O157 E. COLI CARD

For in *Vitro* diagnostic use only

Immunochromatographic rapid test for the detection of O157-E.COLI in human faeces and food samples

I. INTRODUCTION AND INTENDED USE

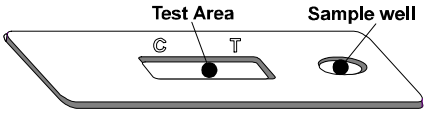
The O157 E. COLI CARD is a rapid test for the qualitative detection of *Escherichia coli (E. coli)* antigens in food and in human faeces samples, to aid in the diagnosis of *E. coli* infections.
O157:H7 E. coli is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless, this strain produces a powerful toxin that can cause severe illness. O157:H7 E. coli has been found in the intestines of healthy cattle, deer, goats, and sheep.
O157:H7 E. coli was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers and vegetables Since then, more infections in all over the world have been caused by eating undercooked ground beef than by any other food.

II. PRINCIPLE OF THE TEST

The O157 E. coli card is a qualitative immunochromatographic assay for the determination of Escherichia coli in food and in faeces samples. The membrane is pre-coated with monoclonal antibodies against O157E. coli antigens on the test line region. During testing, the sample reacts with the particle coated with anti- O157E. coli antibodies which was pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate a coloured line. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains:



1. O157 E. coli card (25 card)
2. Extraction buffer (20 mL x 1 Vials)
3. Droppers (5)
4. Instruction for use (1)

Required materials (not supplied)

Testing tubes. Specimen collection container, Disposable gloves and container, Plastic pipette and Timer.
O157 E. COLI Enrichment media: ECBroth (Cat.551425), Bagmixer 1 (Cat.Biolife Ref 7221230). Incubators +37°C ± 1°C and Purified water.

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The kit is for in vitro diagnosis only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another lot.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMENS COLLECTION FOR STOOL SAMPLES

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. PROCEDURE FOR STOOL SAMPLES

To process the collected stool samples:

Use a separate swab or stick, dropper and testing tube or vial for each sample. Dispense 0.7 mL (or 14 drops) of extraction buffer into a testing tube. Collect the stool sample with the tip of the collection device by dipping in two different places of the same stool specimen. Verify to transfer a small portion (200-300 mg) of stool. Put the collection device back into the testing tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 200-300 µl of each stool into a testing tube with extraction tube (dispense 0.7 mL (or 14 drops). Mix carefully, then vortex 15 seconds.

Test Procedure

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the card from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops or 100uL into the specimen well. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

VIII. SPECIMENS COLLECTION FOR FOOD SAMPLES

Food samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 ºC) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise sample as thoroughly as possible prior to preparation.

Sample enrichment:

- Mix 25 g of solid sample or 25 ml of liquid sample with 225 ml enrichment medium Enrichment media: ECBroth ; if necessary, homogenize with a homogenizer for 2 min. (Bagmixer 1) .
- Incubate for 18-24 hours at 37°C ± 1°C.

IX. PROCEDURE FOR FOOD SAMPLES

Allow the tests, enrichment samples to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open the pack with strips until ready to perform the assay.

1. Place 1 or 2 mL of enrichment samples in a testing tube and cover it. Only bring to room temperature the number of tests required to assay before opening it.
2. Use *E. coli O157* Card as soon as possible when opening the pack..
3. Use a separate test card for each sample. Extract some liquid from the topside with a dropper and dispense 150 µL into the specimen well s. Start the timer.
4. Read the result at **5 minutes** after dispensing the sample.

X. INTERPRETING THE RESULTS

NEGATIVE: Only one GREEN control band appears across the central window in the site marked with the letter C (control line).

POSITIVE: In addition to the GREEN control band across the central window in the site marked with the letter C (control line), a RED band (test line) also appears in the site marked with the letter T (result region).

INVALID: A total absence of the control coloured band. Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.

XI. INTERNAL QUALITY CONTROL AND INTERPRETATION OF RESULTS

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.
The intensity of the red coloured band in the result line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

XII. PERFORMANCE

A. EXPECTEDVALUES

O157:H7 *Escherichia coli* is a leading cause of foodborne illness. Based on a 1999 estimate, 73,000 cases of infection and 61 deaths occur in the United States each year.
Negative results are expected in non- *O157 E. coli* contaminated food and in well cooked food was reached a minimum internal temperature of 160ºF (70ºC).

B. SENSITIVITY, SPECIFICITY AND ACCURACY

O157 E. COLI CARD was evaluated to determine sensibility in selective enrichment cultures, specificity with producer organisms of Shiga toxins, non-Shiga toxins producers and other *Enterobacteriaceae* species (Reference Laboratory for *Escherichia coli* (LREC)
The detection of O157 E. COLI CARD showed a >99% of concordance in sensitivity; a 85% of concordance in specificity.
PPV showed a 70% and NPV showed a 100%. The detection of E. coli O157 showed a 94% of concordance in exactitude.

C. CROSS-REACTIVITY AND INTERFERENCES

It was performed an evaluation to determine the cross reactivity of **O157 E. COLI CARD**. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *H. pylori, Campylobacter spp, Listeria monocytogenes, Salmonella,Giardia lamblia, Adenovirus and Rotavirus*

XIII. LIMITS OF THE KIT

1. The test must be carried out within 2 hours of opening the sealed bag.
2. An excess of sample could result in wrong results (brown bands appear or absence of the control coloured band). Dilute the sample with the buffer and repeat the test
3. Stool from some stool samples can decrease the intensity of the control line.
4. Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
5. This **O157 E. COLI CARD** will only indicate the presence or absence of *Escherichia coli* in the specimen (qualitative detection) and should be used for the detection of *E. coli O157* antigens in food and human faces samples only. Neither the quantitative value nor the rate of increase in *E. coli* antigens concentration can be determined by this test. All results must be interpreted together with other clinical information and laboratory findings available to the physician.
6. 6.If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *E. coli* infection.

XIV. REFERENCES

1. Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M. & Swerdlow, D. L. 2005 Epidemiology of *Escherichia coli O157:H7* outbreaks, United States, 1982–2002. Emerg. Infect. Dis. 11, 603–609.
2. Griffin, P.M. “The Epidemiology of infections caused by *Escherichia coli O157:H7*, other enterohemorrhagic E. coli, and the associated haemolytic uremic syndrome”. Epidemiol. Rev , 1991, 13:60-98.
3. Grant, Michael A. “ Improved Laboratory Enrichment for Enterohemorrhagic Escherichia coli by exposure to Extremely Acidic

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 (25 tests)

O157 E. COLI CARD
Extraction Buffer
Droppers
Instruction for use

Ref. VC1010

25 Devices (Card)
1 vials x 20 mL
5 items
1 item

EDMA Code 15 01 15 01 CE IVD