NEW TRANSPORT MEDIUM FOR SHIPMENT OF CLINICAL SPECIMENS

I. FECAL SPECIMENS

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ABSTRACT

CARY, SYLVIA G. (Walter Reed Army Institute of Research, Washington, D.C.), and EUGENE B. BLAIR. New transport medium for shipment of clinical specimens. I. Fecal specimens. J. Bacteriol. 88:96-98. 1964.—A new transport medium for the collection and shipment of clinical specimens is described. Preliminary studies indicate that, with initial fecal specimens, Salmonella and Shigella can be recovered for as long as 49 days, Vibrio comma for 22 days, and Pasteurella pestis for at least 75 days.

Stuart, Toshach, and Patsula (1954) devised a transport medium for the shipment of specimens to a central laboratory for the isolation of gonococci. Crookes and Stuart (1959) used this medium for a field study and found that 5% of the specimens collected were overgrown with Escherichia coli and Aerobacter aerogenes. By adding 10 units per ml of Aerosporin (polymyxin B sulfate) to inhibit the coliforms, their recovery of gonococci was increased. Huet and Solange (1960), using Hajna’s holding medium, conducted an enteric survey in Tunisia; 452 specimens were collected, stored at 37, 30, 20, and 4°C for varying periods, and subcultured. They found that the maximal time allowing recovery of Salmonella from this medium was 9 days at 30°C. Survival of Shigella in this medium was poor. Cooper (1957) using Stuart’s medium found enteric pathogens in fecal specimens surviving for as long as 56 days.

Recently, we shipped freshly prepared Stuart’s medium and charcoal-treated swabs to K. Koberi, Komagome Hospital, Tokyo, Japan, for the collection of rectal swabs from clinical cases of dysentery to be returned to us for culture. All specimens were also cultured in Japan for comparison with the number of Shigella isolated after shipment. From 40 specimens received, two strains of S. flexneri 2 and one strain of S. flexneri 4 were recovered. The rest were overgrown with E. freundii, members of the Klebsiella-Aerobacter group, and E. coli. The overgrowth by E. coli, E. freundii, and A. aerogenes observed by us and Crookes and Stuart (1959) could be due to the utilization of the glycophosphate as substrate by specific glycophosphate dehydrogenases produced by these organisms. Quastel (1954) has reported over 50 different dehydrogenation reactions for E. coli. Certain anaerobic dehydrogenases are linked with coenzyme I (Salle, 1943) which can be furnished by yeasts or coliforms. Pyocyanine (Thimmann, 1955) produced by Pseudomonas aeruginosa is a coupler for certain dehydrogenases. All these organisms are often found in fecal material. Therefore, if a transport medium is to facilitate laboratory diagnosis, it is desirable that components of the medium do not promote growth of the nonpathogens but are adequate for survival of the etiologic disease agents. The superiority of Stuart’s medium over Hajna’s medium is probably due in part to a higher initial pH (7.4 compared with Hajna’s pH 7.0) and to a low oxidation-reduction potential. Venkatraman and Ramakrishnan (1941) found that Vibrio comma (Bergey’s Manual of Determinative Bacteriology, 7th ed.) could be isolated from a sea-salt medium for as long as 92 days if the initial pH was adjusted to 9.2. Previously described holding media which allowed recovery of enteric pathogens for considerable periods of time were characterized by one or more of the following: (i) low nutrient content, (ii) low oxidation-reduction potential, and (iii) a high pH. The new media described in this report were designed to incorporate all of these factors. In addition, Stuart’s transport medium (Difco) and a modification of the Stuart formula were tested in parallel with the new formulas.

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MATERIALS AND METHODS
Media were prepared in chemically clean glassware rinsed with 0.067 M Sorensen's buffer (pH 8.1). The two new media were designated A and B. Medium A contained sodium thioglycolate, 1.5 g; Na₂HPO₄, 1.1 g; and NaCl, 5 g; added to 991 ml of demineralized distilled water in the order listed. Difco agar (5 g) was added, and the medium was heated only until clear. After cooling to 50 C, 9 ml of freshly prepared 1% CaCl₂ were added, and the pH was adjusted to 8.4. Excessive heating was avoided; 7 ml were added immediately to previously rinsed and sterilized 9-ml screw-cap vials. The vials were then steamed for 15 min, cooled, and the tops tightened. To prevent evaporation during storage at room temperature, transparent clear cellophane (Celon Co., Division of Thatcher Glass Mfg. Co., Inc., Muscatine, Iowa) were placed over the caps. Medium B was prepared in the same manner as A except 1 g per liter of sodium taurocholate was added before the agar. Medium C was Stuart's with 5 g per liter of sodium taurocholate substituted for sodium glycerophosphate. Medium D was commercially prepared Stuart's (Difco).

Cotton swabs were dipped into Sorensen's buffer (pH 8.1) previously heated just to boiling, drained, and placed in glassine syringe envelopes [2 1/4 by 6 3/8 in. (5.7 by 16.8 cm), self-seal]. They were then sterilized at a pressure of 15 psi for 15 min and stored without drying at room temperature. All swabs were dipped into fecal material and placed in vials of transport medium.

Fecal specimens from five clinically normal chimpanzees, proven carriers of Shigella flexneri 2, were used to test the four media. For these preliminary tests, only one storage temperature, 28 C, was used. Additional fecal material from the same animals were also inoculated with 1,000 cells per ml and 10,000 cells per ml of V. comma (ogawa type) strains N-1 and 1449 for testing.

RESULTS AND DISCUSSION
The survival of S. flexneri 2 in the four media is summarized in Table 1. There was an 80% recovery for as long as 18 days. Since the highest recovery rate was obtained with medium A, this was selected to study the survival of V. comma. From this study, it was observed that cholera vibrios could be recovered for up to 22 days at 28 and 35 C.

While these preliminary tests were being run, a human infection due to S. flexneri 2 became available. A specimen was obtained, and swabs of fecal material were placed in vials of medium A and stored at 28 and 35 C. Colony counts were done in triplicate. Suspensions from swabs were made in distilled water, diluted in tenfold increments, and colony counts were done by use of pour plates of alkaline agar (Felsenfeld et al., 1951). The initial count was approximately 854,000 S. flexneri 2 per swab. At a holding temperature of 35 C, the count dropped to 669,000 per swab after 7 days and to 310 per swab at 20 days. After 20 days at 28 C, the count was 123,000 per swab, indicating that 28 C was the better holding temperature. No subcultures were made after 20 days.

Field studies currently in progress with clinical specimens show that Salmonella and Shigella can be recovered from medium A after storage at room temperature for as long as 49 days. In a similar field trial, nonagglutinable V. comma strains, Heiberg groups III and IV, have been isolated after 22 days. Preliminary work with Pasteurella pestis in blood indicates these organisms remain viable for at least 75 days.

LITERATURE CITED

TABLE 1. Survival of Shigella flexneri 2 at 28 C

<table>
<thead>
<tr>
<th>Time of subculture</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>3/5</td>
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* Number positive per total number.
† Not done.


