

Importance of STEC Determination

Shiga toxin-producing *E. coli* (STEC) are food-borne pathogens that cause gastrointestinal illness, hemorrhagic colitis and hemolytic uremic syndrome. *E. coli* O157:H7 is the STEC serotype most often implicated in outbreaks; however, there are numerous other STEC serotypes that have caused serious human illness and outbreaks. STEC that cause human illness were included as notifiable pathogens to the Nationally Notifiable Diseases Surveillance System in 2000. From 2000-2010, 7695 cases were reported; 5688 were associated with the serogroup O157, and the 83% of the other STEC were serogroups with O26, O45, 103, O111, O121 and O145. However, other serogroups, including O91, O113, O104, have also caused serious human. *E. coli* O157 has been found in ground beef and in cattle hides, and in feces. Bovine feces can be a source of environmental contamination (soil, water) which can lead to secondary contamination of produce growing in fields.

It is difficult to distinguish pathogenic *E. coli* O157 strains from non-pathogenic *E. coli* strains because the former rarely possess any distinguishing phenotypic or biochemical characteristics from the latter. Therefore, methods such as the latex agglutination test described in this Instructions for use have been developed by the USDA-Agricultural Research Service Eastern Regional Research Center (USDA-ARS-ERRC) to help on the identification of these STEC strains. This latex agglutination method is part of the testing protocol utilized and mandated by the FSIS for testing ground beef and beef trim and described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga-Toxin Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".

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E. coli O157:H7 Latex Test Kit

Latex Agglutination Test for the Detection of *E. coli* O157:H7

Product No. 541070

1. General Description

The *E. coli* O157 Test is a rapid latex agglutination test, designed solely for the presumptive identification of *Escherichia coli* serogroup O157:H7 cultured on Rainbow agar plate. The *E. coli* O157:H7 Latex Test Kits could be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga-Toxin Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".

2. Safety Instructions

Biological waste should be decontaminated by autoclaving or by using another effective method. Discard samples according to local, state and federal regulations.

3. Storage and Stability

The *E. coli* O157:H7 Latex Test Kit should be stored between 2-8°C (**do not freeze**) and may be used until the last day of the month as indicated by the expiration date on the label. All reagents and samples to be analyzed should be at room temperature before use.

4. Test Principle

The polystyrene latex particles provided in the kit are coupled to antibodies against *E. coli* serotype O157:H7 (according to Medina et al). When the latex particles are mixed on a test card with fresh colonies of *E. coli* O157, the bacteria will bind to the antibody causing the latex particles to agglutinate (positive reaction). Bacteria that are not *E. coli* O157:H7 will not bind to the antibody and will not agglutinate the latex particles (negative reaction).

5. Limitations of the *E. coli* O157:H7 Latex Test

If a positive result is obtained on an unknown organism, further test such as PCR should be carried out for confirmation. Apply good judgment to any test result, particularly when preliminary positive results are observed.

6. Warning and Precautions

- This product is for research purposes only; not for diagnostic or *in vivo* use.
- Do not freeze reagents.
- Do not allow reagents to become contaminated by using dirty transfer pipettes.
- Use reasonable judgment when interpreting the test results.
- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- Avoid cross-contamination of samples by using a new sample stick for each sample.
- Use only the *E. coli* test reagents from one kit lot** (do not mix with other lots), as they have been adjusted in combination.
- Specimens may contain pathogenic organisms, handle with appropriate precautions.
- Ensure that reagent bottle caps are tight after each use to prevent drying of reagents.
- Reagents contain 0.05-0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.

7. Sample Collection and Handling

Colonial growth removed from the agar surface of modified Rainbow Agar (mRBA) or tryptic soy agar with 5% sheep blood (SBA) plates is best suited for this testing procedure.

8. Control Procedures

The control reagents provided should be used to check the correct working of the latex reagents each day before routine tests are performed.

The Positive Control suspension must cause visible agglutination with the Antibody-Latex Reagent within one (1) minute.

The Negative Control Suspension should cause no agglutination within one (1) minute.

Do not use the test kit if reactions with the control suspensions are incorrect.

