E. coli-Stat Latex Agglutination Test for Presumptive Identification of *E. coli* O157:H7

Directions for Use

Intended Use:

E. coli-Stat is a rapid latex agglutination slide test for the presumptive identfication of *Escherichia coli* serogroup O157:H7.

Summary and explanation:

E. coli serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins (verotoxin, shiga-like toxin) are closely related or identical to the toxin produced by *Shigella dysenteriae*.

E. coli O157:H7 was first recognized as the cause of human illness in 1982 with the outbreaks in Oregon and Michigan in the United States. Following sporadic outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS) studies were undertaken to isolate and characterize the responsible organism. After the *E. coli* outbreak in the northwestern US in 1993 in which some small children died from eating hamburgers from a fast food restaurant, the meat industry in that country introduced additional inspection programs to eliminate or prevent pathogenic bacteria from contaminating the meat supply. However, while original outbreaks of *E. coli* O157:H7 were associated with undercooked ground beef, later outbreaks were traced to apple cider, vegetables, and drinking or swimming in contaminated water. The recent massive outbreak in Japan (more than 11,000 cases) has been linked to white radish seeds imported from the United States.

E. coli O157:H7 is not usually detected with the methods normally used for isolating and identifying traditional enteric pathogens. Strains of *E. coli* O157:H7 ferment lactose rapidly and are indistinguishable from other *E. coli* strains on lactose containing media, which could result in a report of "negative for enteric pathogens". There are two main approaches to the isolation of *E. coli* O157:H7: to use MacConkey-sorbitol agar or to screen colonies from MacConkey-sorbitol agar for sorbitol fermentation or agglutination with O157 serological reagents. In contrast to most *E. coli* strains or serotypes, O157:H7 ferment D-sorbitol slowly or not at all. Recently, new types of chromogenic agar have come into use that give presumptive identification of *E. coli* O157 through the distinctive color of this organism's colonies on the agar. After growth on agar presence of the O157:H7 serotype can be confirmed by use of specific antisera.

Latex agglutination provides a rapid and convenient method for immunological confirmation of isolates after growth on MacConkey-sorbitol or other agars.

Principle of the procedure

This is a rapid slide test in which latex particles coated with specific antibody react with the corresponding *E. coli* antigen.

A small portion of a suspect colony is suspended in a drop of dilution buffer on each of the wells of the agglutination slide. A drop of Test Latex reagent or a drop of Control Latex is added. A positive result is indicated when agglutination (clumping) occurs in the Test Latex reagent, while the Control Latex reagent remains smooth and free of agglutination.

KIT CONTENTS (30 determinations) – R-5115

Reagent A - Sample-diluting solution, 1 vial with dropper containing glycine buffered saline with detergent and 0.1 % NaN_3 **6 ml**

Reagent B - O157 Test Latex, 1 vial with dropper. Latex particles coated with immunoglobulin fraction of rabbit anti-*E. coli* O157 antigen in buffer solution with 0.1 % NaN_3. **1.5 ml**

Reagent C - H7 Test Latex, 1 vial with dropper. Latex particles coated with immunoglobulin fraction of rabbit anti-*E. coli* H7 antigen in buffer solution with 0.1 % NaN_3. **1.5 ml**

Reagent D - Control latex, 1 vial with dropper. Latex particles coated with immunoglobulin fraction of normal rabbit serum in buffer solution with 0.1% NaN_3 . **3 ml**

Reagent E - O157 positive control antigen, 1 vial with dropper. O157 antigen in phosphate buffered saline (PBS) 0.01 M, pH 7.35 with 0.1% NaN_3 **0.5 ml**

- Disposable slides **60**
- Disposable mixing sticks **120**
- Directions for use **1**

WARNINGS AND PRECAUTIONS

1) Do not use kit or components beyond expiration date.
2) Infectious agents may be present in test specimens. Therefore all specimens should be regarded and handled as being potentially biohazardous.
3) Do not allow latex suspensions to cool below 2°C.
4) Reagents contain sodium azide, which can react with lead and copper plumbing to form explosive metal azides. Azide build-up may be avoided by flushing with large volumes of water following disposal of reagents.
5) Do not mix reagents from kits of different lots.
6) Do not use kit if indications of instability are evident.
7) Incubation times or temperatures other than those specified may give erroneous results.

Indications of Instability

The Test Latex and Control Latex reagents should contain uniform milky suspensions of latex particles. Sample Diluting Solution and Positive Control Antigen should be clear solutions, free of gross particulate matter. The kit should not be used if:

(a) Sample Diluting Solution or Positive Control shows marked turbidity.
(b) Clumping in Test Latex or Control Latex is evident and cannot be removed by gentle mixing.
(c) Positive Control Antigen does not react with Test Latex or does react with Control Latex..

Storage of Reagents

Refrigerate all reagents. DO NOT FREEZE!

Latex suspensions begin to clump irreversibly below 2°C.

