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A new enrichment medium for salmonellae*

By S. BANIČ
The Institute of Microbiology, Medical Faculty, Ljubljana, Yugoslavia

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The so-called enrichment media are of great importance in the isolation of salmonellae from faeces and other material. Two of them, namely the tetrathionate broth according to Mueller–Kauffmann (Kauffmann, 1930–31) and the selenite F broth according to Leifson (1936), are most frequently used. The first appears to be superior in the isolation of *Salmonella typhi*, the second in the isolation of other salmonella types.

Some of the coliform organisms, especially *Proteus* species, *Pseudomonas aeruginosa* and *Aerobacter aerogenes*, are not always inhibited in the enrichment media mentioned. As a consequence of this, these media sometimes give negative results. For this reason the attempts to develop a new enrichment medium for the isolation of salmonella types appeared justified to the present author. As a result of a laborious investigation a medium was developed which proved, in experiments with pure cultures of enteric bacteria, to be very selective for salmonellae. The medium was then tested in routine diagnosis and compared with other standard media used in this laboratory. The purpose of this paper is to report the results obtained.

MATERIALS AND METHODS

Media. The new enrichment medium (called MS = magnesium chloride–selenite medium) is composed of nutrient broth Difco + 0·2 % yeast extract Difco + 0·05 % sodium hydrogen selenite ($NaHSeO_3$) Merck + 3·4 % $MgCl_2$.

To make 300 ml. of the medium, put 600 mg. of yeast extract and 150 mg. of sodium hydrogen selenite in a sterile bottle, and add 274·5 ml. of sterile nutrient broth. When these are dissolved, adjust to pH 7·5, first with 2–3 drops of 10 N-NaOH and then with N-NaOH, using an electric pH-meter. Then add 25·5 ml. of 40 % $MgCl_2$ solution. The medium is sterilized by standing in boiling water for 10 min., and is distributed aseptically in 10 ml. amounts in sterile test tubes. The final pH of the medium is about 6·8. The medium should be kept at 4° C. until use.

The tetrathionate broth was prepared according to the formula given by Kauffmann (1941).

The Wilson–Blair agar and the SS agar were prepared according to the Yugoslav *Manual of the Microbiological Methods* (1953).

A total of 605 specimens of faeces were examined for salmonellae. Each specimen was first seeded on a direct Wilson–Blair and SS agar plate. After that about 1 g. of the faeces was emulsified in 10 ml. of distilled water, and four drops of this suspension were dropped into a tube containing 10 ml. of tetrathionate broth and

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into a tube containing the same amount of the new enrichment medium. After incubation for 24 hr. a large loopful (4 mm. internal diameter) of each of the enrichment media was plated on to Wilson–Blair and SS agar. The plates were incubated overnight and then examined together with the direct plates. Two or three suspected colonies from each plate were selected and sub-cultured on the screening medium (double sugar agar). The inoculated double sugar agars were incubated overnight and then examined for salmonellae according to the standard methods.

RESULTS

From 605 specimens of faeces 95 salmonella strains were isolated. Among them the following salmonella types were identified: 8 strains of *S. typhi*, 41 strains of *S. paratyphi B* (including *S. java*), 27 strains of *S. typhi-murium*, 7 strains of *S. enteritidis*, 6 strains of *S. derby*, and 6 strains of *S. blockley*.

The efficiency of the new enrichment medium in comparison with other media used in the study is shown in Tables 1–3.

Table 1. *Comparison of efficiency of various selective media for the isolation of salmonella types*

(From 605 specimens of faeces, 95 salmonella strains were isolated.)

Name of medium	No. of salmonella strains isolated
Wilson–Blair, direct plate	37
SS agar, direct plate	21
Mueller–Kauffmann medium	82
MS medium	79

Table 2. *Number of salmonella strains isolated on only one of the four selective media*

Name of the medium	No. of salmonella strains isolated
Wilson–Blair, direct plate	0
SS agar, direct plate	1
Mueller–Kauffmann medium	14
MS medium	7

Table 3. *Comparison of the efficiency of various selective media for the isolation of* Salmonella typhi

(The distribution of positive cultures from the eight specimens of faeces from which *S. typhi* was isolated.)

	Patient							
Medium	1	2	3	4	5	6	7	8
Wilson–Blair, direct	+	−	+	+	+	+	+	−
SS agar, direct	+	−	+	+	+	+	+	−
Mueller–Kauffmann	+	−	−	−	+	−	+	−
MS medium	+	+	+	−	+	−	+	+

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The new enrichment medium described contains two inhibitory substances, magnesium chloride and sodium hydrogen selenite.

Magnesium chloride was used for the first time for the preparation of an enrichment medium by Rappaport, Konforti & Navon (1956) and Rappaport & Konforti (1959). In an appropriate concentration it is inhibitory for coliform organisms, including *Proteus* species and *Pseudomonas aeruginosa*. The magnesium chloride–malachite green (MM) medium described by Rappaport *et al.* contains magnesium chloride in a 4 % concentration. In our medium 3·4 % of $MgCl_2$ was found to be the optimal concentration.

The second characteristic of our MS medium is that it contains sodium hydrogen selenite in a very low concentration (0·05 %). Such a low concentration was chosen because it was found that higher concentrations may be inhibitory for many salmonella strains. The combined action of 0·05 % sodium hydrogen selenite and 3·4 % $MgCl_2$ makes the medium sufficiently selective.

The instructions for the preparation of the new MS enrichment medium must be strictly followed. The only satisfactory broth found was Difco nutrient broth, and it cannot therefore be replaced by any other nutrient broth or by infusion broth. The same is true for the sodium hydrogen selenite Merck.

The MS medium is very cheap and very easy to prepare. The results obtained indicate that the new medium is an efficient enrichment medium for the isolation of *S. typhi* as well as for the isolation of other salmonella types. From Table 3 it can be seen that the MS medium is in fact superior to the Mueller–Kauffmann medium when dealing with the isolation of *S. typhi*. In the isolation of other salmonella types the MS medium is, however, a little inferior to the Mueller–Kauffmann medium.

From 95 salmonella strains 7 were isolated only on the MS medium. It is clear that this gain would have been much higher if the study had been done in a province in which *S. typhi* is prevalent, as the MS medium compares more favourably with the Mueller–Kauffmann medium in the isolation of *S. typhi* than in the isolation of other salmonella types.

Subcultures from Mueller–Kauffmann and MS medium were made in the present study on Wilson–Blair and SS agar plates. The analysis of the results showed that subcultures on Wilson–Blair medium were more efficient.

SUMMARY

A new enrichment medium for the isolation of salmonella types from faeces is described.

The inhibitory substances for coliform organisms and enterococci in this medium are magnesium chloride and sodium hydrogen selenite.

The new medium was found in routine diagnostic work to be superior to the Mueller–Kauffmann medium in the isolation of *Salmonella typhi* and only a little inferior in the isolation of other salmonella types.

When used in addition to the standard media the new medium increases the number of positive results.

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