Enrichment Culture Coagglutination Test for Rapid, Low-Cost Diagnosis of Salmonellosis

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Specific diagnosis of salmonellosis by conventional culture and identification methods usually requires 2 to 4 days. Since Salmonella may be disseminated from infected individuals during this period, this amount of time required for diagnosis may be too slow to aid in epidemic control. To obtain earlier diagnoses of salmonellosis, a coagglutination test was used for rapid, simplified detection of Salmonella oranienburg antigens in enrichment broth cultures of fecal specimens from infants involved in a nursery outbreak. Two selective enrichment broths were used, selenite cystine and dulcitol selenite. These were compared in parallel for efficiency by subculture on deoxycholate lactose sucrose, MacConkey, xylose lysine deoxycholate, and tryptic soy lactose teepol agars. These overnight enrichment broth cultures of stool specimens were also examined by a coagglutination slide test with stabilized protein A-containing staphylococci sensitized with antisera for Salmonella antigens C₁, E, and Vi. Of 113 diarrhea stool specimens tested, 86 were positive by conventional culture, 82 were positive by dulcitol selenite-coagglutination, and 55 were positive by selenite cystine-coagglutination. All these tests were negative on 50 stool specimens from infants in a noninfected nursery. Salmonellae were specifically detected in stool cultures within 20 h by the coagglutination technique. This early detection of Salmonella antigens provided a useful adjunct to culture for rapid diagnosis of salmonellosis.

Salmonellosis is a major public health problem throughout the world. It can be an especially severe problem in hospitals in which there are concentrations of those most susceptible to severe Salmonella infections: infants, children, and debilitated patients (1, 11). Although salmonellosis in infants can be very severe, resulting in a high mortality rate (3, 6), it can also be mild or even unsuspected (14). Once salmonellae are established in a hospital, it is generally agreed that their primary, continuing source usually becomes the infected patients themselves (6, 8, 11, 12, 14). Thus, if a salmonellosis epidemic in a hospital is to be controlled, it is essential that these sources of salmonellae be identified quickly and isolated, because they contribute large numbers of salmonellae to the environment (3, 6, 14).

During studies aimed at controlling endemic salmonellosis in the newborn nursery of a hospital in Jakarta, Indonesia, dulcitol selenite enrichment broth (DUL-SEL) (19) and a coagglutination (COAG) test for identification of Salmonella colonies on agar plates (2), both already under study in our laboratory at the time, appeared to offer promise for simplified, rapid specific detection of salmonellosis. Therefore, a study was designed to evaluate application of these techniques in the clinical situation. The purposes of this study were to: (i) apply the COAG test to detect and identify salmonellae in selective enrichment broth culture, (ii) compare selective enrichment broth-COAG tests and conventional methods for specificity and sensitivity, (iii) compare the relative efficiency of two selective enrichment broths, and (iv) compare the estimated costs and logistic factors for several approaches to surveys for salmonellosis.

MATERIALS AND METHODS

Source of specimens. Fecal samples were obtained from infants in two hospital nurseries. There were 113 subjects sampled in the infected nursery and 50 in the normal control nursery. There had been a long-standing endemic salmonellosis in the infected nursery due to a strain of Salmonella oranienburg that was resistant to ampicillin, cephalothin, tetracycline, kanamycin, and neomycin (10). Freshly passed stool specimens were obtained from diapers. Stool specimens were usually moderately to severely diarrheic, often containing mucus. The stool samples from the second, or control, nursery were also diaper specimens, but they were well-formed, had the normal appearance of young infant stools, and some contained mucin. None of these control infants displayed any signs or symptoms of gastroenteritis. Infants in the first nursery ranged in age from 1 to 90 days, but

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most were 1 to 5 days old. All infants in the normal control nursery were 1 to 5 days old.

**Culture media.** Two *Salmonella* selective enrichment broths were used, selenite cystine broth (SEL-CYS; BBL Microbiology Systems) and DUL-SEL broth (9). DUL-SEL broth consisted of the following: proteose peptone, (Difco Laboratories) 0.4%; yeast extract, 0.15%; dulcitol, 0.3%; sodium selenite, 0.5%; Na2HPO4, 0.125%; and KH2PO4, 0.125%. The pH was adjusted to 6.9, and the broth was distributed at 10 ml per screw-capped test tube (16 by 125 mm). These tubes were then steamed for 20 min at atmospheric pressure. Four plating agars representing varying degrees of selectivity were used: deoxycholate lactose sucrose agar, MacConkey Agar, xylose lysine deoxycholate agar (all BBL Microbiology Systems), and tryptic soy teepol agar (Sanborn, unpublished data). Tryptic soy teepol agar consisted of tryptic soy agar (Difco Laboratories); teepol (3; Shell Oil Company), 1%; lactose, 0.5%; and 40 mg of bromothymol blue per liter; this was adjusted to pH 6.9 and sterilized at 121°C for 15 min before being poured into petri plates. Xylose lysine deoxycholate agar was prepared from XL agar base (BBL Microbiology Systems) with the indicator and deoxycholate solutions added separately (13).

