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Semisolid Selective-Motility Enrichment Medium for Isolation of Salmonellae from Fecal Specimens

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A semisolid selective-motility enrichment medium for the isolation of salmonellae from fecal specimens was developed which was based on Rappaport enrichment broth. During a 7-year period more than 30,000 stool samples were tested. The medium showed a high specificity (95.1%) and sensitivity (80.3%) when compared with MacConkey agar, SS agar, and brilliant green agar (after Selenite-F Enrichment [BBL Microbiology Systems]). Furthermore, our isolation rate of *Salmonella* species from fecal samples showed an increase of 22.3% when this semisolid medium was added to the routine culture media. Growth could easily be interpreted. The medium has a bias toward the isolation of *Salmonella paratyphi* B, but it is unsatisfactory for detecting the nonmotile strains *Salmonella typhi* and *S. paratyphi* A.

Successful isolation of salmonellae can be a complex, multifactorial procedure. Little attention has been made to the magnesium chloride-malachite green enrichment broth described by Rappaport et al. (11, 12). The authors assumed that magnesium chloride and malachite green synergistically inhibited gram-negative contaminants of fecal specimens, whereas they did not affect the growth of salmonellae. However, the medium was unsuitable for the isolation of *Salmonella typhi*, but it has been used successfully by Collard and Unwin (4), Hooper and Jenkins (8), and Iveson and co-workers (9, 10). During the 1960s, the use of semisolid media as enrichment for isolating salmonellae on the basis of their selective motility was studied (1, 5, 6, 13). The workers noted that after passing samples through the semisolid media followed by direct plating onto MacConkey agar, salmonellae were often isolated in a pure or nearly pure culture state. Finally, Chau and Huang (2, 3) developed a 1-day procedure, in which the migrated growth was checked directly for salmonella O and H antigenicity. The formula of the semisolid enrichment medium that they were using closely resembled the composition of the medium described by Rappaport et al. (11, 12). This gave the idea to one of us (G. Wauters) in 1976 to use Rappaport enrichment broth as a semisolid selective enrichment medium. In this paper, for the first time, we report the results of our experience with this medium over a period of 7 years.

Culture media. The original Rappaport broth is made of Tryptone (6 g; Difco Laboratories, Detroit, Mich.)–NaCl (8 g, art. 6404; E. Merck AG, Federal Republic of Germany)–KH_2PO_4 (1.6 g, art. 4873; Merck)–40% $MgCl_2 \cdot 6H_2O$ (100 ml, art. 5833; Merck)–2% malachite green oxalate (6 ml, art. 1398; Merck)–distilled water (1,000 ml) (total volume, 1,106 ml). Distilled water was used to prepare the 40% $MgCl_2 \cdot 6H_2O$ and 2% malachite green oxalate solutions. After autoclaving, the broth was dispensed in 5-ml amounts into upright tubes (15 by 150 mm) which were stored in the refrigerator and used within a month.

For the preparation of the semisolid Rappaport medium, a 0.8% agar (Difco) solution in distilled water was made and autoclaved. The Rappaport broth was mixed with the agar solution, after being cooled to 50°C, in a proportion of 4 volumes of agar solution to 6 volumes of Rappaport broth. The medium was then poured into small sterile petri dishes (diameter, 50 mm). The media were prepared twice weekly. Plates were kept at room temperature. The other media which have been used for comparative studies are brilliant green agar (Bio-Merieux, Charbonnières les Bains, France); MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.); Salmonella-Shigella agar (Bio-Merieux); and Selenite-F Enrichment broth, according to Leifson (art. 7717; Merck), supplemented with novobiocin (40 mg per liter of Selenite-F Enrichment broth). All media were prepared as recommended by the different companies.

Specimens. A total of 33,674 stool specimens for routine culture of enteric pathogens were examined (382 were submitted to the Department of Microbiology of the St. Luc University Clinic, and 33,292 were submitted to the Department of Microbiology of the St. Pieters University Hospital).

