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Sorbitol-MacConkey Medium for Detection of *Escherichia coli* O157:H7 Associated with Hemorrhagic Colitis

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***Escherichia coli* serotype O157:H7 is a recently recognized human pathogen associated with hemorrhagic colitis. Unlike most *E. coli* strains, *E. coli* O157:H7 does not ferment sorbitol. Therefore, the efficacy of MacConkey agar containing sorbitol (SMAC medium) instead of lactose as a differential medium for the detection of *E. coli* O157:H7 in stool cultures was determined in comparison with MacConkey agar. The relative frequency of non-sorbitol-fermenting (NSF) organisms other than *E. coli* O157:H7 in feces was low at 10 to 20% (95% confidence limits), and NSF organisms also occurred mostly in small numbers. In a field trial involving over 1,000 diarrheal stools, *E. coli* O157:H7 was isolated from 18 stools, all of which were from patients with bloody diarrhea. In every instance, the growth of *E. coli* O157:H7 on SMAC medium was heavy and occurred in almost pure culture as colorless NSF colonies in contrast to fecal flora, which are mostly sorbitol fermenting and hence appear pink on this medium, whereas on MacConkey agar cultures, the growth of *E. coli* O157:H7 was indistinguishable from fecal flora. SMAC medium permitted ready recognition of *E. coli* O157:H7 in stool cultures. Detection of *E. coli* O157:H7 on SMAC medium had a sensitivity of 100%, a specificity of 85%, and an accuracy of 86%. SMAC medium stool culture is a simple, inexpensive, rapid, and reliable means of detecting *E. coli* O157:H7, and we recommend routine use of SMAC medium especially for culturing bloody stools.**

Escherichia coli O157:H7 is a newly recognized human pathogen associated with hemorrhagic colitis, a recently described syndrome characterized by watery diarrhea progressing rapidly to grossly bloody diarrhea with little or no fever (11, 14). Although the initial evidence implied *E. coli* O157:H7 as a rare agent of human disease, subsequent reports have indicated that this organism and the associated diarrheal illness may not be uncommon, at least in the United States and Canada (1, 4, 5, 8, 10, 13); in addition to several sporadic cases of hemorrhagic colitis, there have also been outbreaks of this syndrome since its description in 1982 (1, 3, 6, 8a, 12). The mounting evidence linking *E. coli* O157:H7 and hemorrhagic colitis with hemolytic uremic syndrome has lent further clinical, epidemiologic, and bacteriologic importance to *E. coli* O157:H7 (1, 4, 5, 8, 13).

Recent reports have presented evidence to consider routine culturing of diarrheal stools, especially those with blood, for *E. coli* O157:H7, (8, 10). Detection of *E. coli* O157:H7 on routine enteric media is time consuming and expensive. Unlike most *E. coli* strains, serotype O157:H7 does not ferment sorbitol, and testing for sorbitol fermentation, therefore, has been suggested as a simple means to screen for this organism (8, 14). This is, however, slow and tedious when several colonies from primary stool cultures obtained on routine enteric media are individually tested. An earlier report suggested the use of MacConkey agar containing sorbitol (SMAC agar) instead of lactose to differentiate sorbitol-negative *E. coli* strains (9). The potential application of this medium to detect *E. coli* O157:H7 in stool cultures was recently indicated (10). In view of the above and the emerging importance of *E. coli* O157:H7, we determined the usefulness of sorbitol as a strain marker to aid in the detection of *E. coli* O157:H7 in stool cultures by using SMAC agar as a primary isolation medium in comparison with MacConkey agar. This study was carried out in two stages: (i) the initial laboratory analysis series to determine the relative frequency of non-sorbitol-fermenting (NSF) organisms in fecal flora and assess the reliability of SMAC medium to permit recognition of *E. coli* O157-H7 in stool cultures, and (ii) the field trial series to test the efficacy of SMAC medium for the detection of *E. coli* O157:H7 in routine stool cultures.

