichment broths is discussed. The medium of Rappaport, Konforti & Navon (1956) is not suited to incubation at 43° C.

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