Method. In a first study carried out in 1976 by G. Wauters, 382 stool specimens were examined for the presence of salmonellae by comparing the semisolid Rappaport medium with the Rappaport enrichment broth subcultured onto brilliant green agar. The semisolid Rappaport medium was inoculated with a loopful of feces placed into the medium at the border of the agar plate and incubated for 18 h at 37°C. Migration of organisms through the medium was then checked. The liquid Rappaport was inoculated with 1 drop of stool sample and incubated for 18 h at 37°C. A droplet taken with a 3-mm loop was streaked into four quadrants on brilliant green agar, which was then incubated at 37°C for another day. In a second study from December 1977 to February 1979 at St. Pieters University Hospital, the degree of migration of *Salmonella* species through the semisolid medium incubated for 18 h at 37°C was tested on 7,482 stools. In a third study from September 1979 to November 1983 at St. Pieters University Hospital, a comparison was made between the semisolid Rappaport medium and some other previously used routine culture media for *Salmonella* species, i.e., MacConkey agar, SS agar, and Selenite-F Enrichment broth subcultured onto brilliant green agar. Stool specimens (25,810) were tested. All the media were incubated at 37°C for 18 to 24 h.

Results. In the first study carried out in 1976, a total of 63

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TABLE 1. Comparison between the semisolid Rappaport, MacConkey, SS, and brilliant green agar (after Selenite-F Enrichment)[a]

Medium[b]	No. of salmonellae (%)
R + M + S + B	95 (5.5)
R + M + S	17 (0.9)
R + M + B	27 (1.6)
R + S + B	247 (2[c]) (14.3)
R + M	15 (0.8)
R + S	75 (4.3)
R + B	524 (2[c]) (30.4)
R only	390 (1) (22.5)
M + S + B	15 (3[d]) (0.8)
M + B	3 (0.2)
M + S	12 (6[d]) (0.7)
S + B	34 (4[d]) (2.0)
M only	8 (0.5)
S only	26 (8[d], 1[e]) (1.5)
B only	242 (2[d], 4[e]) (14.0)

[a] A total of 25,810 stool specimens were tested.
[a] Abbreviations: R, Rappaport medium; M, MacConkey agar; S, SS medium; B, brilliant green agar.
[c] Number of *S. paratyphi* B included.
[d] Number of *S. typhi* included.
[e] Number of *S. paratyphi* A included.

stool samples were found to be positive by either one or both procedures. However, the same number of stool samples, namely 58, reported a positive culture with either the semisolid or liquid Rappaport medium. All the isolates were *Salmonella nontyphi*. In the second study, 331 *S. nontyphi* were isolated with the semisolid Rappaport medium. The results show clearly that all salmonellae migrated at least 20 mm from the point at which the feces were inoculated. At the same time, the specificity of the medium was determined. It was found that of 348 migrated organisms, 17 (4.9%) gave false-positive results, which were defined as ≥20-mm migration through the medium (11 were *Citrobacter* sp. and 6 were *Enterobacter* sp.). The results of the third study are expressed in Table 1. The study includes 62 different serotypes.

Discussion. Rappaport et al. (11, 12) developed a hypertonic solution as an enrichment medium. A combination of malachite green and magnesium chloride was found to produce a satisfactory enrichment broth for salmonellae. One of us (G. W.), decided to change the Rappaport broth into a semisolid medium by adding 0.32% agar. In this way, he was able to use it as a selective enrichment medium based also on the motility of the salmonella (Fig. 1). In a first study in 1976, no differences were found in the isolation rate between the classical Rappaport broth and the semisolid medium. After the second study, it was decided that only those organisms which had migrated ≥20 mm should be suspected as being salmonellae. The medium showed a specificity of 95.1%.

