**INSTRUCTIONS FOR USE**

# O157 E. COLI CARD

**RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF *O157-E. COLI* IN HUMAN FAECES, FOOD SAMPLES AND IN SUSPECTED COLONIES**

## 1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Infection with *Escherichia coli O157:H7* (Enterohemorrhagic Escherichia coli, EHEC) presents with a wide spectrum of clinical manifestations, including asymptomatic carriage, nonbloody diarrhoea, haemorrhagic colitis, the haemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Not only is *Escherichia coli O157:H7* an important agent for haemorrhagic colitis, it is also one of the leading causes of bacterial diarrhoea.
Transmission of *Escherichia coli O157:H7* is primarily food-borne. Undercooked meat is the most common culprit, dairy products and secondary person-to-person spread are also important. The organism produces at least two Shiga-like toxins. These toxins are thought to have direct pathogenic significance in *Escherichia coli O157:H7* infection. This infection is usually diagnosed from a positive stool culture, from the presence of Shiga toxins, or both. Timely collection (within 7 days of illness onset) of a stool sample for culture is imperative for a high recovery rate.
O157 E. COLI CARD is a manual, rapid immunochromatographic test for the qualitative detection of *Escherichia coli O157:H7* in food and in human stool samples, to aid in the diagnosis of *E. coli* infections. It is possible to detect the *E. coli O157:H7* from suspected colonies in stool culture. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of *Escherichia coli O157:H7* infection and it could be used to identify of suspected isolates of *E. coli O157:H7* from selective media.

## 2 - PRINCIPLE OF THE METHOD

O157 E. COLI CARD is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of *Escherichia coli O157:H7* in food specimens, in stool samples and *E. coli O157:H7* suspected colonies in stool culture.
The strip consists of a nitrocellulose membrane pre-coated with antibodies on the test line (T), in the results window, against *Escherichia coli O157:H7* and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (antibodies anti-*Escherichia coli O157:H7*) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming coloured conjugate complexes.
If the sample is positive for *Escherichia coli O157:H7,* the antigens of the diluted sample react with the red-coloured conjugate complex (anti-*Escherichia coli O157:H7* antibodies-red polystyrene microspheres) which was previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-*Escherichia coli O157:H7* antibodies present on the membrane (test line) capture the coloured conjugate and the red line will be visible. This band is used to interpret the result.
If the sample is negative, there is no *Escherichia coli O157:H7* antigen presence and yet, the antigens may be present in a concentration lower than the detection limit value, for which the reaction will not take place with the red-coloured conjugate complex. The anti-*Escherichia coli O157:H7* antibodies present on the membrane (test line) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red line will not appear. Whether the sample is positive or not, the mixture continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-specific protein antibodies present on the membrane will capture control green-conjugate complex and the control line will always appears. The presence of this green line serves as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

## 3 - MATERIALS PROVIDED – PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| O157 E. COLI CARD<br>CND: W0105011501;<br>EDMA: 15.01.15.01;<br>RDM: 1465288/R | Immunochromatographic test | **VC1010**<br>(25 tests) | 25 sealed in foil pouch containing the device, with dessicant.<br>1 plastic bottle with dropper tip containing the extraction liquid. (1 x 20mL).<br>5 plastic pipettes.<br>Secondary packaging: cardboard box. |

## 4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Tubes for test, Disposable gloves, Timer. Incubators +37°C ± 1°C and Purified water.
O157 E.COLI Enrichment media: ECBroth (Ref. Biolife 551425), Bagmixer 1 (Ref. Biolife 7221230), Sorbitol MacConkey Agar (Biolife Ref. 541669S).

## 5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- O157 E. COLI CARD is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- This product is not classified as dangerous according to current European legislation.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test device is for single use only.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use, and the test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- The presence of yellow lines in the results window (control and test line zone) that are visible before using the test are completely normal. That not means failure on test functionality.
- Wear gloves when handling the sample.
- Disposable gloves, extraction buffer, test tubes, and used devices in a proper biohazard container.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website. www.masciabrunelli.it.
- If the device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case is recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.

