

O157 Coli-Strip CE

(C-1011)

In vitro Rapid Diagnostic Test for the detection of E.coli O157 bacteria in stool specimen after enrichment in mTSB + Novobiocin

**FOR IN VITRO DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY**

EN

Reference: C-1011, 20 tests per kit.

CE/98/79 registration number : BE/CA02/683/03

I. INTRODUCTION

Amongst the enterovirulent *Escherichia coli* (EEC), 4 classes are recognised to cause gastroenteritis in humans: Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), and Enterohemorrhagic *E. coli* (EHEC).

E. coli O157 belongs to the enterohemorrhagic group. The infection is acquired by eating food containing the bacteria and can also be associated with undercooked beef, water, raw milk, unpasteurized apple juice, lettuce, handling potatoes, yoghurt, alfalfa sprouts, etc.

Symptoms include abdominal cramps and watery diarrhea that can develop into bloody diarrhea (haemorrhagic colitis). Fever and vomiting may also occur. Most patients recover within 10 days.

Children under 5 years of age, elderly, and people with compromised immune systems are at particular risk. Serious complication called Hemolytic Uremic Syndrome (HUS) occurs in 2 to 10% of cases, which can be fatal in about 3-5% of the cases.

The illness is often misdiagnosed, and expensive diagnostic procedures may be performed.

There is no evidence that antibiotics improve the course of disease and moreover it has been shown that treatments with some antibiotics may precipitate kidney complications. Antidiarrhoeal agents should also be avoided. It is important to drink plenty of fluids to prevent dehydration.

On a yearly basis, about 10,000 to 20,000 people are affected in the United States, including 500 deaths.

II. PRINCIPLE OF THE TEST

This is a ready-to-use test that is based on the use of a homogeneous immunochromatographic system with colloidal gold. Broth enriched sample must be diluted in the dilution buffer that is supplied with the test. A nitrocellulose membrane is sensitized with antibody to *E. coli* O157

The test's specificity is ensured by a monoclonal antibody specific to *E. coli* O157 LPS and O polysaccharide moiety. It is conjugated to colloidal gold particles and dried on a borosilicate glass sheet.

When the stick is dipped into the sample suspension, the solubilized conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the anti-*E. coli* O157 monoclonal antibody adsorbed onto the nitrocellulose. If the sample contains *E. coli* O157, the conjugate complex remains bound to the anti-*E. coli* O157 reagent. The result – in the form of a dark red line that forms on the strip – is visible within 15 minutes. The solution continues to migrate to encounter a second reagent that will produce a second dark red line.

The test is aimed at the detection of *E.coli* O157 in stool after enrichment for minimum 8 hours in mTSB + Novobiocin (TSB = Tryptic Soy Broth)

III. REAGENTS AND MATERIALS

Each kit contains:

1. O157 Coli-Strip (20 items)

Each stick is sensitized with a mouse anti-*E. coli* O157 monoclonal antibody that is specific to the *E. coli* O157 LPS and O polysaccharide moiety and a specific reagent for the control line. The specific reagent is purified on protein A and adsorbed onto nitrocellulose. The anti-*E. coli* O157 conjugate is produced using a monoclonal antibody directed against the LPS and O polysaccharide moiety and coupled to colloidal gold particles after purification on protein A.

These strips come in a bottle with a desiccant.

2. Dilution buffer (15 ml)

Salt solution buffered to pH 7.5 with Tris and containing EDTA, NaN₃ (<0.1%), a detergent, and charged proteins.

3. Instruction for use (1)

Required materials not supplied:

- 3 or 5 ml test tubes
- inoculation loops for taking faecal samples
- O157 coli Positive Control (C-1091)
- broth enrichment (mTSB + Novobiocin)

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The O157 Coli-Strip is for *in vitro* diagnosis only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Dispose of gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another kit.
- The bottle containing the sensitized strips must be recapped as soon as the necessary number of the strips for the operation has been removed, since the strips are sensitive to humidity. Make sure that desiccant sachet is present.
- Two green lines indicate the antibody adsorption sites. They disappear during the course of the test.
- Discard the buffer solution if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life dates or if the reagents are stored under inappropriate conditions.
- Specimens should be collected and handled following recommended guidelines.