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MATERIALS AND METHODS

Media. SMAC medium was prepared as follows with MacConkey agar base (Difco Laboratories, Detroit, Mich.), which is identical to MacConkey agar but contains no lactose. MacConkey agar base (40.0 g) and D-sorbitol (10 g) (Difco) were mixed in 1 liter of distilled water, autoclaved, and poured into plates which were stored and used per standard practice. MacConkey agar (Difco) was prepared as usual.

Frequency of NSF organisms in fecal flora. To determine the frequency of NSF organisms other than *E. coli* O157:H7 in feces, randomly picked nonbloody stools were cultured on SMAC medium, and organisms yielding NSF colonies were identified by standard methods (7).

Laboratory evaluation of SMAC medium. The reliability of SMAC medium to facilitate the recognition of *E. coli* O157:H7 in stool cultures was determined next in comparison with MacConkey agar, the routinely used differential medium for *E. coli*. Fourteen randomly picked stools were seeded with strains of *E. coli* O157:H7 (approximately 10^3 to

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TABLE 1. Validity of detection of *E. coli* O157:H7 on SMAC medium[a]

NSF organisms	No. of stool cultures	
	Without *E. coli* O157:H7	With *E. coli* O157:H7
Present	36	14
Absent	204	0

[a] Diagnostic parameters (2): sensitivity, 14/14 = 100%; specificity, 204/240 = 85%; positive predictive value, 14/50 = 28%; negative predictive value, 204/204 = 100%; accuracy, 218/254 = 86%; prevalence, 14/254 = 5.5%.

10^4 CFU/ml per stool), and 10 μl of each stool suspension was cultured on SMAC and MacConkey media. The plates were incubated overnight at 36°C. Six NSF colonies were picked at random from each of the SMAC medium cultures, and an equal number of colonies typical of *E. coli* was picked from each MacConkey agar culture. The colonies were individually identified by conventional biochemical tests (7); the *E. coli* isolates were then serotyped with *E. coli* O157 and H7 antisera (Laboratory Centre for Disease Control, Ottawa, Canada).

Field trial of SMAC medium. The usefulness of SMAC medium to detect *E. coli* O157:H7 in routinely processed stools was determined during a local surveillance study of hemorrhagic colitis. Over a period of 20 months, a total of 1,043 diarrheal stools, 99 of which were from patients with bloody diarrhea, were cultured as before on SMAC and MacConkey media for *E. coli* O157:H7. Presumptive colonies were picked and identified likewise. The *E. coli* O157:H7 isolates were tested for verotoxin production as previously described (8).

RESULTS

Of 240 stools processed to determine the frequency of NSF organisms in fecal flora, 36 (15%) yielded cultures with NSF colonies. The breakdown on the frequency of NSF organisms was: *E. coli* O157:H7, 0%; *E. coli* other than O157:H7, 6%; *Proteus* sp., 4%; *Morganella* sp., 2%; other coliform bacteria and *Pseudomonas* sp., 3%. This observation confirmed that only a small percentage of *E. coli* other than serotype O157:H7 failed to ferment sorbitol and indicated that the frequency of NSF organisms that might interfere with the detection of *E. coli* O157:H7 on SMAC medium cultures was relatively low at 15% (95% confidence limits of 10 to 20%). These data supported the potential application of SMAC agar as a differential medium for *E. coli* O157:H7 (specificity, 85%).

In the laboratory evaluation of SMAC medium with seeded stools, *E. coli* O157:H7 was detected in all 14 cultures obtained on SMAC medium as well as on MacConkey agar. However, of the total of 84 colonies tested from the 14 SMAC medium cultures, 76 (90%) were identified as *E. coli* O157:H7, whereas, only 43 (51%) of 84 colonies tested from the MacConkey agar cultures were found to be *E. coli* O157:H7. Being NSF organisms, all test strains of *E. coli* O157:H7 yielded colorless colonies on SMAC medium. These colonies were similar in appearance to non-lactose-fermenting colonies on MacConkey agar and contrasted well with bright pink colonies of sorbitol-positive organisms of the fecal flora. *E. coli* O157:H7 colonies, therefore, were easily recognizable on SMAC medium culture, whereas they were indistinguishable from fecal flora in cultures obtained on MacConkey agar. The above data indicated that, although the sensitivity was the same for both media, SMAC medium could permit ready detection of *E. coli* O157:H7 visually and through testing of fewer colonies per culture, with positive identification 9 of 10 times in contrast to about 50% on MacConkey agar.

