

Latex Agglutination Assays for Detection of Non-O157 Shiga Toxin–Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145†

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MS 11-430: Received 19 September 2011/Accepted 20 January 2012

ABSTRACT

Latex agglutination assays utilizing polyclonal antibodies were developed for the top six non-O157 Shiga toxin–producing *Escherichia coli* (STEC) serogroups. Rabbit antisera were affinity purified through protein A/G columns, and the isolated immunoglobulins (IgGs) were covalently immobilized onto polystyrene latex particles. The resulting latex-IgG complex had a protein (IgG) load of 0.20 to 0.28 mg/ml in a 1% latex suspension. Optimum conditions for the agglutination assay consisted of utilizing 20 µl of latex-IgG reagent containing 2.0 to 2.8 µg IgG in a 0.5% latex suspension. Agglutination or flocculation was observed almost instantly after mixing the colonies with the latex-IgG, indicating STEC strains. More than 100 target and nontarget strains were tested in more than 3,000 test replicates. All target organisms produced positive results, but three antisera (anti-O26, anti-O103, and anti-O145) cross-reacted with some other STECs. The anti-O103 and anti-O145 latex reagents cross-reacted with O26 strains, and the anti-O26 cross-reacted with O103 strains. The latex-IgG reagents are stable for at least 1 year and are easy to prepare. These agglutination assays can be used for identification of presumptive non-O157 STEC colonies from agar media. The techniques used to prepare the latex reagents also can be utilized for testing other STEC serogroups, other *E. coli* serotypes, or other pathogens to ensure safe foods to consumers.

Shiga toxin–producing *Escherichia coli* (STEC) strains are important foodborne pathogens that have been responsible for numerous outbreaks of hemorrhagic colitis and hemolytic uremic syndrome worldwide. STEC strains possess a number of virulence factors, which play a role in their pathogenicity (2, 12, 30), including two major types of Shiga toxins (Stx1 and Stx2). *E. coli* O157:H7 is the most commonly recognized STEC in the United States; however, many other STEC serogroups responsible for human illness have been isolated from animals and food. Recent estimates from the Centers for Disease Control and Prevention (CDC) indicate that non-O157 STEC infections are more common than illnesses caused by *E. coli* O157:H7 (23). Non-O157 STEC serogroups associated with sporadic cases and outbreaks of foodborne illness worldwide include O26,

O103, O111, and O145 (4, 6, 9, 16–18, 22, 29). A review of the records for non-O157 STEC isolates forwarded by state public health laboratories to the CDC's reference laboratory between 1983 and 2002 revealed that six serogroups, O26, O45, O103, O111, O121, and O145, of the 61 serogroups identified accounted for 71% of the isolates recovered in the United States (5).

Cattle and other ruminants are the most important reservoirs for *E. coli* O157:H7 and other STEC strains, and food of bovine origin or food and water contaminated with animal feces have been linked to hemorrhagic colitis and hemolytic uremic syndrome (15, 26). Bosilevac and Koohmariae (3) tested 4,133 commercial ground beef samples over a 2-year period and detected genes encoding for Shiga toxins in 1,006 (24.3%) of the samples. The U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 an adulterant in beef products in 1994 and began a verification sampling program to test for the pathogen in samples collected from federally inspected establishments and retail stores. The “top six” non-O157 STEC serogroups found in the United States (O26, O45, O103, O111, O121, and O145) have caused outbreaks and illness as severe as those caused by O157:H7. Therefore, methods to detect and identify these

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pathogens in food samples, clinical specimens, and environmental isolates are crucial for assessing the risk for food safety.

There is genetic diversity among non-O157 STEC strains, and generally these pathogens do not have phenotypic features that can be utilized with commercially available agar media to distinguish them easily from nonpathogenic *E. coli*. Therefore, methods for detection and isolation of important non-O157 STEC serogroups are needed to determine their prevalence in food and their association with human infections. Culture- and PCR-based methods have been used to detect and isolate non-O157 STEC in beef (1, 10, 11, 27, 28); however, isolation remains problematic because of the lack of suitable selective and differential agar media. After enrichment and plating, numerous presumptive STEC colonies often are selected from plates and retested by PCR assays and/or other methods to confirm their identity. This process is costly and time-consuming; therefore, an alternative approach to identification of the colonies by latex agglutination is needed.

Latex agglutination reagents for *E. coli* O157:H7, prepared using O157-specific antisera, are commercially available; however, such reagents are lacking for the top six non-O157 STEC serogroups that are prevalent in the United States and are of interest to the FSIS. The objective of the current study was to use polyclonal antisera to develop latex agglutination reagents specific for the top six non-O157 STEC serogroups and to attach the immunoglobulins (IgGs) covalently to the latex particles. Previously reported latex agglutination assays for pathogen detection utilized latex particles coated with antisera through adsorption (7, 8, 24, 25). Huang et al. (14) and Hajra et al. (13) developed latex agglutination assays for detection of *E. coli* strains including STECs. However, binding of antibodies by adsorption could result in nonuniform coating of the latex beads, yielding a nonuniform load of antibody onto the latex. Covalent immobilization also can lead to greater stability of reagents compared with the adsorption technique. In this study, the IgG fractions were isolated through protein A/G affinity columns and covalently immobilized to polystyrene latex particles to provide more uniformly coated beads. Agglutination assay conditions were optimized for the identification of the top six non-O157 STEC strains.

