PRO-LAB DIAGNOSTICS

E. coli O157
LATEX TEST REAGENT KIT
(for *in vitro* diagnostic use)

PRODUCT CODE PL.070B (50 Tests)
PRODUCT CODE PL.071B (100 Tests)

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INTENDED USE
PRO-LAB *E. coli* O157 LATEX TEST REAGENT KIT is an agglutination test kit for the presumptive identification of *E. coli* serogroup O157 antigen on laboratory culture media.

SUMMARY AND EXPLANATION
Escherichia coli serotype O157:H7 is a verotoxin producing (VT-producing) pathogen[1,2]. This serotype has been reported as an etiological agent in sporadic and outbreak cases of haemorrhagic colitis.[3,4,5] It is also associated with haemolytic uraemic syndrome.[6] Certain *E. coli* serotypes other than O157:H7 also produce verotoxin.[7,8,9] However, the diarrhoea caused by these other serotypes is not usually bloody. Additionally, *E. coli* serotype O157:H7 does not ferment sorbitol whereas the majority of other serotypes do ferment sorbitol.[10,11] Therefore, if Sorbitol-MacConkey agar medium is used as a primary screen, the colonies of *E.coli* serotype O157:H7 appear colourless (non-sorbitol fermenting colonies- NSFC) while colonies of other serotypes appear characteristically pink (sorbitol fermenting colonies-SFC).[11]

PRINCIPLE OF THE PROCEDURE
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 (positive reaction). Bacteria which are not O157 serotype will not bind to the antiserum and will not result in agglutination (negative reaction).

REAGENTS AND MATERIALS AVAILABLE
PRO-LAB *E. coli* O157 Latex Test Reagent Kits contains:

a) one dropper vial of *E. coli* 0157 Latex Reagent containing latex particles coated with purified rabbit IgG which reacts with *E. coli* serotype O157. Sufficient for:
PL.072B 50 tests 3.1 ml (PL.070B)
PL.073B 100 tests 6.2 ml (PL.071B)

b) one dropper vial of Positive Control Suspension containing *E. coli* serotype O157:H7 antigen. *E. coli* serotype O157:H7 colonies are grown on agar medium, harvested and inactivated to produce the positive control. Sufficient for:
PL.074B 50 tests 1.5 ml (PL.070B)
PL.075B 100 tests 3.0 ml (PL.071B)

c) one dropper vial of Negative Control Latex containing latex particles coated with purified rabbit IgG which does not react with *E. coli* serotype O157. Sufficient for:
PL.077B 50 tests 1.5 ml (PL.070B)
PL.076B 100 tests 3.0 ml (PL.071B)

d) test cards

e) mixing sticks

FOR IN VITRO DIAGNOSTIC USE

STORAGE
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.

EQUIPMENT AND REAGENTS NOT SUPPLIED
1. Normal saline.
2. Culture tubes.
3. Sterile loops or culture swabs.
4. Sterile pasteur pipettes.

SPECIMEN COLLECTION
Clinical specimens should be cultured on Sorbitol-MacConkey medium. Non-sorbitol fermenting colonies (NSFC) may be subcultured on non-selective agar medium. Colonies from overnight growth must be cleanly removed from the agar surface for testing using a sterile loop or sterile culture swab. Young, fast growing cultures will yield typical test reactions.

PRECAUTIONS
1. PRO-LAB reagents are intended for In Vitro Diagnostic use only.
2. Do no use reagents after expiry date shown on product.
3. Reagents contain 0.098% sodium azide. Sodium azide can react explosively with copper or lead if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal large quantities of water should be used when flushing used reagent down the sink.
4. Specimens should be considered potentially infectious and precautions appropriate to microbiological hazards must be observed.
5. Do not use the Latex Reagents if autoagglutination is visible. Autoagglutination is defined as agglutination of *E. coli* O157 Latex Reagent in the absence of added test specimen or agglutination of the Negative Control Latex Reagent in the presence of Positive Control Antigen or test specimen. Autoagglutination may indicate that contamination or reagent deterioration has occurred.
6. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.
7. Some reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.

TEST PROCEDURE
Allow all reagents to come to room temperature before use.

The *E. coli* O157 Latex Reagent and Negative Control Latex Reagent must be tested with the Positive Control Antigen prior to running test specimens. The *E. coli* O157 Latex Reagent must show positive agglutination and the Negative Control Latex Reagent must show no agglutination within two minutes. This indicates that the reagents retain their activity.

