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Evaluation of Commercial Latex Reagents for Identification of O157 and H7 Antigens of *Escherichia coli*

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Agglutination reactions obtained with three commercial latex reagents for detecting *Escherichia coli* O157 antigen (Oxoid Diagnostic Reagents, Hampshire, England; Pro-Lab Inc., Richmond Hill, Ontario, Canada; and Remel Microbiology Products, Lenexa, Kans.) and one for detecting H7 antigen (Remel) were compared with those obtained by standard serologic methods by using the Centers for Disease Control and Prevention (CDC) reference antisera for O157 and H7 antigens. For 159 strains of *E. coli* and related organisms, the Oxoid, Pro-Lab, and Remel O157 latex reagents each had a sensitivity and specificity of 100% compared with the CDC reference antiserum. For 106 strains of *E. coli* and related organisms that were not enhanced for motility through semisolid medium, the Remel H7 latex reagent had a sensitivity of 96% and a specificity of 100% compared with the standard tube agglutination method with CDC H7 antiserum. Measures to enhance motility were needed for some strains to detect the H7 antigen. Our findings demonstrate that the commercial latex reagents are good alternatives to standard serologic methods for identifying the O157 and H7 antigens of *E. coli*.

Escherichia coli O157:H7 is now recognized as an important human pathogen of public health concern. Since its recognition in 1982, it has caused many outbreaks and sporadic cases of diarrheal disease. Infection with *E. coli* O157:H7 may be inapparent or may result in a spectrum of disease ranging from mild, nonbloody diarrhea to hemorrhagic colitis, which is characterized by severe abdominal cramping, bloody diarrhea, and little or no fever (11). In some individuals, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura may develop after infection (3, 4, 9, 11). The ability of this organism to produce cytotoxins, referred to as Shiga toxins or Verocytotoxins, is believed to play an important role in the pathogenesis of hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura (3, 4).

E. coli O157:H7 infection is usually diagnosed by isolation of the organism from stool specimens. Unlike approximately 80% of other *E. coli* organisms, *E. coli* O157:H7 ferments sorbitol slowly or not at all. This trait has been used by many to selectively test isolates for the O157 antigen. When *E. coli* O157:H7 was first recognized as an enteric pathogen, screening of sorbitol-negative isolates from sorbitol MacConkey (SMAC) agar plates for the O157 antigen was performed with O157-specific antiserum in slide or tube agglutination assays. In recent years, the development of latex reagents for the detection of the O157 antigen has greatly facilitated the screening of isolates for this antigen (7).

Although an *E. coli* isolate may be presumptively identified as serotype O157:H7 after identification of only the O157 antigen, the complete identification of *E. coli* O157:H7 requires testing for both the O157 and the H7 antigens. Testing for the H7 antigen is routinely performed by using H7-specific antiserum in a tube agglutination assay. Recently, a latex reagent for the detection of the H7 antigen was developed. This reagent would enable laboratories to make a complete identification of *E. coli* O157:H7 by a slide agglutination assay. The objective of the study described here was to assess the sensitivities and specificities of several commercial latex reagents for the identification of the O157 and H7 antigens of *E. coli* O157:H7.

Included in the study were two latex reagent kits for the identification of the O157 antigen (Oxoid Diagnostic Reagents, Hampshire, England; Pro-Lab Inc., Richmond Hill, Ontario, Canada) and one latex reagent kit for the identification of both the O157 and the H7 antigens (Remel Microbiology Products, Lenexa, Kans.). The agglutination reactions obtained with these latex reagents were compared with those obtained by standard serologic methods with O157 or H7 reference rabbit antisera (Centers for Disease Control and Prevention [CDC], Atlanta, Ga.). A total of 159 strains (Table 1) were tested. These strains were isolated from human stool specimens and were identified by standard biochemical and serologic tests (2). We randomly selected from our culture collection *E. coli* strains, received between 1989 and 1993, that possessed either the O157 or the H7 antigen, or both. We also selected strains that possess cross-reacting antigens (1, 10) or that were not able to ferment sorbitol within 48 h and that could potentially be selected for screening from SMAC agar (6).