MATERIALS AND METHODS

Reagents and materials. Formalin and the Bradford protein assay method with gamma globulin, bis-acrylamine, ammonium persulfate, and EDTA were purchased from Bio-Rad Laboratories (Hercules, CA). Polystyrene carboxylated latex microparticles (COOH/2, 10% (wt/vol); PC03N/6499, 0.92- μ m diameter, 10.3) were obtained from Bangs Laboratories (Fisher, IN). *N*-Hydroxysuccinimide (NHS), ethyl-*N'*-(dimethylaminopropyl) carbodiimide (EDC), Hepes (free acid), EDTA, sodium phosphate, sodium azide, glycine, citric acid, sodium citrate, glycine, Tris, Tween 20, Brij surfactants, ethanolamine, and protease-free bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). Veal infusion broth was obtained from BD (Franklin Lakes, NJ), and protein A/G Ultralink columns (3 ml), bovine gamma globulin protein standard, and Slide-A-Lyzer were purchased from Pierce Biotechnology/Thermo Scientific (Rockford, IL). Immunolon 4

HBX strip microwells and strip holders were from Thermo (Milford, MA). The Synergy HT Multi-Label Plate Reader was from Biotek (Winooski, VT). The Sonicator 3000 was from Misonix (Farmingdale, NY), and the Tomy MTX50 centrifuge was from Peninsula Laboratories, Inc. (Belmont, CA). The IKA Vibrax VXR Shaker was purchased from Cole-Palmer (Vernon Hills, IL).

Antibody production. *E. coli* strains from the World Health Organization Reference Collection (21) were used for the production of antibodies against the six O serogroups. Isolated single colonies belonging to serogroups O26 (H311b), O45 (H702c), O103 (H515b), O111 (Stoke W), O121 (39w), and O145 (E1385) (3) were grown overnight in 50 ml of veal infusion broth at 37°C. The cells were harvested by centrifugation at 12,000 \times g for 10 min, resuspended in 50 ml of phosphate-buffered saline (PBS), and heated at 100°C for 2 h. The suspension was centrifuged at 12,000 \times g for 10 min, and the pellet was washed twice with 0.5% saline and resuspended in 20 ml of 0.5% saline. Formalin was added to a final concentration of 0.5%. The antisera were generated in a commercial facility approved by the Public Health Service and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Inoculation of rabbits. New Zealand White rabbits were dosed with antigen via subcutaneous intramuscular injection. Antigen (0.5 ml) of each serogroup was diluted with 0.5 ml of Freund's adjuvant, and each rabbit was inoculated with one serogroup. The rabbits were given booster injections on days 3, 6, 9, 13, 17, 21, and 24 after the initial inoculation. The boosters consisted of 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 ml of inoculum, respectively. On day 27, the rabbits were exsanguinated and the antisera were collected. To preserve the antisera, one part of undiluted serum was diluted with two parts of glycerol.

Isolation of IgG. Rabbit antisera (3 to 5 ml) were diluted 1:2 to 1:4 with 100 mM PBS. The diluted antisera were loaded onto protein A/G Ultralink columns that were preequilibrated with five column volumes of 100 mM PBS. After the diluted antisera were loaded, the columns were washed with 10 column volumes of 100 mM PBS. IgGs were eluted with either 0.1 M glycine-HCl or 0.1 M citric acid, and the eluates were immediately neutralized with phosphate buffer. The glycine-eluted antibodies were further dialyzed in 10 mM NaAc, pH 7, using a Slide-A-Lyzer to remove the glycine before conjugation onto the latex particles and diluted to 0.2, 0.3, and 0.5 mg/ml. The citric acid-eluted antibodies were used without further preparation before conjugation at approximately 0.3 mg/ml. The IgG concentrations were assessed using the Bradford method with gamma globulin (Bio-Rad Laboratories) as the standard, and the purity was assessed on a 10% nonreducing sodium dodecyl sulfate (SDS)-polyacrylamide gel. The isolated IgGs for covalent attachment to the latex particles were preserved with thimerosal.

Immobilization of anti-*E. coli* IgG to latex particles. Covalent attachment of the anti-STECS IgG to the latex utilized a procedure previously described (19, 20). Two milliliters of affinity-purified anti-*E. coli* IgGs were diluted to 0.25, 0.3, and 0.5 mg/ml in 10 mM sodium acetate buffer, pH 4.5. Aliquots (0.2 ml) were set aside for quantification of the original IgG protein concentration for determination of the IgG load on the latex. An optimum concentration of 0.3 mg/ml IgG was utilized in subsequent covalent linkage of the antisera to the latex. The carboxy latex particles were sonicated for 15 s (power 3, continuous), and 0.2 ml was transferred with a 1-ml pipette into 12-ml conical polypropylene centrifuge tubes. The carboxyl groups were activated with NHS and EDC prepared at 11.5 and 75 mg/ml, respectively, in

TABLE 1. Volumes and concentrations of reagents utilized for covalent immobilization of six non-O157 STEC IgGs onto latex particles^a

| Reagent | Anti-O26 | Anti-O45 | Anti-O103 | Anti-O111 | Anti-O121 | Anti-O145 |
|--|----------|----------|-----------|-----------|-----------|-----------|
| IgG concn (mg/ml) ^b | 0.473 | 0.570 | 0.522 | 0.385 | 0.647 | 0.488 |
| IgG vol (ml) | 2.50 | 5.50 | 6.00 | 4.25 | 3.00 | 3.00 |
| Total diluent vol (ml) | 1.44 | 4.97 | 4.45 | 1.19 | 3.47 | 1.88 |
| Total IgG vol (ml) | 3.74 | 10.26 | 10.25 | 5.24 | 6.27 | 4.68 |
| Latex (100 µl/ml) vol (µl) | 374 | 1,025 | 1,025 | 524 | 627 | 468 |
| EDC-NHS (25 µl/ml) vol (ml) | 93.4 | 256 | 256.2 | 131.0 | 156.8 | 117 |
| HBS wash buffer (1 ml/ml) vol (ml) | 3.74 | 10.26 | 10.25 | 5.24 | 6.27 | 4.68 |
| Ethanolamine (25 µl/ml) vol (µl) | 93 | 256 | 256 | 131 | 157 | 117 |
| HBS storage buffer (1 ml/ml) vol (ml) ^c | 3.74 | 10.26 | 10.25 | 5.24 | 6.27 | 4.68 |