Based on the data obtained in the above two laboratory analysis series, i.e., 240 stool cultures without *E. coli* O157:H7 and 14 stools seeded with *E. coli* O157:H7 and cultured, the validity of detection of *E. coli* O157:H7 on SMAC medium stool cultures was determined (Table 1).

In the field trial of SMAC medium, *E. coli* O157:H7 was isolated from 18 of 99 stools obtained from patients with bloody diarrhea and from none of the remaining 944 stools which were from patients with nonbloody diarrhea. This difference in the association of *E. coli* O157:H7 with nonbloody diarrheal illness was significant ($P < 0.01$) (Table 2). In all 18 positive stool cultures, the growth of *E. coli* O157:H7 on SMAC medium was heavy and was obtained in almost pure cultures as colorless NSF colonies. Of over 1,000 stools which were negative for *E. coli* O157:H7, only about 10% grew any NSF colonies on SMAC medium (Table 2). But, unlike the growth of NSF colonies of *E. coli* O157:H7 in stool cultures of patients with bloody diarrhea, this growth was mostly light to moderate and mixed with heavy growth of sorbitol-fermenting fecal flora; in only about 1.5% of the cases did the growth of fecal NSF resemble that of *E. coli* O157:H7 on SMAC medium cultures. In contrast to SMAC medium cultures, the growth of *E. coli* O157:H7 on MacConkey agar was indistinguishable from fecal flora, and its detection in MacConkey agar cultures involved a systematic search of all stool cultures, with testing of up to six individual colonies per culture, resulting in considerable work, time, and expense. Moreover, MacConkey agar did not readily permit quantitation of the growth of *E. coli* O157:H7 when this was present. Nonetheless, *E. coli* O157:H7 could be detected on MacConkey agar stool cultures in all instances (data not shown). All 18 isolates of *E. coli* O157:H7 produced verotoxin and, on primary isolation, failed to ferment sorbitol for up to 48 h.

The initial laboratory analysis series involving 240 stool cultures without *E. coli* O157:H7 and 14 stools seeded with *E. coli* O157:H7 indicated a positive predictive value of 28% and a negative predictive value of 100% at an accuracy of 86% for detection of *E. coli* O157:H7 on SMAC medium cultures (Table 1). The low positive predictive value is due to the low prevalence of 5.5% in the analysis caused by the low number of *E. coli* O157:H7-positive cultures in this series. In the field trial series, which was a random sample of the population, the prevalence of *E. coli* O157:H7 was 18% (18 of 99) in stools from patients with bloody diarrhea (Table 2). This prevalence is 3.3 times higher than that used to calculate the positive predictive value of 28% in Table 1. If the population prevalence of 18% were applied, the positive predictive value would in all likelihood become 56%.

TABLE 2. Results of field trial of SMAC medium stool cultures

Nature of stool	No. of stools tested	No. (%) of stools yielding NSF organisms	No. (%) of stools yielding *E. coli* O157:H7
Bloody	99	28[a] (28)	18[b] (18)
Nonbloody	944	88[a] (9.3)	0

[a] Mostly mixed growth with sorbitol fermenters except when *E. coli* O157:H7 was present.

[b] All obtained in almost pure cultures as NSF organisms. Significantly different from zero ($P < 0.01$).

