



REF

M44 MICROSCREEN® E. COLI 0157



50

IVD

INTENDED USE

Microscreen® E. coli 0157 is a rapid latex agglutination test intended for confirmatory identification of *E. coli* serogroup 0157 cultured on selective solid media from human faecal samples. The test allows the rapid differentiation of *E. coli* 0157 from other *E. coli* serotypes and organisms isolated from the faeces of patients with diarrhoea. The kit is intended for professional laboratory use only.

PRINCIPLE OF THE TEST

Latex particles are coated with antibodies raised against the lipopolysaccharide 0157 antigen of *E. coli* 0157:H7. When sensitised latex particles are mixed with a suspension containing *E. coli* 0157 antigens, a sensitive and specific immunochemical reaction takes place causing the finely dispersed latex particles to agglutinate into aggregates that are easily visible to the naked eye.

CONT

KIT PRESENTATION

REAG

TEST

M44a Test Latex Reagent: 2.5mL

Latex particles coated with rabbit antibodies to *E. coli* 0157. Preserved with 0.099% sodium azide. (**Blue** cap)

REAG

CONTROL

M44b Control Latex Reagent: 2.5mL

Latex particles coated with rabbit antibodies non-reactive to *E. coli* 0157. Preserved with 0.099% sodium azide. (**Black** cap)

NaCl

0.85%

M40 0.85% Isotonic Saline: 5mL

Preserved with 0.099% sodium azide. (**White** cap)

Instructions for Use
Disposable agglutination slides
Disposable mixing sticks

Additional Requirements:

- Bacteriological loops
- MacConkey agar plates containing 1% D-sorbitol instead of lactose (Sorbitol MacConkey agar).

WARNINGS AND PRECAUTIONS

Safety:

- The reagents supplied in this kit are for *in vitro* diagnostic use only
- Sodium azide, which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
- Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralised before treatment.

Procedural:

- Microscreen® E. coli should be used according to the kit instructions.
- Allow all reagents to reach room temperature before use.
- Do not dilute any of the kit reagents
- Do not intermix reagents from different batches of kits.
- Do not freeze any of the kit reagents
- Do not allow the latex reagent dropper to touch the bacterial samples.
- Ensure the agglutination slide is clean and dry prior to use.
- Ensure adequate attention is paid to the section on "Quality Control".
- Be careful only to record agglutination. Reactions that are "curdy" or "stringy" may not be true agglutination.

STORAGE AND SHELF LIFE

Microscreen® E. coli should be stored at 2-8°C when not in use. The kit should not be used after the expiry date printed on the carton label.

SPECIMENS

The patient's sample (bloody stool specimen) should be inoculated onto Sorbitol MacConkey agar (containing 1% D-sorbitol instead of lactose). Incubate aerobically for 18-24 hours at 35-37°C. Potentially toxicogenic strains of *E. coli* 0157:H7 appear as colourless colonies morphologically similar to other *E. coli*.

PROCEDURE

Quality Control:

The following checks should be performed each time the kit is used to confirm that the reagents are functioning correctly:

- Reagent Control**
Add 1 drop of Microscreen® E. coli 0157 Test Latex (M44a) and 1 drop of Control Latex (M44b) to 2 separate wells on an agglutination slide. Add 1 drop of saline solution (M40) to each drop of latex and mix each latex/saline suspension separately spreading liquid over the entire surface of the well. Rock the slide gently for 30 seconds and observe for agglutination in both wells. If agglutination is observed, then either the latex or the saline is giving non-specific agglutination and should be discarded.
- Positive Control**
Prepare a smooth suspension of a known *E. coli* 0157 on two wells of an agglutination slide (see Test Procedure below). Rock the slide gently for 30 seconds and observe for autoagglutination. If there is no autoagglutination in either well, add 1 drop of Microscreen® E. coli 0157 Test Latex (M44a) to one well and one drop of Control Latex (M44b) to the other. Rock the slide gently for 2 minutes and observe for agglutination. The well containing the test latex should show obvious agglutination, whereas the well containing control latex should show no agglutination. If this reaction pattern is not seen, the reagents may have deteriorated or become contaminated and should be discarded.