^a Volumes of reagents in this table were derived proportionally from the concentrations and volumes as described in the text and were utilized to prepare batch 3 of latex-IgG. Because of different IgG protein concentrations for each serogroup, the volumes of each starting serogroup antiserum also differed. The IgG concentrations before immobilization are shown in Table 2.

^b Protein (IgG) concentration for antisera of the six non-O157 STEC after dialysis of the purified eluted with glycine-HCl buffer.

^c Total volume of latex-IgG prepared containing 1% latex suspension.

water and stored at -80°C in 0.2-ml aliquots. Immediately before immobilization, the contents of each vial were mixed, 50 µl of the EDC-NHS mixture was added to the latex particles, and the solution was gently mixed on a vortex mixer for 30 min at room temperature. Anti-STE C IgG (1.8 ml) was added dropwise to each tube with continuous vortex mixing for 60 min at room temperature. The derivatized latex-*E. coli* IgG mixtures were centrifuged at $8,225 \times g$ for 5 min at 4°C , and the supernatants were decanted to polypropylene tubes for protein analysis. The supernatants and original IgG preparations were assayed for protein content to determine the latex conjugation efficiency as described below. The protein content of the supernatants was compared with the initial protein concentration, and the protein uptake by the latex was measured. The derivatized latex particles were resuspended in 1 ml of 10 mM HBS wash buffer, pH 7 (1.19 g Hepes free acid, 150 mM NaCl [4.38 g], and 3.4 mM EDTA [0.63 g] diluted in 500 ml distilled water) and sonicated by 10 pulses at power 3. The unreacted active carboxyls were capped (neutralized) by addition of 50 µl of 1 M ethanolamine and further mixed gently for 45 min at room temperature. The latex-IgG was separated by centrifugation at 10,000 rpm (8,225 RCF) for 10 min, the supernatant was discarded, and the reconstituted pellet was sonicated by 10 pulses (power 3). The derivatized latex-IgG was stored in 2 ml of HBS storage buffer (HBS wash buffer, pH 7, containing 0.015% Brij surfactant and 0.1% BSA), resulting in a 1% (solids) latex concentration. The latex derivatives were used in subsequent agglutination assays. Tables 1 and 2 include the details of preparation of immobilized latex-IgG in larger amounts than those described here. All reagents were proportionally adjusted according to the original volumes of reagents discussed previously.

Protein analysis. The protein IgG concentrations were measured with the BioRad-protein assay and calibrated against the Pierce protein gamma globulin standard. Triplicate aliquots (20 µl) of the original IgG before and after immobilization were transferred to microtiter wells, 200 µl of the protein assay reagent was added, and the mixture was incubated for 15 min at room temperature. The optical density was measured at 595 nm, and the sample concentrations were determined with the Biotek data analyzer. The protein IgG uptake (load) by the latex was determined as concentration (milligrams per milliliter) of IgG before immobilization minus concentration after immobilization (% load = protein loss divided by preimmobilization \times 100). Protein load is the amount of IgG (milligrams) per milligram of latex or per milliliter of suspension. In a 1% latex suspension, protein load is equivalent to milligrams of protein IgG per 10 mg of latex in 1 ml.

Latex agglutination assay reagents. Tris-buffered saline (TBS) buffer contained 50 mM Tris, pH 8 (1.51 g), 0.1% BSA (protease free) (0.25 g), 5 mM EDTA (0.4 g), and 0.2% sodium azide (0.5 g) in 250 ml, adjusted to pH 8. TBST assay buffer contained TBS and 0.5% Tween 20 (0.5 g/100 ml). Latex-antibody conjugate suspension was sonicated before use for 15 s at power level 3. The latex anti-STE C preparation was diluted 1:1 with TBST buffer resulting in a 50% latex-IgG concentration (0.5% solids). In a 0.5% latex suspension, the protein load is micrograms of IgG protein per 0.1 mg of latex per 20 µl of suspension.

Bacteria. *E. coli* strains ($n = 32$) used in this study and their sources are listed in Table 3. The bacterial strains were streaked onto tryptic soy agar plates and incubated at 37°C overnight (18 h). The latex agglutination reagents were tested with target and

TABLE 2. Immobilization efficiency and protein IgG load (concentration) on the latex particles in the batch 3 latex-IgG reagents^a

| Anti-STE C IgG | Preimmobilized protein IgG concn (mg/ml) | % IgG protein latex uptake | IgG protein load/ml latex (mg/ml) | IgG protein load (µg/0.02 ml) | IgG protein load/ml (µg/0.02 ml, 1:1 dilution) |
|----------------|--|----------------------------|-----------------------------------|-------------------------------|--|
| O26 | 0.241 | 85 | 0.205 | 4.10 | 2.0 |
| O45 | 0.270 | 96 | 0.259 | 5.18 | 2.6 |
| O103 | 0.310 | 77 | 0.238 | 4.76 | 2.4 |
| O111 | 0.290 | 95 | 0.275 | 5.50 | 2.8 |
| O121 | 0.320 | 85 | 0.272 | 5.44 | 2.7 |
| O145 | 0.276 | 81 | 0.223 | 4.46 | 2.2 |

^a The IgGs were isolated using glycine-HCl elution buffer, dialyzed before immobilization, and covalently linked to the latex as described in Table 1.