For evaluation of the O157 latex reagents, bacterial cultures frozen at −70°C were inoculated onto blood agar plates (BAPs) and were then subcultured onto SMAC agar. A single colony of bacterial growth from the SMAC agar plate was then either tested in latex reagents for the O157 antigen or inoculated into broth for testing in the tube agglutination assay with CDC O157 reference antiserum. The latex agglutination assays were performed according to their manufacturers' instructions. A positive result with the O157 latex reagents was interpreted as large clumps of agglutinated latex and bacteria with partial or complete clearing of the background latex within 1 to 2 min. The tube agglutination assay was performed as described by Ewing (2), except that it was performed with fivefold less reagent in the microtiter plates. A positive result by the standard serologic microtiter method with CDC O157 antiserum at its

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TABLE 1. Results for strains tested with commercial latex reagents and CDC antisera for the identification of the O157 and H7 antigens of *E. coli*

Species	Sorbitol fermentation[a]	No. of strains tested for O157	No. of strains agglutinated by O157 reagents		No. of strains tested for H7	No. of strains agglutinated by H7 reagents	
			Oxoid, Pro-Lab, and Remel O157[b]	CDC O157 antiserum		Remel latex H7[c]	CDC H7 antiserum[d]
E. coli O157:H7	−	60	60	60	60	58	60
E. coli O157:NM[e]	−	14	14	14	14	0	0
E. coli O157:H12	+	1	1	1	1	0	0
E. coli O157:H16	−	3	3	3	3	0	0
E. coli O157:H45	+	4	4	4	4	0	0
E. coli O157:H45	−	1	1	1	1	0	0
E. coli O21:H7	+	1	0	0	1	1	1
E. coli O27:H7	+	2	0	0	2	2	2
E. coli O50:H7	+	1	0	0	1	0	1
E. coli O149:H7	+	1	0	0	1	0	1
E. coli Ound[f]:H7	+	1	0	0	1	1	1
E. coli non-O157:non-H7	−	2	0[g]	0	2	0	0
E. hermannii	−	10	0	0	10	0	0
C. freundii	+	1	1	1	1	0	0
Salmonella O group N (O30)	+	4	4	4	4	0	0
S. enterica subsp. *indica*	−	10	0[g]	0			
Edwardsiella tarda	−	1	0	0			
Hafnia alvei	−	1	0	0			
Proteus spp.	−	2	0	0			
Vibrio cholerae	+	10	0	0			
Shigella spp.	−	29	0	0			
Total strains tested		159			106		

[a] Sorbitol fermentation phenotype on SMAC agar after 24 h of incubation at 35°C.
[b] A single colony from SMAC agar was tested.
[c] Sweep of bacterial growth from a BAP not enhanced for motility.
[d] Tested after two to three passages through motility medium.
[e] NM, nonmotile (no growth throughout motility medium after 7 days at 35°C).
[f] und, undetermined.
[g] These strains were positive in test and control latex reagents and required heating to give interpretable results.

routine test dilution (≥1:640) was interpreted as a mat of cells in which at least 50% of the cells in the well were agglutinated. The results of these tests are summarized in Table 1. The test results obtained with all three O157 latex reagents were in complete agreement with those obtained with the CDC reference O157 antiserum. Both the latex reagents and the CDC reference O157 antiserum reacted with a strain of *Citrobacter freundii* and all four strains of *Salmonella* O group N (O30), which have previously been reported to possess antigens that are serologically and chemically related to the O157 antigen (1, 5, 8). Although cross-reactions between some strains of *Escherichia hermannii* and *E. coli* O157 (10) have been noted previously, they were not observed with any of the O157 latex reagents or the CDC reference O157 antiserum. We did observe nonspecific agglutination (i.e., agglutination of both the test latex and the control latex) with two *E. coli* strains (sorbitol negative, non-O157:non-H7) and 10 *Salmonella enterica* subsp. *indica* strains. After boiling for 10 min, according to the manufacturers' directions, we obtained negative results for these 12 strains.

