Department of Microbiology
PRO-LAB *E. coli* O157 Latex Test Procedure

I. Test Principle

Latex particles are coated with an antiserum against *E. coli* O157 antigen. When the coated latex particles are mixed with fresh colonies of *E. coli* serotype O157 the bacteria will bind to the antiserum, causing the latex particles to visibly agglutinate resulting in a positive reaction. Bacteria which are not O157 serotype will not bind to the antiserum and will not result in agglutination yielding a negative reaction.

II. Specimen Information

Clinical specimens should be cultured on BBL CHROMagar O157 medium. Incubate plates aerobically at 35 ± 2°C for 18–24 h in an inverted position (agar-side up). Plates should not be incubated beyond the 24 h time period prior to reading. Mauve-colored colonies should be tested to confirm or rule out *E. coli* O157.

III. Reagents & Equipment

A. Kit Components - Reagents should be stored at 2° to 8°C. DO NOT FREEZE. Reagent stored under these conditions will be stable until the expiry date shown on product label.
- One dropper vial of *E. coli* 0157 Latex Reagent containing latex particles coated with purified rabbit IgG which reacts with *E. coli* serotype O157.
- One dropper vial of Positive Control Suspension containing *E. coli* serotype O157:H7 antigen.
- One dropper vial of Negative Control Latex containing latex particles coated with purified rabbit IgG which does not react with *E. coli* serotype O157.
- Test cards
- Mixing sticks

B. Other Materials Required
- Normal saline
- Culture tubes
- Sterile loops or wooden applicators
- Sterile pasteur pipettes

IV. Procedure

A. Allow all reagents to come to room temperature before use.
B. Verify that quality control testing has been performed on the current kit lot and/or shipment prior to use for clinical testing.
C. Select mauve-colored colonies from the CHROMagar medium surface.
D. Resuspend the colonies in 0.2 ml normal saline in a culture tube (12 x 75 mm or equivalent) to a turbidity corresponding to a McFarland 3-5.

E. Place one drop of *E. cloi* 0157 Latex Reagent onto a test circle on one of the test cards provided. Using a sterile pasteur pipette add one drop of the test specimen (colony suspension) to the test circle, then mix with the Latex Reagent using one of the mixing sticks provided. DO NOT ALLOW THE TEST SPECIMEN TO COME INTO CONTACT WITH THE REAGENT BOTTLES.
F. Rock the card gently and examine for agglutination over a two-minute period. Specimens showing positive agglutination within two minutes must be examined further. Test positive specimens again by repeating the procedure using the Negative Control Latex Reagent.

V. Interpretation

A. Positive
If the test suspension produces agglutination with the Latex Reagent within a two-minute period and does not produce agglutination with the Negative Control Latex Reagent, the test is interpreted as presumptively positive for presence of *E. coli* serotype O157. Confirmatory testing must be performed with biochemical identification of the isolate to prove that it is an *E. coli*.
B. Negative
If the test suspension does not agglutinate within a two-minute period the test is interpreted as negative. If the test suspension agglutinates in Latex Reagent and the Negative Control Latex Reagent, the test should be interpreted as negative. This indicates the absence of *E. coli* serotype O157 and the presence of an auto-agglutinating or cross-reacting strain.

VI. Quality Control

Quality control is performed with each new kit and each new lot number using the positive and negative controls included with the kit. For test results to be considered valid the O157 Latex Reagent must show positive agglutination and the Negative Control Latex Reagent must show no agglutination within two minutes when tested with the Positive Control Antigen. If control results do not give expected results, the kit must not be used and the supervisor must be notified.

VII. Limitations

A. If a positive result is obtained with a test isolate, biochemical tests must be performed to confirm that the organism is *E. coli*.
B. Neither the CHROMagar O157 medium nor the *E. coli* O157 Latex Test confirms the isolate as a toxin producing strain.
C. Strains of *Escherichia hermanii* cross-react with *E. coli* O157 sera and the Latex test due to a shared antigen.

VIII. Test Verification

In July, 2008, the test kit was modified by the manufacturer. The latex reagents were changed from white to blue. The new kit was evaluated with 8

clinical isolates of *E. coli* O157 and 5 non-O157 strains. All 8 (100%) of the known O157 isolates produced positive results and all of the non-O157 strains produced negative results with the new kit. The number of isolates used for this comparison study was limited due to the lack of available reagent from the old kit.

In December, 2009, MacConkey Sorbitol agar was replaced with BBL CHROMagar O157. Refer to the CHROMagar O157 Procedure for the details of this verification study. A total of 18 isolates of *E. coli* O157 were used to evaluate the medium. All 18 of the isolates (100%) yielded positive test results with the PRO-LAB *E. coli* O157 Latex reagent.

IX. References

A. Pro-Lab Diagnostics, Inc. Ontario, Canada Revision: 7/2007.
B. Scotland, S., Day, N., and Rowe, B. (1980), FEMS Microbiol. leH, 7, 15-17.

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Reviewed by Microbiology Director, Dr. Ann Robinson: 03/29/2000
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Reviewed by Microbiology Supervisor, Jerry Claridge: 03/2000, 04/2001, 01/2002, 03/2003, 04/2004, 11/2005, 11/2006, 10/2007, 05/2008, 05/2009, 06/2010, 04/2011, 03/2013, Jason Ammons 05/2015
Updates and Revisions: 06/03/2010 Updated culture medium from MAC to BBL CHROMagar O157.