TABLE 3. Serotypes of STEC strains used to test the specificity of the latex agglutination assays at the ERRC^a

| STEC serotype | Strain | Source ^b |
|------------------|---------|---------------------|
| Negative control | K-12 | ATCC 29455 |
| O26:H11 | SJ1 | CDC |
| O26:H11 | SJ2 | CDC |
| O26:H11 | SJ3 | CDC |
| O26:H11 | 00971 | FDA |
| O26:H11 | 05-6544 | PHAC |
| O45:H2 | SJ7 | CDC |
| O45:H2 | SJ8 | CDC |
| O45:H2 | SJ9 | CDC |
| O45:H2 | 05-6545 | PHAC |
| O103:H2 | 04-2446 | PHAC |
| O103:H2 | 99-2076 | PHAC |
| O103:H2 | SJ10 | CDC |
| O103:H25 | SJ11 | CDC |
| O103:H11 | SJ12 | CDC |
| O103:H6 | 04162 | FDA |
| O103:H11 | 04-3973 | PHAC |
| O111:NM | 00-4748 | PHAC |
| O111:NM | 98-8338 | PHAC |
| O111:H8 | 01387 | FDA |
| O111:NM | SJ13 | CDC |
| O111:H8 | SJ14 | CDC |
| O111:NM | SJ15 | CDC |
| O121:H19 | SJ16 | CDC |
| O121:H19 | 08023 | FDA |
| O121:H19 | SJ18 | CDC |
| O121:H19 | 03-2832 | PHAC |
| O121:NM | 03-4064 | PHAC |
| O145:NM | SJ23 | CDC |
| O145:NM | SJ24 | CDC |
| O145:H18 | 07865 | FDA |
| O145:NM | 03-4699 | PHAC |

^a U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center.

^b CDC, Centers for Disease Control and Prevention, Atlanta, GA; FDA, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD; PHAC, Public Health Agency of Canada, Winnipeg, Manitoba, Canada.

nontarget non-O157 STEC strains, and *E. coli* K-12 was used as a negative control. An additional 67 *E. coli* strains and 7 non-*E. coli* bacterial strains also were tested at the FSIS testing laboratories.

Assay procedure. Glass slides were prepared by drawing two or three circles (1.5-cm diameter) with wax pencil or marking pen. The latex-STECS IgG reagents were vortexed gently, and 20 μ l of

latex-antibody conjugate in TBST was transferred to the circles. Two or three colonies of *E. coli* K-12 (negative control) were transferred to three circles in one slide. Similarly, two or three colonies of non-O157 STEC were transferred to two circles on the slide, mixed gently with toothpicks, and also rotated manually. Agglutination was observed after 3 to 10 s. Negative results were indicated by latex dispersion, and positive results were indicated by agglutination and/or flocculation or network formation.

RESULTS AND DISCUSSION

Antibody production and latex-IgG preparation.

Glycine-HCl buffer was utilized to elute the first batch of the purified IgGs from the polyclonal antisera for STEC O26, O45, O103, O111, O121, and O145. The IgG isolates were dialyzed using the Slide-A-Lyzer to remove the glycine to prevent interference in the coupling of the amino group with the COOH groups of the latex particles. There was no apparent loss of the total IgG before and after dialysis. However, the concentrations of the dialyzed IgG decreased because of the increase in the volume of the dialyzed IgG. SDS-polyacrylamide gel electrophoresis (PAGE) revealed one major band for each anti-STECS IgG. Tables 1 and 2 show the reagent concentrations and immobilization efficiency of the latex reagent preparation. These latex-IgG reagents were transferred to the FSIS for testing.

A second batch of purified IgG was prepared by eluting with citrate-phosphate buffer to eliminate glycine from the IgG isolate. The protein concentrations in this batch using the gamma globulin standards were 0.68, 0.33, 0.66, 0.42, 1.07, and 0.62 mg/ml isolated from anti-STECS O26, O45, O103, O111, O121, and O145, respectively, and latex reagents were prepared and tested with this batch. After a successful preliminary test, a third batch of purified IgG was prepared by eluting with citrate buffer. Again, only one major band from each isolated antibody was obtained with SDS-PAGE. This purified IgG was utilized for the preparation of the final latex agglutination reagents (Table 4) and was transferred to the FSIS laboratories for monitoring (27, 28).

Immobilization of STECS IgG to latex particles.

Following the immobilization protocol with reagent volumes shown in Table 1, latex-anti-STECS IgG had a protein load of 2.0 to 2.8 μ g/20 μ l of latex reagent based on the bovine gamma globulin protein standard (Table 2). The working latex-IgG contained 0.5% latex (solids) after a 1:1 dilution of the prepared undiluted latex containing 1% solids.

TABLE 4. Immobilization efficiency and protein IgG load (concentration) on the latex particles in batch 4 latex-IgG reagents^a

| Anti-STECS IgG | Preimmobilized protein IgG concn (mg/ml) | % IgG protein latex uptake | IgG protein load/ml latex (mg/ml) | IgG protein load (μ g/0.02 ml of 1% latex) | IgG protein load/ml (μ g/0.02 ml, 1:1 dilution) |
|----------------|--|----------------------------|-----------------------------------|---|--|
| O26 | 0.286 | 72 | 0.207 | 4.14 | 2.1 |
| O45 | 0.244 | 100 | 0.244 | 4.80 | 2.4 |
| O103 | 0.294 | 87 | 0.256 | 5.12 | 2.6 |
| O111 | 0.250 | 100 | 0.250 | 5.00 | 2.5 |
| O121 | 0.278 | 94 | 0.262 | 5.20 | 2.6 |
| O145 | 0.293 | 91 | 0.265 | 5.30 | 2.6 |

^a The IgGs were prepared using citrate-phosphate elution buffer and utilized for immobilization after dilution to approximately 0.3mg/ml protein IgG concentration.

