PROLEX™ *E. coli* O157 Latex Test Reagent Kit

PL.070B 50 PL.071B 100

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REF PL.070B REF PL.071B REF PL.076B

INSTRUCTIONS FOR USE

INTENDED USE

The Prolex™ *E. coli* O157 Latex Test Reagent Kit is an agglutination test kit for the presumptive identification of *Escherichia coli* serogroup O157.

SUMMARY AND EXPLANATION

Escherichia coli serotype O157:H7 is a Shiga toxin-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 Shiga toxin.[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 most other serotypes do.[10,11] Therefore, if Sorbitol MacConkey Agar is used as a primary screen, the colonies of *E. coli* serotype O157:H7 appear colourless (non-sorbitol-fermenting colonies [NSFC]) while colonies of the other serotypes appear characteristically pink (sorbitol-fermenting colonies [SFC]).[11]

PRINCIPLE OF THE TEST

The blue polystyrene latex particles used in the kit are coated with an antibody against the *E. coli* O157 somatic antigen. When these latex particles are mixed with fresh colonies of *E. coli* serogroup O157, the bacteria will bind to the antibody causing the latex particles to agglutinate (positive reaction). Bacteria that are not *E. coli* O157 will not bind to the antibody and will not agglutinate (negative reaction).

MATERIALS PROVIDED

PL.070B kit is sufficient for 50 tests. PL.071B is sufficient for 100 tests. Materials are supplied ready to use.

- Prolex™ *E. coli* O157 Latex Reagent (PL.072B/PL.073B): One dropper bottle containing 3.1 ml (PL.070B) or 6.2 ml (PL.071B) of latex particles coated with purified rabbit IgG that reacts with *E. coli* serogroup O157. Latex particles are suspended in a buffer containing 0.098% sodium azide as a preservative.
- Prolex™ *E. coli* O157 Positive Control (PL.074B/PL.075B): One dropper bottle containing 1.5 ml (PL.070B) or 3.0 ml (PL.071B) of Positive Control suspension containing *E. coli* serotype O157:H7 antigen produced by harvesting and inactivating *E. coli* serotype O157:H7 colonies grown on agar medium. The antigen is suspended in a buffer containing 0.095% sodium azide as preservative.
- Prolex™ *E. coli* O157 Negative Control Latex Reagent (PL.077B/PL.076B): One dropper bottle containing 1.5 ml (PL.070B) or 3.0 ml (PL.071B) of latex particles coated with purified rabbit IgG that does not react with *E. coli* serogroup O157. Latex particles are suspended in a buffer containing 0.098% sodium azide as a preservative.
- Test cards
- Mixing sticks

MATERIALS REQUIRED BUT NOT PROVIDED

- Normal saline (0.85% sodium chloride) or Phosphate Buffered Saline (PBS)
- McFarland Standard 3.0, 4.0 or 5.0
- 12 x 75mm test tubes

- Inoculating loop or needle
- Pasteur pipettes

STABILITY AND STORAGE

Reagents should be stored at 2-8°C. Reagents stored under these conditions will be stable until the expiration date shown on the product label. **Do not freeze.**

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. Do not use the reagents after the expiration date shown on the product label.
3. The reagents contain ≤ 0.098% sodium azide. Sodium azide can react explosively with copper or lead plumbing if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used if reagents are flushed down the sink.
4. Universal precautions should be taken in handling, processing and discarding all clinical specimens. All test materials should be considered potentially infectious during and after use and should be handled and disposed of appropriately.
5. Do not use the latex reagents if autoagglutination is visible. This would appear as agglutination of the Prolex™ *E. coli* O157 Latex Reagent in the absence of a test isolate or agglutination of the Negative Control Latex Reagent in the presence of Positive Control Antigen or the test isolate.
6. The procedures, storage conditions, precautions, and limitations specified in these directions must be followed to obtain valid test results.
7. Reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.

PREPARATION OF CULTURES

Clinical specimens should be cultured on Sorbitol MacConkey Agar. Non-sorbitol-fermenting colonies (NSFC) may be tested directly or from a subculture on a non-selective agar medium. Colonies from an overnight culture (18-24 hrs) must be cleanly removed from the agar surface for testing using a sterile loop or needle. Young, fast-growing cultures typically give the best results.