1. Test material may be obtained by culturing clinical specimens and using either:
   a) Non-sorbitol fermenting colonies (NSFC) from Sorbitol MacConkey agar medium.
   b) Subculture of NSFC from non-selective agar medium.
2. Select suitable colonies from the agar medium surface.
3. 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.
4. Place one drop of *E.coli* O157 Latex Reagent on to 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.

5. Rock card gently and examine for agglutination over a two minute period.
6. 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.

INTERPRETATION OF RESULTS
1. For test results to be considered valid the *E. coli* 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.

   Agglutination of Latex Reagents with the Positive Control Antigen is interpreted as shown below:

O157 LATEX REAGENT	NEGATIVE CONTROL LATEX REAGENT	REMARKS
+	-	Reagent performance is satisfactory.
-	-	Potency too low. Discard reagents.
+	+	Autoagglutination Discard Reagents

2. Agglutination of Latex Reagents with test specimen is interpreted as shown below:

O157 LATEX REAGENT	NEGATIVE CONTROL LATEX REAGENT	REMARKS
+	-	Presumptive for presence of *E. coli* serotype O157.
+	+	Indicates absence of *E. coli* serotype O157. Autoagglutinating or cross-reacting strain present.
-	not done	Indicates absence of *E. coli* serotype O157.
stringy or mucoid appearance	not done	Uninterpretable. Make fresh suspension of colonies in saline and allow clumps to settle out. Retest supernatant.

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LIMITATIONS

1. Only pure cultures from Sorbitol-MacConkey media, and which show typical *E. coli* colony morphology should be tested.
2. Tests must be interpreted by an individual experienced in microbiology, the identification of bacterial colony morphology and examination of latex agglutination.
3. Positive test results should be biochemically confirmed. Conventional serological testing, using *E. coli* O and *E. coli* H antisera, should be used to confirm the serotype of a latex agglutination positive culture.
4. PRO-LAB reagents were developed to detect the presence of *E. coli* serogroup O157 antigen. Most non-sorbitol fermenting colonies (NSFC on Sorbitol-MacConkey medium[11]) giving a positive result in this test are presumptively identified as *E. coli* O157:H7. Some other *E. coli* O157 strains (e.g. H16) which are non-sorbitol fermenting may also produce a positive reaction in this test.[1,12,13]
5. Although this test has been specifically developed to reduce the normal cross-reactivity of *Escherichia hermanii* (12), uncommon strains may cross react. Cellibiose growth in the presence of KCN and yellow pigmentation (may be delayed) may be used for differentiation.

PERFORMANCE CHARACTERISTIC

Clinical performance of the PRO-LAB test was evaluated at a hospital microbiology laboratory. Blood-stained stool specimens from 474 patients diagnosed with diarrhoea, haemorrhagic colitis or haemolytic uraemic syndrome were cultured.

Of these 474 specimens, 47 produced sorbitol-negative colonies and were tested as positive for *E. coli* strain 0157 by a commercially available latex test. These results were confirmed by conventional biochemical testing.

All 47 of the confirmed specimens gave a positive result when tested using the PRO-LAB *E. coli* O157 Latex Reagent Kit (47\47 = 100% sensitivity)

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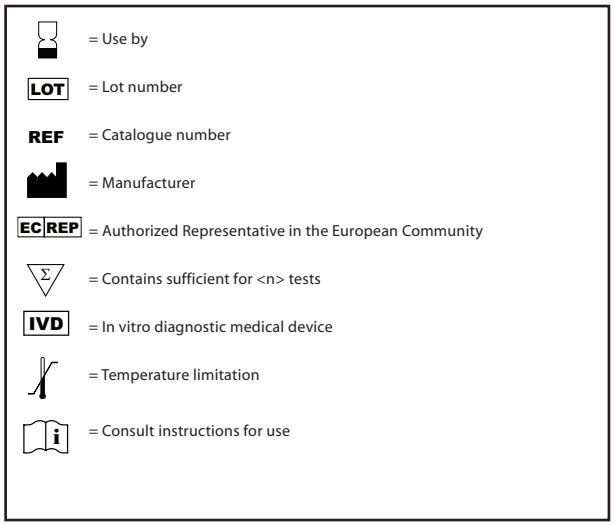

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