TABLE 5. Latex agglutination test results on *E. coli* strains listed in Table 2^a

| Sample no. | Strain tested | Specific antibody response to STEC <i>E. coli</i> strain (target) ^b | Antibody cross-reactions (nontarget) |
|------------|------------------|--|--------------------------------------|
| 1 | K-12 ATCC 29425 | | |
| 2 | O26:H11 05-6544 | +++ , +++ | Not tested |
| 3 | O26:H11 SJ1 | +++ , +++ | O145, O103 |
| 4 | O26:H11 SJ2 | +++ , +++ | O145, O103 |
| 5 | O26:H11 SJ3 | +++ , +++ | O145, O103 |
| 6 | O26:H11 00971 | +++ , +++ | O145, O103 |
| 7 | O45:H2 05-6545 | ++ , ++ | |
| 8 | O45:H2 SJ7 | ++ , ++ | |
| 9 | O45:H2 SJ8 | ++ , ++ | |
| 10 | O45:H2 SJ9 | ++ , ++ | |
| 11 | O103:H11 SJ12 | +++ , +++ | O26 |
| 12 | O103:H11-04-3973 | +++ , +++ | O26 |
| 13 | O103:H2 04-2446 | +++ , +++ | O26 |
| 14 | O103:H2 99-2076 | +++ , +++ | O26 |
| 15 | O103:H2 SJ10 | +++ , +++ | O26 |
| 16 | O103:H25 SJ11 | +++ , +++ | O26 |
| 17 | O103:H6 04162 | +++ , +++ | O26 |
| 18 | O111:H8 01387 | +++ , +++ | |
| 19 | O111:H8 SJ14 | +++ , +++ | |
| 20 | O111:NM 00-4748 | +++ , +++ | |
| 21 | O111:NM 98-8338 | +++ , +++ | |
| 22 | O111:NM SJ13 | +++ , +++ | |
| 23 | O111:NM SJ15 | -, -, - | |
| 24 | O121:H19 SJ18 | ++ , ++ | |
| 25 | O121:H19 03-2832 | ++ , ++ | |
| 26 | O121:H19 SJ16 | ++ , ++ | |
| 27 | O121:NM 03-4064 | + , + | |
| 28 | O121:TW 08023 | ++ , ++ | |
| 29 | O145:NM SJ23 | +++ , +++ | |
| 30 | O145:NM 03-4699 | +++ , +++ | |
| 31 | O145:NM SJ24 | +++ , +++ | |
| 32 | O145:H18 07865 | +++ , +++ | |

^a Testing was carried out at the Agricultural Research Service Eastern Regional Research Center. These results are identical to those of two other batches of latex reagents.

^b Each batch was tested in three separate trials, and each bacterial strain was tested in duplicate except for sample 23, which was tested in triplicate. STEC O111:NMSJ15 (sample 23) from the CDC was negative with anti-O111 latex reagent. + + +, instant agglutination; + +, slow agglutination; +, very slow agglutination; -, no agglutination.

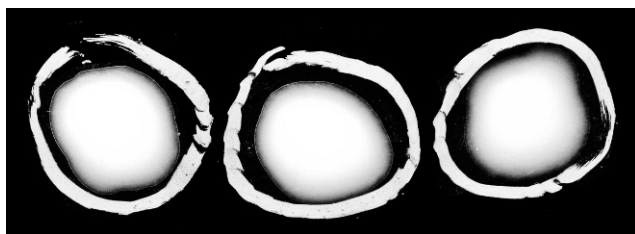
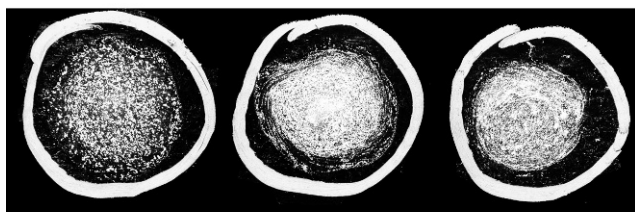
A. Negative with *E. coli* K12B. Positive with *E. coli* O26

FIGURE 1. Typical latex agglutination results with latex anti-O26 STEC.

Latex agglutination assays. Three separate conjugations were prepared from the antisera eluted with citrate-phosphate buffer. The latex reagents were tested with the 31 STEC strains (Table 3) at the Agricultural Research Service Eastern Regional Research Laboratory (ERRC), and the typical results are shown in Table 5. All of the latex-anti-STECS reagents tested positive against the target serogroup and strains except for sample 23 (strain O111:NM SJ15), which tested negative with the anti-O111 latex reagent. Three latex-IgG reagents (anti-O26, anti-O103, and anti-O145) had some cross-reactions, indicating false-positive results for the specific serotype. Anti-O26 cross-reacted with O145 and O103 strains, and anti-O103 cross-reacted with O26 strains. These results were consistent in tests of the three batches of the latex-IgG reagents. The negative control, *E. coli* K-12, produced negative results. The typical latex agglutination assay positive and negative results are shown in Figure 1. Overnight cultures that had been stored more than 24 h at 4°C sometimes produced various results; therefore, the agglutination tests should be performed on freshly cultured organisms.