**Mascia Brunelli** S.p.A., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@masciabrunelli.it; export@masciabrunelli.it; web: www.masciabrunelli.it

- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

## 6 - Storage conditions and shelf life

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Do not freeze.

## 7 – Specimens Collection and Procedure for Stool Samples

**Faecal samples:** Stool samples should be collected in clean and dry containers. The samples can be stored in the refrigerator (2-8ºC) for **1-2 days** prior to testing. For longer storage, maximum **1 year**, the specimen must be kept frozen at -20ºC. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize stool sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended.

**Samples preparation**

Use a separate swab or stick, dropper and testing tube or vial for each sample. Dispense 0,7 mL (or 14 drops) of the extraction buffer into a testing tube. Collect the stool sample with a stick by dipping in *four* different places of the same stool specimen. Verify to transfer a small portion of stool (approx. 125 mg). Put the stick into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Repeat the operations just to obtain a dark yellow-brown solution, if necessary.

The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow. For **liquid or semi-solid samples,** use a transfer pipette, taking a quantity of faeces from the sample itself. Dispense 125µL of the faecal sample into a tube containing the extraction liquid (0.7 mL or 14 drops). Close the tube containing the diluent and stool sample. Shake the tube in order to assure good sample dispersion.

**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 test card from the protective pouch. Identify the plastic cassette with the patients data.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops into the specimen well. Start the timer.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn´t work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

## 8 - Specimens Collection and Procedure 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.

**Test Procedure**

**Allow the tests, 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. 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 a separate device for each sample. Extract some liquid from the topside with a dropper and dispense 150 µL into the specimen wells. Start the timer.
3. Read the result at 5 minutes after dispensing the sample.

## 9 - Procedure for Plate Culture

**Suspected *E. coli O157:H7* colonies in stool culture.** The Sorbitol MacConkey Agar (Biolife Ref. 541669S) is the method of choice for the isolation of *E.coli O157:H7* (aerobic atmosphere, 24 hours/37°C). After 24 hours of incubation in selective media the typical *E. coli O157:H7* colonies will be colourless.

1. Examine the plates after 1 day incubation. Select *E. coli O157:H7* typical colonies. Dispense 0,7 mL (or 14 drops) of extraction buffer in a collection tube. Use an inoculating needle to pick up 3 or 4 suspected *E. coli O157:H7* colonies and add them to the collection tube.
2. Close the tube with the extraction buffer and suspected colonies. Shake the tube in order to assure good sample dispersion.

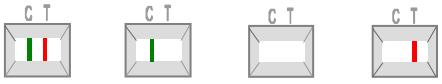
**Test Procedure**

**Allow the tests 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 test card from the protective pouch. Identify the plastic cassette with the patients data.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops into the specimen well. Start the timer.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

## 10 – Reading and Interpretation

Interpret the results as follow:




Positive Negative Invalid

| | E. coli O157:H7 | Interpretation of results |
|---|---|---|
| 1. | - | There is no *E. coli O157:H7* presence. No infection caused by *E. coli O157:H7*. Negative result. |
| | GREEN | |
| 2. | + | There is *E. coli O157:H7* presence. *E. coli O157:H7* infection, presents with a wide spectrum of clinical manifestation, including asymptomatic carriage, nonbloddy diarrhoea, haemorrhagic colitis, the haemolytic-uremic syndrome (HUS) and thrombocytopenic purpura (TTP). |
| | RED-GREEN | |
| 3. | OTHER RESULTS | Invalid result, we recommend repeating the assay using the same sample with another test. |

INVALID: Total absence of any control coloured line (GREEN) regardless the appearance or not of the test line (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

### NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured band in the test line (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

### 11 - INTERNAL QUALITY CONTROL

The Internal Quality Control procedure is included in the test. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

### 12 – EXPECTED VALUES

*Escherichia coli O157:H7* causes 73,000 illnesses in the United States annually. That means 8.598 cases, 17% require hospitalizations, 4% haemolytic uremic syndrome cases and 0,5% deaths. The main transmissions routes are: 52% foodborne, 21% unknown, 14% person to person, 31% waterborne, 3% animal contact and 0,3% laboratory related.