**Bacteriology.** Specimens were transported to the laboratory in Cary and Blair medium and were planted directly on plates of the four agar media. Swab samples from the specimens were also placed in both enrichment broths. After 18 to 20 h of incubation in the broths at 36°C, these swabs were planted on fresh sets of the same four agar media. Agar plates were also incubated at 38°C for 20 to 24 h. Identification of isolates was done by selecting isolated colonies and planting them into Kligler iron agar, lysine iron agar, and motility-indole-ornithine medium (4; all Difco Laboratories). The identity of biochemically presumptive positive salmonellae was confirmed by standard slide agglutination tests with commercial antisera (BBL). Isolates were serologically screened only for groups C, E, and E and for Vi antigens, because *S. oranienburg* and *Salmonella welleucreden* were already known to be in the nursery environment, and *Salmonella typhi* was the most commonly found *Salmonella* in Jakarta.

**COAG test.** The COAG reagents were prepared as previously reported (2). Commercial *Salmonella* O agglutinating antisera (BBL Microbiology Systems) were used to sensitize the stabilized *Staphylococcus* cells. We used a *Staphylococcus* cell concentration of 10%, although it was later determined that 5% was equally, or possibly more, satisfactory. For testing purposes, Pasteur pipettes were used to place 1 drop each of 15- to 20-h selective enrichment broth cultures and COAG reagent in the well of an agglutination slide. The drops were mixed with an applicator stick, and then the slide was rocked to and fro until clumping occurred. Reactions were considered negative if no clumping occurred within 2 min. Weak reactions were read with the aid of a dissecting microscope. COAG reagents sensitized with antisera to *Salmonella* groups C, E, and Vi antigens were the only ones used for the same reasons stated above.

**Cost estimates.** Based on our experience in processing the 163 stool specimens reported here, selected logistic factors and costs were estimated for processing 100 stool specimens by each method, conventional culture isolation and identification versus the enrichment-COAG technique. For comparison purposes, costs of media and reagents were calculated at 1979-1980 prices. Labor costs were estimated for convenience at $7.50/h for technicians doing technical benchwork and at $4.00/h for laboratory aides preparing culture media and reagents. Also for comparison purposes, two approaches were considered for conventional culture diagnosis of enteric bacterial infections. The first was similar to that recommended by Ewing and Martin (5). In this approach, termed "recommended," both direct plate streaking and enrichment broth culture methods were included. Three agar plates of varying degrees of selectivity were considered to be employed at each step. For comparison purposes, three colonies were considered to be picked from each positive plate for biochemical and serological identification by the same methods that we used in the actual study. In the second approach, termed "minimum," it was considered that only one agar plate would be employed with a selective enrichment broth; although this approach would be expected to reduce costs, a reduction in isolation efficiency could also be anticipated.

**RESULTS**

There were 86 specimens culture positive for *S. oranienburg* (group C; antigen) and 1 for *Shigella flexneri*. All of these were obtained from infants in the infected nursery. Direct plating yielded only 42 isolations of *S. oranienburg* (49%), whereas SEL-CYS and DUL-SEL broth enrichments yielded 72 (84%) and 85 (99%) isolations, respectively. The difference between direct plating and either enrichment method was highly significant, *P* < 0.001, as was also the difference between isolations from the enrichment media themselves (7).

The results of culture and COAG tests on 163 fecal samples are compared in Table 1. There was one specimen from a symptomatic infant that was positive for *Salmonella* group C; antigen only by the DUL-SEL enrichment-COAG procedure. The specimen yielding *S. flexneri* was negative in all *Salmonella* COAG tests.

Among the 86 specimens culture-positive for *Salmonella*, the SEL-CYS-COAG system was positive for 55 (64%), whereas the DUL-SEL-COAG system was positive in 82 (95%). The difference between the two COAG test systems was highly significant, *P* < 0.001 (7). The difference between culture isolation and COAG from DUL-SEL broth was not significant. No other salmonellae were isolated from either nursery, and the COAG tests for group E and Vi antigens were universally negative.

The COAG test was negative on 4 DUL-SEL broths and 14 SEL-CYS broths that yielded *S.
oranienberg by culture. Other minor discrepancies were also noted (Table 2).

There was a considerable difference in the logistics involved in detecting salmonellae by conventional culture methods as compared with the enrichment-COAG method (Table 3). Enrichment-COAG yielded a diagnosis in one-third the time required for conventional culture. Furthermore, the amount of working time for both technicians and laboratory aides was reduced with the COAG test.