DISCUSSION

All *E. coli* O157:H7 strains isolated to date have been found to produce verotoxin and are known to ferment sorbitol either slowly over a period of days or not at all (8, 10, 14). All our isolates of *E. coli* O157:H7 were verotoxin positive and failed to ferment sorbitol. Our observation indicates that, because *E.coli* O157:H7 does not ferment sorbitol, colonies of this organism on SMAC medium stool cultures are colorless and hence readily recognizable, whereas they are indistinguishable from fecal flora in cultures obtained on MacConkey agar. *E. coli* O157:H7 seems to be excreted in stools in large numbers mixed with little or no fecal flora, especially during the acute stage of illness, as evidenced by the heavy growth of this organism in almost pure cultures on SMAC medium in every instance in which it was isolated from a clinical case. This type of characteristic growth of *E. coli* O157:H7, which is readily recognizable on SMAC medium, permits presumptive identification of this agent with a low false-positive rate. Our data also indicate that the distinction of NSF colonies of *E. coli* O157:H7 on SMAC medium makes confirmation of presumptive colonies by additional tests simple and easy. Since the frequency of other NSF organisms in feces is 15% (found to be ≈10% as expected in the field trial), the great majority of stool cultures that are negative for *E. coli* O157:H7 can be readily dismissed as negative by a glance at SMAC medium cultures. In this connection, we reiterate our observation that the growth of all our clinical isolates of *E. coli* O157:H7 was heavy and occurred in almost pure cultures as NSF colonies on SMAC medium, whereas only rarely did stools negative for *E. coli* O157:H7 yield this type of growth on SMAC medium. On the average, no more than 10% of SMAC medium cultures would need to be screened with specific tests to exclude fecal NSF organisms as opposed to testing all cultures obtained on MacConkey agar in the detection of *E. coli* O157:H7. The fecal samples included in the survey were mostly collected during the acute stage of illness, and the heavy growth of *E. coli* O157:H7 in all positive stool cultures may be a direct result of this. The importance of early stool collection to detect *E. coli* O157:H7 has been emphasized by other workers (8, 10).

Prior exposure to sorbitol is known to induce sorbitol-positive mutants among *E. coli* O157:H7. This obviously does not pose problems in the primary isolation of *E. coli* O157:H7 on SMAC medium, even when cultures are incubated beyond 48 h, but exposure to SMAC medium may favor emergence of sorbitol-positive mutants subsequently. Therefore, SMAC medium cultures of *E. coli* O157:H7 may not be reliable for repeat testing of sorbitol fermentation which, however, is not required in routine practice.

E. coli O157 and H7 antisera have not yet become available commercially, and it is likely that most hospital laboratories are not testing for *E. coli* O157:H7. Because this organism is culturally indistinguishable from other fecal coliform bacteria on routine enteric media, it will be almost certainly missed when the standard stool culture protocol is used or if there is no specific request for this agent. Not all physicians may be aware of hemorrhagic colitis, which is a recently recognized syndrome, and it is probable that a specific request for *E. coli* O157:H7 may not always be made. Recent reports have indicated the widespread prevalence of hemorrhagic colitis and emphasized the need to consider *E. coli* O157:H7 in bloody diarrheal illness (1, 3, 4, 8, 10). In this regard, it appears worthwhile for clinical laboratories to consider culturing all bloody stools for *E. coli* O157:H7, at least on an experimental basis. SMAC medium is ideally suited for this purpose. A similar medium was reported to have been used in a recent surveillance study of hemorrhagic colitis associated with *E. coli* O157:H7 (10). The occurrence and significance of *E. coli* O157:H7 in nonbloody diarrheal illness and in asymptomatic individuals are not fully known, and routine use of a simple medium such as SMAC medium may also help us to understand the spectrum of illness associated with this agent and its prevalence and epidemiology.

Based on our results, we conclude that heavy growth of NSF colonies on SMAC medium stool cultures may be presumptive of *E. coli* O157:H7, especially when cultured from bloody stools; SMAC medium is a useful, rapid, and reliable screening aid for the detection of this agent in stool specimens. It is also a simple, inexpensive medium to include in the routine work-up; its inclusion as a primary isolation medium is more economical than testing for NSF colonies in stool cultures obtained on routine enteric media in the attempt to detect *E. coli* O157:H7. In smaller laboratories, inclusion of SMAC medium can also facilitate ready recognition and selection of NSF colonies for referral. During the course of this study, we also found evidence that hemorrhagic colitis may be a common disease, and there is a definite need to consider *E. coli* O157:H7, especially in bloody diarrheal illness (9a).

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