### 13 - PERFORMANCES CHARACTERISTICS

**Analytical sensitivity (detection limit)**

Detection limit value of O157 E.COLI CARD is $1.87x10^4$ CFU/mL.

**Clinical sensitivity and specificity**

O157 E.COLI CARD was evaluated to determine sensibility in selective enrichment culture and samples, specificity with producers organisms of Shiga toxins, non-Shiga toxins producers and other Enterobacteriaceae species (Reference Laboratory for Escherichia coli – LREC).
14 STEC strains (O157:H7 antigen); 4 Non STEC strains (O157); 9 STEC strains (non O157); 4 other *Enterobacteriaceae spp*.
The results show: >99% of sensitivity, 85% of specificity, PPV 70% and NPV >99%.

**Cross reaction and Interferences**

It was performed an evaluation to determine the cross reactivity of O157 E.COLI CARD. There is not cross reactivity against gastrointestinal pathogens occasionally present in faeces: *Campylobacter coli, Campylobacter jejuni; Citrobacter freundii; C. difficile; E. coli O22:H8, O91:H-, O103:H2, O111:H21, O145:H-, O171:H2, O174:H8; Klebsiella pneumoniae; H. pylori; Listeria monocytogenes; Morganelle morganii; Proteus mirabilis; Salmonella enteritidis, paratyphi, typhi, typhimurium; Shigella boydii, dysenteriae, flexneri, sonnei; Staphylococcus aureus; Yersinia enterocolitica.*

### 14 - LIMITATIONS OF THE METHOD

- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the extraction liquid and repeat the test.
- O157 E.COLI CARD should only be used on human faecal samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
- A positive result determines the presence of *E. coli O157:H7* in the sample (qualitative determination); nevertheless, a positive result should be followed up with additional laboratory techniques (biochemical method or by PCR) to confirm the results. A confirmed infection should only be made by the physician after evaluation of all clinical and laboratory findings and should be based on correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigens concentration in the stool sample is lower than the detection limit value. If the symptoms or situation still persist, an *E. coli O157:H7* determination should be carried out on a sample from an enrichment culture.

### 15 - REFERENCES

1. THOMPSON, J., HODGE, D. and BORCZYK, A.; "Rapid Biochemical Test to Identify Verocytotoxin-Positive Strains of Escherichia coli Serotype O157"; Journal of Clinical Microbiology, Oct. 1990, Vol. 28, No. 10, pp 2165-2168.
2. VALLANCE B.A. and FINLAY B.B., "Explitation of host cells by enteropathogenic Escherichia coli", PNAS, August 2000, Vol. 97, No. 16, pp. 8799-8806.
3. BLANCO, M. et al. "Escherichia coli Verotoxigénicos (ECVT) en Espana: ECVT O157:H7 y NO-O157 en humanos y alimentos. El Ganado bovino y ovino como reservorio. Técnicas para detección de ECVT" Laboratorio de Referencia de Escherichia coli (LREC).

**TABLE OF APPLICABLE SYMBOLS**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IVD | In Vitro Diagnostic Medical Device | | Temperature limitation | LOT | Batch code (DXXX) | | Manufacturer | | Keep dry | UDI | Unique device identifier |
| | Consult Instructions for use | | Use by (year/month) | REF | Catalogue number | | Do not reuse | | Fragile, handle with care | | Keep away from heat |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Instructions for Use (IFU) - Revision 5 | Updated layout and content | 2023/03 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

**Mascia Brunelli** S.p.A., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@masciabrunelli.it; export@masciabrunelli.it; web: www.masciabrunelli.it