

Latex Agglutination Test for Detection of *Escherichia coli* Serotype O157

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The value of a latex agglutination test (*Escherichia coli* O157 latex test; Oxoid Ltd.) for rapid presumptive detection of *E. coli* serotype O157:H7 was determined by laboratory trials and during an outbreak of hemorrhagic colitis. The latex test was found to be a simple, highly efficient and reliable test in detecting *E. coli* O157:H7 with 100% sensitivity and specificity. It was also found that sorbitol-MacConkey agar cultures were not as useful for food samples as they were for fecal specimens in screening for *E. coli* O157:H7, but the use of the latex screen was particularly efficient in this setting.

Escherichia coli serotype O157:H7 has emerged as an important agent of public health concern, with many outbreaks and numerous sporadic cases of hemorrhagic colitis, hemolytic uremic syndrome, and diarrheal illness in different settings (4, 6-8, 12, 14, 16-18), and has been isolated from cattle and foods of animal origin and shown to be transmitted through contaminated foods (1, 5, 11, 19; J. H. Jessop. Conjoint Meet. Infect. Dis., Calgary, Alberta, Canada, 21 to 23 November 1988, abstr. no. A-8). Although there have been several reports on the laboratory approach to detecting *E. coli* O157:H7, the common practice is to screen specimens on sorbitol-MacConkey (SMAC) medium and test the non-sorbitol-fermenting (NSF) colonies for *E. coli* O157:H7 by biochemical parameters and by serotyping with O157 and H7 antisera (6, 7, 9, 10). Biochemical identification and serotyping are slow and inefficient, especially in outbreak situations and large-scale screening of clinical and food samples. Recently, latex agglutination tests (*Escherichia coli* O157 latex test, Oxoid Ltd., Hampshire, England; Serobact *E. coli* O157, D.P. Diagnostics, Adelaide, Australia) have become commercially available for rapid presumptive detection of *E. coli* belonging to the serogroup O157. We evaluated the *Escherichia coli* O157 latex test (Oxoid Ltd.) initially with known clinical isolates of *E. coli* and subsequently had an opportunity to determine its application and reliability for rapid detection of *E. coli* O157 in fecal specimens as well as in a variety of food samples during a province-wide outbreak of hemorrhagic colitis. The outbreak occurred during the months of July and August 1988 and affected all age groups across the province of Newfoundland; close to 50 cases of bloody diarrheal illness were recorded.

Escherichia hermannii is sorbitol negative and agglutinates in *E. coli* serotype O157 antiserum, and thus it could be mistaken for *E. coli* O157:H7 (2). *E. hermannii* has been isolated from fecal samples (3) and, more importantly, from foods such as raw milk and beef, both known sources of *E. coli* O157:H7 (1, 2, 5, 11, 19). Among other distinctive features (13), *E. hermannii* can be differentiated from *E. coli* O157:H7 by cellobiose fermentation (2, 13). During the outbreak, therefore, we thought it might be useful to screen food samples on cellobiose-MacConkey (CMAC) medium in addition to SMAC medium, especially because *E. hermannii* has been reported to be commonly isolated from food items

(2, 15). We previously reported the value of SMAC medium in detecting *E. coli* O157:H7 from clinical specimens (10), and in this report we include our preliminary observations on the application of SMAC and CMAC medium cultures in detecting *E. coli* O157:H7 from food samples.

Dehydrated SMAC medium was obtained from Oxoid (CM 813, Sorbitol MacConkey agar No. 3), and CMAC was prepared in-house by adding 1% cellobiose (Difco Laboratories, Detroit, Mich.) to MacConkey agar base (Difco) which contains no sugar. *E. coli* serotype O157 and H7 antisera were obtained from Difco. The *Escherichia coli* O157 latex test (DR 620, Oxoid) includes two reagents: test latex, consisting of latex particles sensitized with specific rabbit antibody reactive with the *E. coli* O157 antigen, and control latex, consisting of latex particles sensitized with preimmune rabbit globulins. The test is designed to demonstrate, by slide agglutination, *E. coli* strains possessing the O157 antigen, and it is best used in conjunction with SMAC medium cultures. The control latex is used only if agglutination occurs with the test latex, and it is meant to rule out autoagglutination.

The *Escherichia coli* O157 latex test was initially evaluated by using 200 known strains of *E. coli* serotype O157:H7 and 50 clinical isolates of *E. coli* with serotypes other than O157. The test was carried out in accordance with manufacturer instructions, as follows. Overnight growth of test strains was emulsified in a drop of saline on a slide and mixed with a drop of test latex, and the slide was rocked for a minute and observed for agglutination. If the result was positive, autoagglutination was ruled out by a repeat test with control latex.

Fecal samples were cultured on SMAC medium for *E. coli* serotype O157:H7 as per the established method (10). Up to 10 NSF colonies per culture were screened with the latex test, and those found positive were tested for biochemical parameters, including cellobiose, raffinose, and dulcitol fermentations (13), and confirmed by conventional serotyping. All *E. coli* O157:H7 isolates were also tested for verocytotoxin production as previously described (8). NSF colonies testing negative by the latex screen were examined further to determine their identity to rule out a false-negative latex result.