Test Procedure:

1. Dispense 1 drop (30µL) of isotonic saline (M40) on to two wells of a clean, dry Microscreen® agglutination slide.
2. Using an inoculating loop, remove several suspected *E. coli* colonies from the Sorbitol MacConkey agar plate. Only select colourless colonies whose morphology resembles that of *E. coli*.
3. Emulsify the colonies in the two drops of saline on the test slide to produce a heavy, smooth suspension. Spread the suspension over the entire surface of the wells.
4. Rock the slide gently for 30 seconds and observe for autoagglutination or clumping. If the suspensions remain smooth, proceed to section 5. If the suspension is "stringy" or "granular", the sample is unsuitable for testing with Microscreen® *E. coli* 0157 since it may give a falsely positive agglutination when latex is added. In this event, an alternative test method should be used.
5. Gently shake each latex reagent to ensure a homogeneous suspension.
6. Add 1 drop of Microscreen® *E. coli* 0157 Test latex to one of the bacterial suspensions, and one drop of Microscreen® *E. coli* 0157 Control latex to the other. Do not allow the latex dropper to touch the bacterial suspensions.
7. Mix the suspensions with a fresh mixing stick for each combination.
8. Rock the slide gently for two minutes and observe for agglutination. An agglutination reaction is indicated by visible aggregation of the latex particles.
9. Discard the used slides and mixing sticks into a suitable disinfectant.

INTERPRETATION

Microscreen® *E. coli* 0157 should be interpreted as follows:

Test Latex	Control Latex	Interpretation
+	-	<i>E. coli</i> 0157 present
-	-	<i>E. coli</i> 0157 not present
+	+	Non-specific agglutination
-	+	Inconclusive result

LIMITATIONS OF USE

1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information
2. Only pure cultures from Sorbitol-MacConkey media, and which show typical *E. coli* colony morphology should be tested.
3. Conventional serological testing, using *E. coli* O and *E. coli* H antisera, should be used to confirm the serotype of latex agglutination positive cultures
4. Most non-sorbitol fermenting colonies on Sorbitol MacConkey plates giving a positive result in Microscreen® *E. coli* 0157 are presumptively identified as *E. coli* 0157:H7. However, some other *E. coli* 0157 strains (e.g. H16) which are non-sorbitol fermenting may also be reactive in this test.
5. Whilst Microscreen® *E. coli* 0157 has been developed to specifically reduce the normal cross-reactivity of *E. hermanii*, uncommon strains may cross-react. Cellulose growth in the presence of potassium cyanide and yellow pigmentation (which may be delayed) may be used for differentiation.
6. Culture-derived suspensions which auto-agglutinate cannot be tested by Microscreen® *E. coli* 0157. Alternative methods should be used.

REPRODUCIBILITY

Lot to lot reproducibility has been confirmed and is monitored by testing each batch against a defined panel of specimens as part of the QC release procedure.



Tel : 02 8212 4074
Fax: 02 9423 6692
www.keydiagnostics.com.au
PO Box 1038 Gymea NSW 2227



Microgen Bioproducts Ltd
1, Admiralty way
Camberley
Surrey, GU15 3DT, UK

PERFORMANCE CHARACTERISTICS

The clinical performance of Microscreen® *E. coli* 0157 has been evaluated at a hospital microbiology laboratory. Blood-stained stool specimens from 474 patients diagnosed with diarrhoea, haemorrhagic colitis or haemolytic uraemic syndrome were cultured. 47 cultures produced non-sorbitol fermenting colonies which tested positive for *E. coli* 0157 using both Microscreen® *E. coli* 0157 and another commercially available latex test. All colonies were confirmed as *E. coli* 0157 by conventional biochemical testing.

Sensitivity of Microscreen® *E. coli* 0157 = 47/47 = 100%

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