TEST PROCEDURE

1. Allow all of the reagents to come to room temperature before use.
2. Re-suspend the latex reagent by gently inverting the dropper bottle several times. Examine the dropper bottles to ensure that the latex particles are properly suspended before use. Do not use if the latex fails to re-suspend.
3. Using a pipette, transfer 0.2 ml normal saline or phosphate buffered saline into a 12 x 75 mm test tube.
4. Select sufficient colonies from the plate with a sterile loop or needle and prepare a suspension in the normal saline or phosphate buffered solution to achieve turbidity corresponding to a 3-5 McFarland Standard.
5. Place one drop of the Prolex™ *E. coli* O157 Latex Reagent in a test circle on one of the test cards provided. Using a Pasteur pipette add one drop of the test suspension into the same test circle and mix using one of the mixing sticks provided.
6. Rock the card gently and examine for agglutination for up to two minutes.
7. Isolates that give a positive result with the Prolex™ *E. coli* O157 Latex Reagent must be tested further by repeating the procedure using the Prolex™ Negative Control Latex Reagent.

QUALITY CONTROL

The Prolex™ *E. coli* O157 Latex Reagent and Prolex™ Negative Control Latex Reagent must be tested with the Prolex™ Positive Control before running the test isolates. There must be agglutination with the Prolex™ *E. coli* O157 Latex Reagent within two minutes and no agglutination with the Prolex™ Negative Control Latex Reagent.

INTERPRETATION OF RESULTS

Prolex™ *E. coli* O157 Latex Test Reagent Kit with the test specimen is interpreted as shown below:

***E. coli* O157 Latex Reagent**	**Negative Control Latex Reagent**	**Interpretation**
+	-	Presumptive for *E. coli* O157
+	+	Inconclusive result
-	not done	Indicates absence of *E. coli* O157
Stringy or mucoid appearance	not done	Uninterpretable

Positive Result: Visible agglutination of the blue latex particles within 2 minutes.

Negative Result: No agglutination of the latex particles at 2 minutes.

Inconclusive: Visible agglutination of the latex particles with *E. coli* O157 Latex Reagent and the Negative Control Latex Reagent indicates an agglutinating or cross-reacting strain present. Perform further testing to rule out *E. coli* O157.

Uninterpretable: Stringy or mucoid appearance with the *E. coli* O157 Latex Reagent. Make fresh suspension of colonies in normal saline or phosphate buffered saline and allow clumps to settle out. Retest supernatant.

LIMITATIONS OF THE PROCEDURE

1. Test only colonies that exhibit typical colonial morphology on Sorbitol MacConkey Agar (non-sorbitol-fermenting).
2. Positive test results should be confirmed using routine biochemical testing.
3. This reagent was developed to detect the presence of *E. coli* serogroup O157 antigen. Some other *E. coli* O157 strains (e.g. O157:H16) that are non-sorbitol-fermenting also produce a positive result with this test.[1,12,13]
4. Although this test has been specifically developed to reduce the normal cross-reactivity of *Escherichia hermanii* [12], rare strains can cross-react.

PERFORMANCE CHARACTERISTICS

Clinical performance of the Prolex™ *E. coli* O157 test kit 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 tested positive for *E. coli* strain O157 by a commercially available latex test. These results were confirmed by conventional biochemical testing. All 47 of these isolates gave a positive result when tested using the Prolex™ *E. coli* O157 Latex Reagent Kit (47/47 = 100% sensitivity).

REFERENCES

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SYMBOLS GLOSSARY

Symbol	Meaning
	Manufacturer
	Use-by date
LOT	Lot number
REF	Catalogue number
	Temperature limit
	Consult instructions for use or consult electronic instructions for use
IVD	In vitro diagnostic medical device
Σ	Contains sufficient for <n> tests
CE	Indicates European Conformity
UK CA	Indicates UK Conformity
EC REP	Authorized representative in the European Community / European Union

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