When the present study was initiated, the optimal quantity and type of antigen needed for the H7 latex agglutination assay had not been determined. Using the test procedures recommended by the manufacturer and the criterion that we specified earlier for the interpretation of a positive agglutination reaction with the O157 latex reagents, we evaluated several different antigen preparations from 10 *E. coli* O157:H7 strains to determine which one would be the most suitable for detecting the H7 antigen. First, we tested single colonies from the SMAC agar plates described earlier and obtained negative results for all 10 strains. To determine if these negative results were attributable to poor motility, we passed the strains two times through motility medium (0.4% [wt/vol] agar in nutrient broth [Difco, Grand Rapids, Mich.]), subcultured growth from the bottom of the second motility tube onto a BAP, and retested single colonies, as well as a sweep of growth (≥2.5 mg of growth), in the H7 latex reagent. The single colonies from the motility-enhanced strains were unable to agglutinate the H7 latex reagent, but the sweeps of growth off of the BAP were all positive in the H7 latex reagent; all broth antigens prepared from growth from the second motility tube were positive in the standard tube agglutination assay with CDC H7 antiserum. These findings suggested that the amount of antigen contained in a single colony is not sufficient to afford adequate agglutination of the H7 latex reagent.

Using the same strains, we then evaluated the H7 latex reagent with sweeps of bacterial growth from a BAP before and after enhancement of motility. Nine of 10 strains that were not enhanced for motility and 10 of 10 strains that were enhanced for motility agglutinated the H7 latex.

Because the majority of strains in the preliminary evaluation were agglutinated without enhancement of motility, we performed the evaluation of the H7 latex reagent using the manufacturers' test procedures with a sweep of bacterial growth from a BAP that was not enhanced for motility. Strains giving false-negative results were retested after two to four passages through motility medium. The H7 latex reagent reactions were compared with those obtained by the standard tube agglutina-

tion assay with CDC H7 antiserum (2). Overnight broth antigens prepared from strains passed two to three times through motility medium were tested by the tube agglutination assay with CDC reference H7 antiserum. A positive result with the H7 antiserum at the routine test dilution (1:500) was interpreted as clumping of at least 50% or more of the bacterial cells.

A subset of strains were selected for evaluating the H7 latex reagent because of their reactivity in the O157 latex reagents and/or the presence of the H7 antigen. The results for these tests are summarized in Table 1. Of 106 strains tested with H7 latex reagents, 102 (96%) gave the expected results without measures to enhance their motility; 62 of 66 (94%) strains possessing the H7 antigen agglutinated the H7 latex, whereas 40 of 40 strains lacking the H7 antigen did not. Four *E. coli* strains (O50:H7 [$n = 1$], O149:H7 [$n = 1$], and O157:H7 [$n = 2$]) required four passages through motility medium before they were sufficiently motile to agglutinate the H7 latex. One of these (O157:H7) required three passages through motility medium before it was sufficiently motile to be agglutinated by the CDC H7 antiserum, while the other 65 strains possessing the H7 antigen were positive after two passages. These findings demonstrate that expression of the H7 antigen can be variable and that measures to enhance motility will be needed for some strains to achieve adequate antigen expression for agglutination of the H7 latex reagent.

Since the majority of *E. coli* O157:H7 strains in the present study agglutinated the H7 latex without enhancement of motility, we recommend that an O157-positive colony be subcultured first to blood or an equivalent agar and that a sweep of growth from this medium be tested in the H7 latex assay. An *E. coli* O157 isolate that is initially negative in the H7 latex reagent reaction should be retested after passage through appropriate medium to enhance motility. The number of passages needed to achieve adequate antigen expression will vary from strain to strain. For many strains, two passages are sufficient; however, some *E. coli* O157:H7 strains received in our laboratory have required 10 or more passages to achieve adequate flagellar antigen expression.