TABLE 6. Serogroup inclusivity panel resulting from the latex agglutination assays conducted at FSIS laboratories

| Sample no. | Source ^a | <i>E. coli</i> strain | Latex-IgG reagent | Latex serogroup(s) reactions ^b |
|------------|---------------------|-----------------------|-------------------|---|
| 1 | ARS ERRC | O26:H11 | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 2 | CDC (SJ1) | O26:H11 | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 3 | CDC (SJ2) | O26:H11 | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 4 | CDC (SJ7) | O45:H2 | Anti-O45 | O45 |
| 5 | ARS WRRC | O45 | Anti-O45 | O45 |
| 6 | CDC (SJ8) | O45:H2 | Anti-O45 | O45 |
| 7 | ARS ERRC | O103:H2 | Anti-O103 | <i>O26</i> , O103 |
| 8 | CDC (SJ11) | O103:H25 | Anti-O103 | <i>O26</i> , O103 |
| 9 | CDC (SJ12) | O103:H11 | Anti-O103 | <i>O26</i> , O103 |
| 10 | CDC (SJ13) | O111:NM | Anti-O111 | O111 |
| 11 | ARS ERRC | O111:[H8] | Anti-O111 | O111 |
| 12 | ARS WRRC | O111 | Anti-O111 | O111 |
| 13 | CDC (SJ18) | O121:H19 | Anti-O121 | O121 |
| 14 | ARS WRRC | O121 | Anti-O121 | O121 |
| 15 | CDC (SJ23) | O145:NM | Anti-O145 | O145 |
| 16 | ARS ERRC | O145:H28 | Anti-O145 | O145 |
| 17 | ARS ERRC | O145:Nm | Anti-O145 | O145 |
| 18 | ARS ERRC | O145:H ⁺ | Anti-O145 | O145 |
| 19 | ARS ERRC | O145:H18 | Anti-O145 | O145 |
| 36 | ARS ERRC | O145:NM | Anti-O145 | O145 |
| 37 | CDC (MG1) | O103:H11 | Anti-O103 | <i>O26</i> , O103 |
| 38 | CDC (MG9) | O121:NM | Anti-O121 | O121 |
| 39 | CDC (MG13) | O45:H2 | Anti-O45 | O45 |
| 50 | MSU | O26:NM | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 52 | MSU | O111:H ⁻ | Anti-O111 | O111 |
| 53 | MSU | O111:H ⁻ | Anti-O111 | O111 |
| 54 | MSU | O111:H ⁻ | Anti-O111 | O111 |
| 62 | MSU | O45:H2 | Anti-O45 | O45 |
| 64 | MSU | O121 | Anti-O121 | O121 |
| 65 | MSU | O103:H2 | Anti-O103 | <i>O26</i> , O103 |
| 66 | MSU | O111:H8 | Anti-O111 | O111 |
| 67 | MSU | O45:NM | Anti-O45 | O45 |
| 68 | MSU | O121:H19 | Anti-O121 | O121 |
| 69 | MSU | O26:H11 | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 70 | MSU | O145:[H28] | Anti-O145 | O145 |
| 71 | MSU | O26:N | Anti-O26 | O26 , <i>O103</i> , <i>O145</i> |
| 72 | MSU | O111:NM | Anti-O111 | O111 |
| 73 | MSU | O145:NT | Anti-O145 | O145 |
| 78 | MSU | O145:H16 | Anti-O145 | O145 |

^a ARS ERRC, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA; CDC, Centers for Disease Control and Prevention, Atlanta, GA; ARS WRRC, U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA; MSU, Michigan State University, East Lansing.

^b Positive results of target organisms are shown in bold, and cross-reactions are shown in italics.

The latex agglutination reagents and procedures (protocols) were transferred to the FSIS and were tested in the Eastern Laboratory Outbreaks Section for further verification. Of the 74 strains tested, 39 were target-specific serogroups or strains (Table 6) and 35 were nontarget O serogroup (Table 7). Results in Table 6 demonstrate the cross-reactions of anti-O26 with O103 and O145 serogroups, and of anti-O103 with the O26 serogroup. Table 7 shows cross-reaction of anti-O145 with *E. coli* O177:H25 and *E. coli* O15:NM, although both were PCR negative for the O145 gene clusters (11). These results are similar to those obtained at the ERRC (Table 5), showing the cross-reactions of anti-O26 and anti-O103. Cross-reactions (nonspecific binding) may be reduced by adsorbing the antisera to the cross-reacting serogroups, but this adsorption would diminish the antibody avidity (titer)

and require additional manipulation of the antisera. Future antisera production methods should reduce or eliminate these nonspecific binding cross-reactions.

The results of this study indicate that anti-STEC IgG covalently immobilized onto latex particles can be prepared with a uniform load of antisera, as shown in four separate immobilizations. The latex-IgG reagents were stable after 12 months when tested in our laboratory and in FSIS laboratories.