A. Materials Provided

1. Negative Control. Suspension of inactivated *E. coli* cells (K12) in buffer, 1 vial, 0.5 mL.
2. Positive Control. Suspension of inactivated *E. coli* O157:H7 cells in buffer, 1 vial, 0.5 mL.
3. PBS (1X), 1 vial, 1.0 mL.
4. Antibody-Latex Beads Reagent. A suspension of red or blue latex particles coupled to specific rabbit IgG to *E. coli* O157:H7 serotype, 1 vial with grey cap, 1.5 mL (enough for 50 tests).
6. Control Latex Reagent. A suspension of red or blue latex particles sensitized with pre-immune rabbit globulin, 1 vials with white cap, 1.0 mL.
7. Test Cards. Disposable reaction cards, 5 cards (10 reactions each).
8. Sample Mixing Sticks (100).
9. Transfer Pipettes (20). Color coded (10 green, 5 blue, 5 red).
10. Instructions for use.

B. Additional Materials (not provided with the test)

1. Agar Plates.
2. Disinfectant Solution, e.g. Sodium hypochloride solution >1.3% w/w.

C. Test Preparation

1. All reagents and samples to be analyzed should be at room temperature before use.
2. Thoroughly suspend the latex reagent and controls by agitation.

D. Assay Procedure

1. Bring all reagents and samples to room temperature. Make sure the latex suspensions and control are well mixed by vigorous shaking.
2. Using one of the provided transfer pipettes (green), place one drop of PBS onto one (1) circle on the test card. If more than one sample is being tested use additional circles on the test card.
3. Using one of the sample mixing sticks (or an inoculating loop), pick a portion of a suspect colony from the agar plate and thoroughly emulsify in the drop of PBS contained in one of the circles.
4. Using one of the transfer pipettes (red), add one (1) drop of the Positive Control to a second circle.
5. Using another transfer pipette (blue), add one (1) drop of the Negative Control to a third circle.
6. Dispense one (1) free falling drop (with vial held vertically) of the *E. coli* O157 Latex-Antibody bead reagent onto each circle (Positive, Negative, and sample(s)).
7. Rotate the test card using a complete circular motion (through 3 planes) for up to one (1) minute or until agglutination is evident, whichever occurs first. Record the results.
8. If agglutination with the test reagent does occur, it is necessary to test a further portion of the colony with the Control Latex Reagent to ensure that the isolate is not an auto-agglutinating strain.

NOTE: All mixing sticks, cards, etc. should be disposed in disinfectant or autoclave waste containers.

E. Interpretation of Results

Agglutination of the test latex within one (1) minute is a positive result. This indicates the presence of *E. coli* serogroup O157:H7.

No agglutination occurring within one (1) minute is a negative result. This indicates the absence of *E. coli* serogroup O157:H7.

NOTE: Some strains of *E. coli* are difficult to emulsify in saline and may give a stringy type reaction with the test reagents. This does not look like true agglutination and should be ignored. If this stringiness is found to be too severe for a correct judgment to be made then the colony should be suspended in 1-2 drops of PBS. Allowing the lumps to settle and re-test.

If a positive result is obtained on an unknown organism, further tests such as PCR should be carried out for confirmation. Apply good judgment to any test result, particularly when preliminary positive results are observed.

F. Cross-reactivity Profile

<i>E. Coli</i>	
Serotype	Agglutination
O157:H7	“+++”
O26	“-“
O45	“-“
O103	“-“
O104	“-“
O111	“-“
O121	“-“
O145	“-“
K12	“-“

“+ + +” = Strong positive agglutination

“- “ = Negative agglutination

G. Additional Analysis

Positive samples must be confirmed as described by the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 “Detection, Isolation and Identification of Top Seven Shiga-Toxin Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges”.

H. References

(1) Medina, M., Shelver, W., Fratamico, P., Fortis, L., Narang, N., Cray, W. Jr., Esteban, E., Tillman, G., and Debroy, C. Latex agglutination assays for detection of Non-O157 Shiga Toxin-Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121 and O145. Journal of Food Protection 75(5):819-826.

(2) USDA-FSIS Microbiology Laboratory Guide (MLG) Chapter 5 “Detection, Isolation and Identification of Top Seven Shiga-Toxin Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges”.