The third comparative study (Table 1) needs some comment. The study shows that by adding the semisolid medium, the isolation rate for salmonellae was raised by 22.5%. Furthermore, of the 1,730 positive stool samples for salmonellae, 1,390 (80.3%) were detected on the semisolid Rappaport medium, 1,335 (77.2%) were selected on brilliant green agar, 521 (30.2%) were selected on SS agar, and 192 (11.1%) were selected on MacConkey agar. Thus, the semisolid medium became the most sensitive medium for the isolation

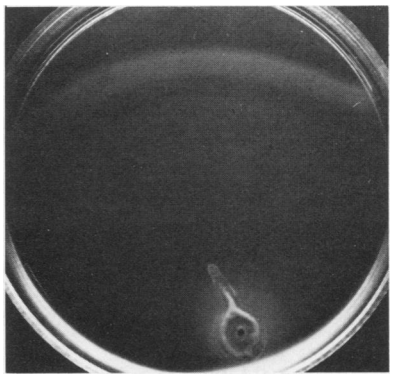

FIG. 1. Semisolid Rappaport medium. Feces were inoculated with a loop (bottom), and a salmonellae migration line can be seen (top) at a distance of 45 mm.

of salmonellae. However, it was unsatisfactory for detecting the nonmotile strains *S. typhi* and *Salmonella paratyphi* A. On the other hand, it appears to have a bias toward the isolation of *S. paratyphi* B. It has been said that the selective-motility technique is not efficient for the isolation of *Salmonella dublin* (7), but of the 35 positive stool samples for *S. dublin*, 27 were detected with the Rappaport medium (from which 6 were found with the Rappaport only). Also, a lactose-fermenting *Salmonella arizonae* isolate was found with the semisolid medium and would have been missed with the other routine media. Finally, a trial will be set up to enhance the specificity of the medium by incubating the plates at more than 40°C.

LITERATURE CITED

1. **Banwart, G. J.** 1968. Glassware apparatus for determining motile bacteria I. Salmonella Poult. Sci. **47**:1209–1212.
2. **Chau, P. Y., and C. T. Huang.** 1974. A one-day selective migration procedure for detecting Salmonellae in faeces. J. Clin. Pathol. **27**:405–407.
3. **Chau, P. Y., and C. T. Huang.** 1976. A simple procedure for screening of Salmonellae using a semi-solid enrichment and a semi-solid indicator medium. J. Appl. Bacteriol. **41**:283–294.
4. **Collard, P., and M. Unwin.** 1958. A trial of Rappaport's medium. J. Clin. Pathol. **11**:426–427.
5. **Fung, F. Y. C., and A. A. Kraft.** 1970. A rapid and simple method for the detection and isolation of Salmonella from mixed cultures and poultry products. Poult. Sci. **49**:46–54.
6. **Harper, J., and K. F. Shortridge.** 1969. A selective motility medium for routine isolation of Salmonella. J. Hyg. **67**:181–186.
7. **Harvey, R. W., and T. H. Price.** 1975. Studies on the isolation of Salm. dublin. J. Hyg. **74**:369–374.
8. **Hooper, W. L., and H. R. Jenkins.** 1965. An evaluation of Rappaport's magnesium chloride/malachite green medium in the routine examination of faeces. J. Hyg. **63**:491–495.
9. **Iveson, J. B., and N. Kovacs.** 1967. A comparative trial of Rappaport enrichment medium for the isolation of Salmonellae from faeces. J. Clin. Pathol. **20**:290–293.
10. **Iveson, J. B., and E. M. Mackay-Scollay.** 1969. Strontium chloride and strontium selenite enrichment media in the isolation of Salmonella. J. Hyg. **67**:457–464.
11. **Rappaport, F., and N. Konforti.** 1959. Selective enrichment medium for paratyphoid bacteria. Inhibitory and growth promoting factors. Appl. Microbiol. **7**:63–66.
12. **Rappaport, F., N. Konforti, and B. Navon.** 1956. A new enrichment medium for certain Salmonellae. J. Clin. Pathol. **9**:261–266.
13. **Stuart, P. F., and H. Pivnick.** 1965. Isolation of salmonellae by selective motility systems. Appl. Microbiol. **13**:365–372.