In summary, the six latex reagents were tested with 32 strains in seven repeat trials with two replicates per trial. Each antibody serotype was tested 384 times, which resulted in 2,304 tests for the six latex-anti-STEC reagents. Some serotypes were retested to verify results, particularly with the cross-reacting reagents and serotypes; thus, a total

TABLE 7. Serogroup exclusivity panel results from the latex agglutination assays conducted at FSIS laboratories

| Sample no. | Source ^a | Organism | Latex serogroup(s) reactions |
|------------|---------------------|--|-------------------------------------|
| 20 | ARS ERRC | <i>E. coli</i> O113:H4 | Negative |
| 21 | ARS ERRC | <i>E. coli</i> O174:H8 | Negative |
| 22 | ARS ERRC | <i>E. coli</i> O157:H7 | Negative |
| 23 | ARS ERRC | <i>E. coli</i> O177:H25 | O145 (cross-reaction; PCR negative) |
| 24 | ARS ERRC | <i>E. coli</i> O91:H21 | Negative |
| 25 | ARS ERRC | <i>E. coli</i> O138 | Negative |
| 26 | ARS ERRC | <i>E. coli</i> K30 | Negative |
| 27 | ARS ERRC | <i>E. coli</i> K6 | Negative |
| 28 | ARS ERRC | <i>E. coli</i> O63:H6 | Negative |
| 29 | ARS ERRC | <i>E. coli</i> O63:H6 | Negative |
| 30 | ARS ERRC | <i>E. coli</i> O128ac:H2 | Negative |
| 31 | ARS ERRC | <i>E. coli</i> O55:H7 | Negative |
| 32 | ARS ERRC | <i>E. coli</i> O41:H26 | Negative |
| 40 | FSIS Eastern Lab | <i>E. coli</i> O157:H7 <i>stx</i> negative | Negative |
| 41 | FSIS Eastern Lab | <i>E. coli</i> | Negative |
| 42 | FSIS Eastern Lab | <i>E. coli</i> O157:H7 <i>stx</i> negative | Negative |
| 43 | FSIS Eastern Lab | <i>Enterobacter aerogenes</i> | Negative |
| 44 | FSIS Eastern Lab | <i>Enterococcus faecalis</i> | Negative |
| 45 | FSIS Eastern Lab | <i>Klebsiella pneumoniae</i> | Negative |
| 46 | FSIS Eastern Lab | <i>Salmonella</i> Paratyphi A | Negative |
| 47 | FSIS Eastern Lab | <i>Salmonella</i> Typhimurium | Negative |
| 48 | FSIS Eastern Lab | <i>Proteus mirabilis</i> | Negative |
| 49 | FSIS Eastern Lab | <i>Lactobacillus plantarum</i> | Negative |
| 51 | MSU | <i>E. coli</i> O15:NM | O145 (cross-reaction; PCR negative) |
| 55 | MSU | <i>E. coli</i> O70:H11 | Negative |
| 56 | MSU | <i>E. coli</i> O104:H21 | Negative |
| 57 | MSU | <i>E. coli</i> O91:H21 | Negative |
| 58 | MSU | <i>E. coli</i> O113:H21 | Negative |
| 59 | MSU | <i>E. coli</i> O156:H21 | Negative |
| 60 | MSU | <i>E. coli</i> O5:N | Negative |
| 61 | MSU | <i>E. coli</i> XO3:NM | Negative |
| 74 | MSU | <i>E. coli</i> O157:H7 | Negative |
| 75 | MSU | <i>E. coli</i> O55:H7 | Negative |
| 76 | MSU | <i>E. coli</i> O55:H7 | Negative |
| 77 | MSU | <i>E. coli</i> O157:H7 | Negative |

^a ARS ERRC, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA; FSIS Eastern Lab, U.S. Department of Agriculture, Food Safety and Inspection Service, Eastern Laboratory, Athens, GA; MSU, Michigan State University, East Lansing.

of 3,036 replicate tests were conducted. FSIS laboratories carried out additional testing. The reagents were stable for more than 12 months when stored at 4°C.

Simple and rapid latex agglutination assays were developed for the detection of non-O157 STEC belonging to serogroups O26, O45, O103, O111, O121, and O145. The latex anti-STEC reagents were prepared by covalent immobilization of the IgGs onto the polystyrene particles, yielding a uniform latex reagent containing 0.20 to 0.28 mg/ml latex-IgG. The latex agglutination assay utilized a latex-IgG preparation containing 2.0 to 2.8 µg/20 µl, and the latex-IgG reagents were stable for more than 12 months. The six latex-antibody reagents were tested with more than 100 *E. coli* and non-*E. coli* bacteria in more than 3,000 replicates. The latex agglutination assay reagents can be prepared with ease, and results indicate reliable recognition of target STECs. The cross-reactions observed can be overcome by modification of antibody production or production of monoclonal antibodies for the cross-reacting

IgG reagents. The latex reagents will be very useful for identification of presumptive non-O157 STEC colonies picked from various types of agar media before or after PCR assays. This method is rapid and less costly than testing colonies by PCR assays.

REFERENCES

1. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
2. Bolton, D. J. 2011. Verotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm. *Foodborne Pathog. Dis.* 8:357–365.
3. Bosilevac, J. M., and M. Koohmariaie. 2011. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* isolates from commercial ground beef in the United States. *Appl. Environ. Microbiol.* 77:2103–2112.
4. Brooks, J. T., D. Bergmire-Sweat, M. Kennedy, K. Hendricks, M. Garcia, L. Marengo, J. Wells, M. Ying, W. Bibb, P. M. Griffin, R. M.