V. STORAGE

An unopened O157 Coli-Strip kit may be kept between 4 and 37°C and used until the shelf-life date on the packaging.

The strips remain stable for 15 weeks after the bottle is opened if they are kept between 4 and 37°C and in a dry environment.

The O157 Coli-Strip kit must not be frozen.

Stability results are available at Coris BioConcept.

VI. SAMPLES

Stool samples must be tested as soon after they are collected. If necessary, they may be stored at 2-8°C for 24 hours or -20°C for longer periods of time.

5 to 10 % of stool are diluted in water + 0,9% NaCl. 1 mL of this suspension should be added to 10 mL of broth made off mTSB + Novobiocin (20 mg/L) and incubated for 8 hours at 37 or 42 °C.

Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.

VII. PROCEDURE

Preparations:

If O157 Coli-Strip is kept at 4°C, let all the reagents warm up to room temperature before proceeding with the test.
Identify each tube with specimen number (foresee one test tube per sample).
Place the marked test tubes in a rack.

Procedure:

1. Add 0.25 ml or 8 drops of the dilution buffer solution to each tube.
2. Add the same volume, i.e. 0,25 ml of broth containing bacteria.
3. Stir to homogenize the solution
4. Dip the sensitized strip in the direction indicated by the arrow.
5. Let react for 15 minutes before to read the results

To avoid diluting the colloidal gold conjugate in the solution, take care not to immerse the strip above the line placed under the mauve arrow.

VIII. INTERPRETING THE RESULTS

The results are to be interpreted as follows:

- 1 upper line = negative**
- 2 lines = positive**
- 0 line = invalid***

* The absence of the control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.
To store the results, let the strip to dry after removing the absorbent material at its base. After drying, a very slight shadow may appear around the test line.

IX. QUALITY CONTROL

In accordance with Good Laboratory Practices, we recommend to check the test's performance regularly according to the laboratory's requirements
Each positive control should be processed after dilution 1:2 (1V:1V) in the dilution buffer.

X. PERFORMANCES:

A. Limit of detection:

The O157 coli-Strip's limit of detection for *E. coli* O157 is off $\pm 5 \cdot 10^4$ CFU/mL after 8 hours of broth culture in mTSB + Novobiocine.

B. Interference:

Tests for cross-reactivity has been checked on purified pathogens and found negative (n = 1):

Acinetobacter lwoffii, *Enrrobacter cloacae*, *Klesiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Samonella typhimurium*, *Serratia marcescens*, *Shigella flexneri*, *Stenotrophomonas maltophilia*, *Yersinia enterocolitica* (serotype O3), *Campylobacter coli*, *Campylobacter jejuni*, *Helicobacter pylor*, *Yersinia enterocolitica* 01, *Yersinia enterocolitica* 09, *Escherichia hermanii*, *E.Coli* ATCC 25922, *E. coli* O117:H7, *E. coli* O55 :H7, *E. coli* O7 :H1, *E. coli* O116 :H-, *Rotavirus*, *Adenovirus*, *Adenovirus* 40-41, *Cryptosporidium parvum*, *E. coli* K99, *Giardia*, *Salmonella enteritidis*.

Tests for cross-reactivity has been checked on purified pathogens and found positive (n = 1):

E. coli O157 broth, *E. coli* O157:H7 (ATCC 35150), *E. coli* O157 VT neg (EH431), *E. coli* O157 VT neg (EH546), *E. coli* : H19, *Staphylococcus aureus*.

Remark : reactivity with *Staphylococcus aureus* come from protein A that binds FC fragments. Positive result was observed with *Staphylococcus aureus* concentration close to 10^8 cfu/ml.

XI. LIMITS OF THE KIT

O157 Coli-Strip kit results must be compared with all other available clinical and laboratory information.
A positive test does not rule out the possibility that other pathogens may be present.
The O157 Coli-Strip is an acute-phase screening test. Stool specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold.

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