Specimen collection and handling

1. For O157 testing select an isolated, sorbitol non-fermenting colony after overnight growth on MacConkey-Sorbitol or other selective agar.
2. For H7 testing use subculture of the sorbitol non-fermenting colony after growth on blood agar (e.g. TSA-5% DSB).

PROCEDURE

Bring kit components and samples to room temp. (15-30°C).

Note: mix Test and Control latexes with **vortex mixer** before use.

The test uses a special disposable slide comprising two wells - one for the Test Latex and one for the Control Latex. Be sure the slides are clean and free of lint before use.

1. **Dispense** one drop (about 50 µl) of the Sample Dilution Solution onto each well.
2. Using an inoculating loop, pick a portion of a suspect colony and thoroughly emulsify in the drop of Sample Dilution Solution on one side of the slide. Repeat on the other side of the slide with another portion of the suspect colony.
3. **Add** one drop of well-mixed O157 Test Latex onto the left-handed well of the slide and one drop of well-mixed Control Latex onto the right-hand well of the slide. Thoroughly mix sample and latex suspension using a separate mixing stick for each well.
4. **Rock** the slide gently for up to 2 minutes. Avoid mixing the contents of the two wells.
5. After reading the results dispose of the slide in disinfectant or autoclave waste container.
6. If a positive result is obtained, streak another portion of the suspect colony onto a blood agar plate. After overnight growth at 37°C, repeat the test in steps 1-6 above with a sweep of the growth from the blood agar plate and substitute the H7 Test Latex for the O157 Test Latex in step 3.

INTERPRETATION OF RESULTS

Positive

A test is positive if agglutination occurs in the test well (left) within 2 minutes, and a milky suspension remains in the negative control well (right).

Negative

A test is negative if a milky suspension remains in both the Test and Control wells. *Any agglutination of the control latex is an indication that the test was performed incorrectly or that the reagents are defective*. In such a case results are invalid.

Result Summary Table

O157 Test Latex (left well)	H7 Test Latex (left well)	Control Latex (right well)	Results
+	-	-	*E. coli* O157, H7 not present
+	+	-	*E. coli* O157, H7 present
-	-	-	Negative
+		+	Not valid*
	+	+	Not valid*

*Any test organism that agglutinates with both the test latex and control latex should be treated as follows:

1. Place 0.5 ml of sterile 0.9% saline into a tube and suspend organisms in this saline to make a homogeneous, turbid suspension (> #1 McFarland Standard).
2. Place the tube into a boiling water bath for 10 min. Allow to cool to room temperature. Add one drop (40 µl) of the boiled suspension to each side of the test slide and retest as described above with the O157 test latex and the control latex.
3. If agglutination occurs with the O157 test latex and not with the control latex, the sample is O157 positive. Continue by repeating the test with the H7 test latex and the control latex.
4. If agglutination occurs with the H7 test latex and not with the control latex, the sample is H7 positive (*E. coli* O157:H7).
5. If agglutination continues to occur with both test and control latex after boiling, the test is considered uninterpretable. If the organism is biochemically identified as an *E. coli*, it should be referred to a reference laboratory for further testing.

QUALITY CONTROL

Ready-for-use Positive Control antigen is supplied with the kit. This control contains O157 antigen only. Good laboratory practice requires running the Positive Control each time the kit is used. If a positive result is not obtained, test results are not valid and the kit should not be used.

LIMITATIONS OF THE PROCEDURE

1. Only pure culture suspensions should be tested. Positive results should always be confirmed biochemically as *E. coli*.
2. Bacteria vary in their expression of flagellar antigens. Therefore, O157 positive samples that are initially negative for H7 should be retested after passage through appropriate motility media (such as TSA-blood agar) to enhance motility before it is concluded that they do not express the H7 antigen.
3. If a strain is identified as O157 positive but H7 negative, it should be evaluated for Shiga-like toxin production to rule out enterohemorrhagic *E. coli*. Some strains of *E. coli* O157 do not produce Shiga-like toxins and have an H antigen other than H7. A few toxin-producing strains of non-motile *E. coli* O157 have been isolated.
4. Neither growth on selective media nor the Novamed E. coli-*stat* test can directly confirm that the suspect organism produces verocytotoxin. Other serotypes of *E. coli* have been identified which produce verocytotoxin, while some isolates of *E. coli* O157:H7 do not produce it.

CROSS-REACTIVITY

The Novamed *E.coli*-stat kit was used to test isolates of the following bacteria after overnight growth on blood agar plates: *Staphylococcus aureus*, Salmonella spp., Shigella spp., Proteus spp., and *E. coli* (not O157:H7). No cross-reactivity was detected. No cross reaction was detected with either *Citrobacter freundii* or *E. hermanii*, previously reported to give false positive reactions with some anti-O157 antisera. In addition: O157 Test Latex did not react when tested against a panel of 15 heterologous O antigens, and H7 Test Latex did not react when tested against a panel of 47 heterologous H antigens.

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