These factors exerted a direct affect on costs of diagnostic testing (Table 4). Estimated costs for 100 tests by the selective enrichment-COAG test were much lower than by conventional methods, varying from 11 to 36% of conventional culture costs, depending on how much culture medium would be used in a conventional procedure. The cost with commercially prepared culture medium was also estimated from current prices. This was found to range from about $300 to $600 for the media required alone, exclusive of technician labor costs, as compared with about $36 for the 100 tubes of enrichment broth that would be used in an enrichment-COAG Salmonella detection procedure.

Visible growth in the two selective broths was observed to be quite different. After overnight incubation of fecal specimens in the broths, most of the SEL-CYS broths exhibited heavy turbidity and precipitation, whether or not salmonellae were subsequently detected in the tubes. On the other hand, most Salmonella-negative DUL-SEL broth tubes were clear or only slightly turbid, whereas Salmonella-positive tubes of DUL-SEL broth contained very turbid growth.

Direct COAG examination of fecal samples emulsified in saline or distilled water was also attempted. Spontaneous agglutination of the reagents was frequent and did not permit satisfactory analysis by this more direct approach.


DISCUSSION

The selective broth enrichment-COAG test incorporating DUL-SEL broth proved to be equally as efficient as an intensive conventional culture procedure (two enrichment broths and direct plating onto four agar media) for detecting S. oranienburg in fecal specimens from infants. The slightly higher detection of salmonellae by the conventional culture methods was not significantly better than that by COAG. Specificity of both methods was equal. The enrichment-COAG test yielded much earlier results than did the conventional methods, taking less than one-third of the time. The enrichment-COAG test was simple to perform and to read. Technicians had no trouble with the slide agglutination procedure used, since mechanically, it was nearly identical with that normally used to serologically identify enteric bacterial cultures.

There was a significant difference in efficiency of the selective enrichment broths used for the tests. DUL-SEL proved to be better than SEL-CYS, both for isolating salmonellae and for producing antigens to be detected by the COAG test. Since there was much more non-Salmonella growth in SEL-CYS, it seemed that these competing bacteria could have been responsible in part for the poorer efficiency of SEL-CYS. Comparison of gross appearance of growth in the two broths was quite striking with these specimens. Since DUL-SEL cultures were seldom cloudy unless salmonellae were present, it was almost possible to predict positive diagnoses simply by observing the tubes. Citrobacter freundii was found in many of those that were cloudy but negative for Salmonella.

One specimen was positive by DUL-SEL-COAG but not by culture. It was not possible to obtain a follow-up specimen from this patient. However, since the patient was symptomatic, it appeared that the culture was a false-negative.

Although the enrichment broth-COAG procedure yielded more rapid diagnosis than the conventional methods, an even more rapid test would have been desirable. Ideally, the diagnosis should be made immediately, directly from the diarrhea stool specimens. This was attempted with both saline and distilled water emulsions of the specimens, but the results were disappointing. Work is continuing on the possibility of a direct diagnostic test, including investigation into means of eliminating spontaneous reactions as well as means of concentrating antigens in the stool specimen.

The enrichment-COAG procedure herein described would be about equivalent in rapid diagnostic speed to the method of picking suspect colonies from primary isolation plates for direct agglutination with specific antisera. However, it offers several advantages over that method. First, only one broth culture, containing more than adequate antigenic material, need be examined. In contrast, it may be necessary to select many colonies for rapid serological identification by the colony agglutination method. The antigenic material in individual colonies is limited, and on many plates, Salmonella colonies may be hidden among those of commensals. Second, directly streaked primary plates must be used as the source of colonies for agglutination to yield a diagnosis within 24 h, whereas picking colonies from enrichment culture plates implies 48 h for a diagnostic result. Third, direct plating is relatively inefficient for isolating salmonellae. In this study, it detected less than 50% of the positive specimens.

This clinical application trial of a selective enrichment broth-COAG test for rapid diagnosis was done in an admittedly special situation, in that only one type of Salmonella was involved, S. oranienburg. Also, the flora in fecal samples from infants is quite different from that of other children and adults. However, since nursery salmonellosis is a problem encountered from time to time, the results of this study indicate that the selective enrichment-COAG test could be a useful adjunct procedure to aid epidemiologists in controlling similar outbreaks.

Successful COAG reagents have been prepared for all major O serogroups of salmonellae, and the preparation method is simple and easily done (2). Since the original report we have found that many, but not all, commercial O antisera, as well as antisera prepared in our laboratory, yield excellent COAG reagents. If general application of this technique is to be realized, further studies in other clinical situations will be necessary. Such a study examining diarrhea stool specimens received from a general population is currently under way in Jakarta.

In summary, the selective enrichment-COAG method described here yielded rapid, valid diagnoses in cases of salmonellosis. It was more cost-effective than conventional culture procedures. It appeared to have potential as a useful clinical and public health diagnostic tool.

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LITERATURE CITED