- Hoekstra, and C. R. Friedman. 2004. Outbreak of Shiga toxin-producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. *Clin. Infect. Dis.* 38:190–198.
5. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J. Infect. Dis.* 92:1422–1429.
 6. Centers for Disease Control and Prevention. 2011. Investigation update: outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) infections associated with travel to Germany. Available at: <http://www.cdc.gov/ecoli/2011/ecoliO104>. Accessed 18 July 2011.
 7. Chang, T. C., and C. H. Chen. 1994. Development of a latex agglutination test for the rapid identification of *Vibrio parahaemolyticus*. *J. Food Prot.* 57:31–36.
 8. Chang, T. C., and S. H. Huang. 1996. Efficacy of a latex agglutination test for rapid identification of *Staphylococcus aureus*: collaborative study. *J. AOAC Int.* 79:661–669.
 9. Ethelberg, S., B. Smith, M. Torpdahl, J. Lisby, J. Boel, T. Jensen, and K. Mølbak. 2007. An outbreak of verocytotoxin-producing *Escherichia coli* O26:H11 caused by beef sausage, Denmark. *Euro Surveill.* 12:E070531.4.
 10. Fratamico, P. M., L. K. Bagi, W. C. Cray, Jr., N. Narang, X. Yan, M. Medina, and Y. Liu. 2011. Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog. Dis.* 8:601–607.
 11. Fratamico, P. M., C. DebRoy, T. Miyamoto, and Y. Liu. 2009. PCR detection of enterohemorrhagic *Escherichia coli* O145 in food by targeting genes in the *E. coli* O145 O-antigen gene cluster and the Shiga toxin 1 and Shiga toxin 2 genes. *Foodborne Pathog. Dis.* 6: 605–611.
 12. Gyles, C. L. 2007. Shiga toxin-producing *Escherichia coli*: an overview. *J. Anim. Sci.* 85(Suppl. E):E45–E62.
 13. Hajra, T. K., P. K. Bag, S. C. Das, S. Mukherjee, A. Khan, and T. Ramamurthy. 2007. Development of a simple latex agglutination assay for detection of Shiga toxin-producing *Escherichia coli* (STEC) by using polyclonal antibody against STEC. *Clin. Vaccine Immunol.* 14:600–604.
 14. Huang, Y. H., H. C. Chang, and T. C. Chang. 2001. Development of a latex agglutination test for rapid identification of *Escherichia coli*. *Eur. J. Clin. Microbiol. Infect. Dis.* 20:97–103.
 15. Hussein, H. S. 2007. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J. Anim. Sci.* 85:E63–E72.
 16. Johnson, K. E., C. M. Thorpe, and C. L. Sears. 2006. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin. Infect. Dis.* 43:1587–1595.
 17. Kaspar, C., and M. E. Doyle. 2009. White paper on non-O157:H7 Shiga toxin-producing *E. coli* from meat and non-meat sources. Available at: <http://amif.org/ht/a/GetDocumentAction/i/56116>. Accessed 15 February 2012.
 18. McCarthy, T. A., N. L. Barrett, J. L. Hadler, B. Salisbury, R. T. Howard, D. W. Dingman, C. D. Brinkman, W. F. Bibb, and M. L. Carter. 2001. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. *Pediatrics* 108:E59.
 19. Medina, M. B. 2006. Developing a fluorescent latex micro-particle immunoassay for detection of staphylococcal enterotoxin B (SEB). *J. Agric. Food Chem.* 54:4937–4942.
 20. Medina, M. B. 2007. Developing a fluorescent latex micro-particle immunoassay using Alexa Fluor 568 for detection of staphylococcal enterotoxin A (SEA). *J. Rapid Methods Autom. Microbiol.* 15:33–48.
 21. Orskov, I., F. Orskov, B. Jann, and K. Jann. 1977. Serology, chemistry and genetics of O and K antigens of *Escherichia coli*. *Bacteriol. Rev.* 41:667–710.
 22. Paton, A. W., R. M. Ratcliff, R. M. Doyle, J. Seymour-Murray, D. Davos, J. A. Lanser, and J. C. Paton. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *J. Clin. Microbiol.* 34:1622–1627.
 23. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
 24. Slotved, H. C., J. Elliott, T. Thompson, and H. B. Konradsen. 2003. Latex assay for serotyping of group B *Streptococcus*. *J. Clin. Microbiol.* 41:4445–4447.
 25. Slotved, H. C., M. I. Kaltoft, I. C. Skovsted, M. B. Kern, and F. Espersen. 2004. Simple, rapid latex agglutination test for serotyping of pneumococci (Pneumotest-Latex). *J. Clin. Microbiol.* 42:2518–2522.
 26. Smith, J. L., and P. M. Fratamico. 2005. Diarrhea-inducing *Escherichia coli*, p. 357–382. In P. M. Fratamico, A. K. Bhunia, and J. L. Smith (ed.), *Foodborne pathogens: microbiology and molecular biology*. Caister Academic Press, Norfolk, UK.
 27. U.S. Department of Agriculture, Food Safety and Inspection Service. 4 November 2011. Detection and isolation of non-O157 Shiga-toxin producing *Escherichia coli* (STEC) from meat products. In *Microbiology laboratory guidebook*. Available at: http://www.fsis.usda.gov/PDF/MLG_nonO157_Flowchart.pdf. Accessed 15 February 2012.
 28. U.S. Department of Agriculture, Food Safety and Inspection Service. 4 November 2011. Detection and isolation of non-O157 Shiga-toxin producing *Escherichia coli* (STEC) from meat products. In *Microbiology laboratory guidebook*. Available at: http://www.fsis.usda.gov/PDF/MLG_5B_01.pdf. Accessed 15 February 2012.
 29. Werber, D., A. Fruth, A. Liesegan, M. Littmann, U. Buchholz, R. Prager, H. Karch, T. Breuer, H. Tschäpe, and A. Ammon. 2002. A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. *J. Infect. Dis.* 186:419–422.
 30. Wickham, M. E., C. Lupp, M. Mascarenhas, A. Vazquez, B. K. Coombes, N. F. Brown, B. A. Coburn, W. Deng, J. L. Puente, M. A. Karmali, and B. B. Finlay. 2006. Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *J. Infect. Dis.* 194:819–827.