In our experience, enhancement of motility by subculturing strains in semisolid medium can be problematic. The amount of moisture and the concentration of agar in the medium are factors that can dramatically influence the effectiveness of this technique for increasing motility and antigen expression. If the amount of moisture is too high or the concentration of agar is too low, a strain may appear to be more motile than it actually is. Testing of strains that are not sufficiently motile can lead to false-negative results. For this reason, we advise having apparently motile *E. coli* O157 strains that do not agglutinate the H7 latex reagent tested for Shiga toxin production. Follow-up serologic testing of Shiga toxin-positive, motile O157 isolates is desirable to identify new serotypes of Shiga toxin-producing *E. coli* strains and to detect problems with procedures to enhance the expression or detection of the H7 antigen. We also strongly advise having *E. coli* O157 strains that are not motile tested for Shiga toxin production, because 94% of such strains submitted to our laboratory over the past 10 years have produced Shiga toxins and were considered to be enterohemorrhagic *E. coli* strains. Since nearly all strains of *E. coli* O157:H7 produce Shiga toxin, we no longer encourage toxin testing of these isolates for diagnostic purposes; however, in light of the recent addition of *E. coli* O157:H7 infection to the list of reportable infections in many states, we recommend that readers consult their state health departments for advice on submitting *E. coli* O157:H7 isolates for further testing.

One limitation of our study for predicting how the latex reagents would perform in a clinical setting is that we evaluated strains stocked in our culture collection rather than freshly isolated bacteria. The percentage of slowly motile strains that we encountered may not reflect what would be seen in clinical specimens.

Compared with the reference method with CDC O157 antiserum, the Oxoid, ProLab, and Remel O157 latex reagents each demonstrated 100% sensitivity and 100% specificity for identifying the *E. coli* O157 antigen. Although some *Salmonella* and *C. freundii* strains possess antigens that cross-react with the O157 antigen of *E. coli*, the identification of *E. coli* O157 should not be compromised by this reactivity because the other bacteria readily ferment sorbitol and can be biochemically distinguished from *E. coli*. By using a sweep of bacterial growth without enhancement of motility, the Remel H7 latex reagent test had a sensitivity of 96% and a specificity of 100% compared with the reference method with CDC H7 antiserum. Enhancement of antigen expression with up to four passages through motility medium increased the sensitivity of the Remel H7 latex reagent test to 100%. Our findings show that these latex reagents, when used as directed by the manufacturers, produce valid results for the strains included in the study and are good alternatives to standard serologic methods for the identification of the O157 and H7 antigens of *E. coli*.

Since the present study was completed, we received three problematic strains for confirmation that appeared to give false-positive reactions with the Remel O157 and H7 latex reagents. It was not clear if single colonies or sweeps of growth from these isolates were used on initial testing. When we tested these isolates we used a lot number of latex reagent different from the one used in the original evaluation. We used single colonies from a SMAC agar plate or a BAP, as recommended by the manufacturer, and found them all to be negative with the Remel O157 latex reagent. However, when using sweeps of growth from a SMAC agar plate or a BAP, these strains did agglutinate in the Remel O157 latex reagent and were negative in the control latex reagent. Since the control latex reagent was negative, a positive result for O157 would have been recorded. When these three strains were tested with CDC O157 antisera, they were negative for O157. This shows that it is important to closely follow the manufacturers' directions when using any latex reagent to avoid false readings; in this case, a single colony is recommended. The three strains were also tested for H7 by using sweeps of growth from a SMAC agar plate or a BAP. The three strains agglutinated with the Remel H7 latex reagent; however, two of the three strains, when tested with CDC H7 antiserum, were not H7. It is therefore important that the H7 latex reagent not be used independently of the O157 latex reagent. Strains should first be tested with the O157 latex reagent, and if they are positive, they should then be tested with the H7 latex reagent to avoid false-positive readings.

As testing for *E. coli* O157:H7 increases, it is likely that organisms that were not anticipated for inclusion in this and prior evaluations will be tested for the O157 and H7 antigens. We would caution readers to be alert to reactions that do not precisely conform to those described by the manufacturer and to seek follow-up testing of isolates that yield atypical results (e.g., stringy type agglutination) compared with the results for controls.

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