Food samples were processed by the method of Doyle and Schoeni (5) with some modifications. Samples were directly plated onto both SMAC and CMAC media and inoculated into a modified Trypticase soy enrichment broth (mTSB)

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(BBL Microbiology Systems, Cockeysville, Md.) (5), and the overnight enrichment was subcultured onto both differential media. Cultures yielding NSF colonies on SMAC medium, cellobiose-negative colonies on CMAC medium, or both were screened by the latex test for *E. coli* serotype O157, and the identity of the cultures was established as described above.

In the initial laboratory evaluation, the latex test was found to be 100% sensitive and specific; it showed a complete and rapid agglutination with all of the 200 *E. coli* serotype O157:H7 strains and a clear-cut negative reaction with the 50 non-*E. coli* O157 strains tested.

During the outbreak, fecal specimens from over 100 persons were cultured for *E. coli* serotype O157:H7 and the fecal extracts were tested for verocytotoxin. *E. coli* O157:H7 was isolated from 30 cases with an additional 8 testing positive for fecal verocytotoxin only. In most instances of positive cultures, *E. coli* O157:H7 was obtained in heavy growth on SMAC medium as NSF colonies. The latex screen facilitated a rapid presumptive identification of all the 30 positive cases with a complete and rapid agglutination of all the colonies of *E. coli* O157:H7 tested. At the same time, in all instances of fecal cultures yielding NSF colonies other than *E. coli* O157 (15%), the latex test gave a clear-cut negative reaction.

During the outbreak, about 400 food samples, most of which were dairy products, ground beef, and poultry, were processed for *E. coli* serotype O157:H7, but the agent was not isolated from any of the samples tested. However, about 130 samples (33%) yielded NSF colonies on SMAC medium. The latex screen again facilitated a rapid presumptive negative culture findings in all instances. Without the latex screen, the routine follow-up work for these cultures would have been considerable, delaying results and hampering the investigation of the outbreak. The 130 cultures with NSF colonies testing negative by the latex screen were further examined to determine their identity. These turned out to be mostly nonfermenting gram-negative rods, *Hafnia alvei*, *Flavimonas oryzihabitans* (formerly CDC group Ve-2), and *Enterobacter* spp.

Only a small number of the food samples cultured on CMAC medium yielded cellobiose-positive colonies, but *E. hermannii* was not detected in any of the foods examined during the outbreak.

We evaluated the *Escherichia coli* O157 latex test by laboratory and field trials for a rapid presumptive identification of *E. coli* serotype O157:H7 in SMAC medium cultures as well as the application of SMAC and CMAC medium cultures in detecting *E. coli* O157:H7 in food samples. The latex test correctly identified every one of about 230 isolates of *E. coli* O157:H7, including 30 clinical isolates during the outbreak, as belonging to the serogroup O157 (100% sensitivity) and gave a clear-cut negative reaction with 50 non-*E. coli* O157 isolates and close to 150 cultures yielding NSF colonies other than *E. coli* O157 (100% specificity). The slide agglutination procedure is simple to carry out and rapid, and the results are clear-cut and highly reliable. We think the use of the latex test in conjunction with SMAC medium cultures can greatly facilitate the search for *E. coli* O157:H7 in fecal specimens and foods. However, the SMAC medium cultures are not nearly as useful for detecting *E. coli* O157:H7 in foods as they are for fecal specimens (9, 10) because of the occurrence of a larger percentage of nonsorbitol fermenters in foods than in feces (33% versus 15%) (10). Also, it is our experience that the majority of stool cultures yield *E. coli* O157:H7 in heavy growth when it is present (10), and this

may not be the case with foods which are likely to have a low contamination level. Under these circumstances, in fact, the use of the latex test to screen NSF colonies is particularly highly efficient. Nonetheless, we emphasize the fact that the latex test provides only a presumptive identification for *E. coli* O157:H7, and further serotyping with H7 antiserum is necessary for definitive identification.

Although we did not isolate *E. hermannii* from about 400 food samples tested during the outbreak, there are indications that it may be frequently isolated from foods (2, 15). If that is the case, the use of the latex screen in conjunction with a combination of SMAC and CMAC cultures should prove to be useful for rapid detection of *E. coli* serotype O157:H7. *E. hermannii* has been reported to cross-react with O157 antiserum (2) and hence is more than likely to cross-react with the latex test, but we could not verify this.

In conclusion, the *Escherichia coli* O157 latex test is a simple, highly efficient, and reliable test in detecting *E. coli* serotype O157:H7 in SMAC medium cultures. A recent communication also indicated that Serobact *E. coli* O157, a product similar to the Oxoid latex test, was a quick, easy-to-perform procedure to provide presumptive results within 24 h (I. Perry, P. C. Kibsey, K. Kowalewska-Grochowska, and L. Mueller, Conjoint Meet. Infect Dis., Calgary, Alberta, Canada, 21 to 23 November 1988, abstr. no. A-10).

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