E.COLI O157 RAPID LATEX TEST KIT

For professional *in Vitro* diagnostic use only

Latex slide agglutination test for the confirmatory identification of *E. coli* O157 colonies

INTENDED USE
E. coli 0157 Rapid Latex Test Kit is a manual rapid latex agglutination test intended for qualitative confirmatory identification of *E. coli* serogroup 0157 cultured on selective solid media from human faecal or food 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 Somatic (cell wall) lipopolysaccharide 0157 antigen of *E. coli* 0157. 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.

REAGENTS AND MATERIALS PROVIDED
REAG TEST EC1: E. coli O157 Latex Reagent: 2.5 mL - Latex particles coated with rabbit antibodies to *E. coli* O157. Preserved with 0.099% sodium azide. (dropper red cap)
REAG CONTROL: Control Latex Reagent: 2.5mL - Latex particles coated with rabbit antibodies non-reactive to *E. coli* 0157. Preserved with 0.099% sodium azide. (Green cap)
SAMPLE DILUENT: 0.9% Isotonic Saline: 5.0mL-Preserved with 0.095% sodium azide. (*yellow* cap)
DISPOSABLE AGGLUTINATION CARDS (SLIDE) : 20 cards, each with 6 black agglutination areas
MIXING STICKS (4x25) : 100 disposable mixing sticks
DISPOSABLE PIPETTE: 1 disposable transfer pipette
INSTRUCTIONS FOR USE

MATERIALS REQUIRED BUT NOT SUPPLIED
Bacteriological loops
Sorbitol MacConkey agar plates
Timer

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 30minutes. Liquid waste containing acid must be neutralised before treatment.

Procedural:
- E. coli 0157 Rapid Latex Test Kit 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
E. coli O157 Rapid 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 label.

SPECIMENS
The patient's sample (bloody stool specimen) should be inoculated on to Sorbitol MacConkey agar (containing 1% D-sorbitol instead of lactose). Incubate aerobically for 18-24 hours at 35-37°C. Potentially toxigenic strains of *E. coli* 0157:H7 appear as colourless colonies (non sorbitol fermenting colonies) which are morphologically distinct from other strains of *E. coli*. It is important to remember that other organisms may grow on this medium and exhibit similar colonial morphology to *E coli* 0157:H7 eg *E. hermani, P. stuartii* (see Limitations of Use).

PROCEDURE
Quality Control:
The following controls should be performed each time the kit is used to confirm that the reagents are functioning correctly:.
1-Reagent Control: Add one drop of **REAG TEST EC1** and one drop of **REAG CONTROL** to 2 separate wells on an agglutination slide. Add 1 drop of **SAMPLE DILUENT** 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.
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 1 drop of **REAG TEST EC1** to one well and one drop of **REAG CONTROL** 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 **SAMPLE DILUENT** on to two wells of a clean, dry 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 **SAMPLE DILUENT** 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 suspension remains smooth, proceed to Step 5. If the suspension is "stringy" or "granular", the sample is unsuitable for testing with E. coli 0157 Rapid 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 1 drop of **REAG TEST EC1** to one of the bacterial suspensions, and one drop of **REAG CONTROL** 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 mixing sticks and slides into suitable disinfectant.

INTERPRETATION

E. coli 0157 Rapid Latex Test should be interpreted as follows:

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

LMITATIONS 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 with E. coli 0157 Rapid Latex Test Kit are presumptively identified as *E. coli* 0157:H7. However, some other *E. coli* O157 strains (e.g. H16) may also grow on this medium as non-sorbitol fermenting colonies and may also produce a positive agglutination with this test.
5. Whilst E. coli 0157 Rapid Latex Test Kit has been developed to specifically reduce the normal cross-reactivity of *E. hermanii*, on rare occasions, strains may cross-react. *E. hermanii* may be differentiated from *E. coli O157* by the performance of Cellibiose Fermentatiuon, growth in KCN (potassium cyanide) broth and the production of a yellow pigment (1)

	E.coli	*E.hermanii*
Cellobiose fermentation	Neg	Pos
Growth in KCN Broth	Neg	Pos
Yellow pigment	Neg	Pos

6. Culture-derived suspensions which auto-agglutinate cannot be tested by E. coli 0157 Rapid Latex Test Kit. Alternative methods should be used.
7. Latex positive isolates should be identified as *E. coli* using an appropriate range of biochemical tests.
8. A positive latex agglutination should not be interpreted as an isolate of *E. coli* O157 which is very verocytotoxic. All such strains should be submitted for appropriate toxin testing.

PERFORMANCE CHARACTERISTICS

The clinical performance of E. coli 0157 Rapid 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 E. coli 0157 Rapid Latex Test Kit and another commercially available latex test. All colonies were confirmed as *E. coli* 0157 by conventional biochemical testing. Sensitivity of E. coli 0157 Rapid 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 part of the QC release procedure.

REFERENCES

1. Borczyk A, Lior H and Cebin B. 1987 False positive identification of Escherichia coli in foods. Int. J. Food. Protection **4**: 347-349

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (50 tests) **REF 271080**

REAG TEST EC1:	2.5 mL (dropper red cap)
REAG CONTROL:	2.5 mL (dropper green cap)
SAMPLE DILUENT	5.0 mL (*yellow* cap)
DISPOSABLE AGGL. CARDS (SLIDE)	20 cards with 6 wells each
MIXING STICKS:	4x25 disposable mixing sticks
DISPOSABLE PIPETTE:	1 disposable transfer pipette
INSTRUCTIONS FOR USE	1 item

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