LK13– HiE.coli 0157™ Latex Test Kit

Product Code	Reagents provided**	LK13
		50 Nos.
LK13a	E. coli Test Latex Reagent	3.0 ml
LK13b	E. coli Control Latex Reagent	3.0 ml
LK13c	Sample Diluent	5.0 ml

**** Agglutination slides and mixing sticks are provided in the kit.**

Intended Use

HiE.coli0157™ Latex Test Kit 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 *in vitro* diagnostic use only. Not for Medicinal Use.

Principle of the Test

Latex particles are coated with antibodies raised against the lipopolysaccharide 0157 antigen of E. coli 0157:H7. When sensitized 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.

Kit Contents

LK13a E. coli Test Latex Reagent:

Latex particles coated with rabbit antibodies to E. coli 0157.Preserved with 0.099% sodium azide.

LK13b E.coli Control Latex Reagent:

Latex particles coated with rabbit antibodies non-reactive to E. coli 0157. Preserved with 0.099% sodium azide.

LK13c Sample diluent (0.85% Isotonic Saline):

Preserved with 0.099% sodium azide.

ISO 9001-2008 CERTIFIED WHO GMP CERTIFIED CE ISO 13485-2003 CERTIFIED

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Instructions for Use

- Disposable agglutination slides
- Disposable mixing sticks

Additional Requirement

- Micropipettes and tips
- Bacteriological loops (PW012 HiFlexiLoop 2)
- MacConkey Agar plates containing 1% D-sorbitol instead of lactose (MacConkey Sorbitol Agar M298).

Warnings

Safety:

1. The reagents supplied in this kit are for *in vitro* diagnostic use only. Not for Medicinal Use.

2. 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.

Specimen Collection and Handling

The patient's sample (human faecal sample) should be inoculated on to MacConkey Sorbitol agar (M298). Incubate aerobically for 18-24 hours at 35-37°C. Potentially toxigenic strains of E. coli 0157:H7 appear as colorless colonies morphologically similar to other E. coli.

After use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Procedure

1. HiE.coli0157™ Latex Test Kit should be used according to the kit instructions.

2. Allow all reagents to reach room temperature before use.

3. Do not dilute any of the kit reagents.

4. Do not intermix reagents from different batches of kits.

5. Do not freeze any of the kit reagents.

6. Ensure the agglutination slide is clean and dry prior to use.

7. Ensure adequate attention is paid to the section on "Quality Control".

8. Be careful only to record agglutination. Reactions that are "curdy" or “stringy" may not be true agglutination.

Storage and Shelf Life

HiE.coli 0157™ Latex Test Kit 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.

Quality Control

Organism	Agglutination with control latex Reagent	Agglutination with test latex Reagent
E.coli 0157	-	+
Key : + is agglutination , - is no agglutination		

Performance & Evaluation

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

1. Reagent Control

Add 20µl of HiE.coli 0157 Test Latex Reagent (LK13a) and 20µl of Control Latex (LK13b) to 2 separate wells on an agglutination slide. Add 20µl of saline solution (LK13c) to each 20µl 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.

2. 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 20µl of E.coli Test Latex Reagent (LK13a) to one well and 20µl of Control Latex (LK13b) 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 20µl of isotonic saline (LK13c) on to two wells of a clean, dry HiE.coli 0157 Latex Test Kit agglutination slide.

2. Using an inoculating loop, remove several suspected E. coli colonies from the MacConkey Sorbitol agar plate (M298). Only select colorless 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 HiE.coli 0157 Latex Test Kit 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 20µl of Test Latex Reagent (LK13a) to one of the bacterial suspensions, and 20µl of Control Latex (LK13b) to the other.

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

HiE.coli 0157 Latex Test Kit should be interpreted as follows:

Test Latex	Control Latex	Interpretation
+	-	E.coli 0157 is present
-	-	E.coli 0157 is absent
-	+	Non-specific agglutination
+	+	Inconclusive result

Key : + is agglutination , - is no agglutination

Limitation 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 MacConkey Sorbitol agar (M298), 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 MacConkey Sorbitol agar plates (M298) giving a positive result in HiE.coli 0157 Latex Test Kit 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 HiE.coli 0157 Latex Test Kit has been developed to specifically reduce the normal cross-reactivity of E.hermanii, uncommon strains may cross-react. Cellibiose 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 HiE.coli 0157 Latex Test Kit. Alternative methods should be used.

Precautions

Read the procedure carefully before starting the experiment.

Performance Characteristics

The clinical performance of HiE.coli 0157 Latex Test Kit 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 HiE.coli 0157 Latex Test Kit and another commercially available latex test. All colonies were confirmed as E.coli 0157 by conventional biochemical testing.

Sensitivity of HiE.coli 0157 Latex Test Kit = 47/47 = 100%

Reproducibility

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

Disposal

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 neutralized before treatment.

Technical Assistance

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In vitro diagnostic medical device

CE Marking

Consult instructions for use

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