

Biocard™ EHEC Test

20.05.2007

Cat.no. 6-001-000

Biocard™ EHEC Test is a rapid immunochromatographic test for the convenient and qualitative detection of *Escherichia coli* O157 and *Escherichia coli* O111 in food.

Read the instructions for use carefully before testing. Perform the test in a well-illuminated place

Introduction

Enterohaemorrhagic *E. coli* (EHEC) in particular serotype O157:H7 have in recent years been associated with numerous high profile outbreaks of food and with many sporadic cases of infection worldwide. EHEC results in serious illness in about a third of all cases and chronic complications occur in smaller number of cases. Complications including haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombo-cytopaenic purpura can occur. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin, e.g. meat and dairy products. The reservoir for EHEC is in faeces of cattle, sheep and goats. These micro-organisms can enter food during the processing of meat, dairy and vegetable products if hygienic conditions are inadequate. *E. coli* O157 is gram-negative, indole-positive, oxidase negative, lactose-positive, rod shaped bacterium. *E. coli* O157 is distinct among *E. coli* due to its inability to ferment sorbitol.

The drastic increase in the incidences of food poisoning caused by EHEC bacteria calls for a reliable and rapid method of its detection. Biocard™ EHEC Test is an immunological screening test based on the lateral flow principle and is designed to avoid time-consuming and intensive work steps.

Materials provided with the kit:

20 pcs Biocard™ EHEC Tests
20 pcs Disposable pipettes
20 pcs filtering tubes
20 pcs filter tips
1 ml Positive Control
1 ml Negative Control
Instructions for use

Material needed but not provided:
Timer

Storage and shelf life

Controls and Biocard™ test units shall be stored at ambient temperature (+10 ... +27 °C). Biocard™ test units shall be stored in original unopened foil pouches; the tests are sensitive to moisture. The shelf life of the Biocard™ test package is 24 months from the date of manufacture. Expiry date is labelled on the box, on the foil pouch and on the labels of control reagents.

Sample Preparation Protocol

Homogenise (e.g. in a Stomacher for 2 minutes) 25 g / ml of solid or liquid sample in 225 ml modified Trypticase-Soy Broth + Novobiocin or EC Broth +Novobiocin. Incubate the enrichment broth either on a rotatory shaker (100 RPM) at +37 °C - +42 °C for 6-8 hours, or static at +37 °C - +42 °C for 18-24 hours.

Test procedure

1. Remove the Biocard™ test unit, filter tip and the pipette from the pouch (one for each specimen) and place the test unit on the bench.

2. Using the disposable pipette, draw up a sample from the enriched material. Hold the pipette in a vertical position and dispense 10-20 drops of sample in the filtering tube.



3. Place the filter tip on the filtering tube.



4. Turn the vial upside down. Let the liquid fall down and wait for 1-2 seconds.

5. Press the filtering tube in order to dispense three (3) free falling drops (approx. 110 µl) through the filtering tip, into the circular sample window (S) on the device.



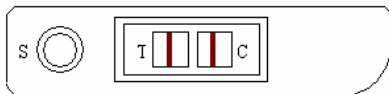
6. Read the test result 5 minutes after the application of the sample.

Colony confirmation

Biocard™ EHEC Test can be also used for identification / confirmation of suspect colonies. Pick up 1-5 suspect S-shape colonies and suspend colonies in 0.4 ml phosphate buffered saline in a tube. Perform the testing as described in Test procedure.

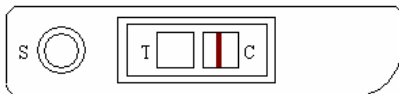
Interpretation of results

Observe the test result 5 minutes later. A positive result is indicated as a distinctly visible red line (from faint to dark) in the test indicator window and control window.



Note that the positive result can be read as soon as the test and the control line is distinctly visible.

A negative result is indicated by the absence of a distinctly visible red line in the test indicator window and the presence of a red line only in the control indicator window.



The absence of a distinctly visible red line in the control indicator window is a sign that either the test unit is damaged, or the instructions have not been followed. In such a case repeat the testing with a new test unit, filtering tube and filter tip.

Controls

Proper performance of the Biocard™ EHEC Test can be

checked by means of Positive and Negative Controls.

Perform the testing as described in Test procedure. When tested according to the instructions for use, the Positive control shall lead to a positive result (two visible lines) and the negative one shall lead to a negative result (one visible line).

Sensitivity and Spesificity

The sensitivity of Biocard™ EHEC Test is 1×10^5 CFU.

Following strains have been tested with Biocard™ EHEC and gave negative results:

Yersinia enterocolitica

ATCC 27729

Yersinia frederikseni

Salmonella typhimurium

ATCC 14020

Salmonella enteritidis

Salmonella idikan

Escherichia coli ATCC 8739,

ATCC 25927, ATCC 21432

Proteus mirabilis

Pseudomonas aeruginosa

ATCC 9027

Klebsiella pneumoniae

Hafnia alvei

Citrobacter freundii

Phaselous vulgaris

Precautions and limitations

Biocard™ EHEC Test shall be used only for *in vitro* detection of *E. coli* O157:H7, *E. coli* O157:NM, *E. coli* O157:non-H7 and *E. coli* O111 in accordance with the instructions for use and by obeying the rules of good laboratory practise.

General laboratory procedures and precautions shall be followed in handling and disposal of samples and testing material.

Do not reuse Biocard™ test units, filtering tubes, filter tips or pipettes.

Do not use expired tests or tests from a lot not showing proper performance when tested by controls. Do not use a test from a pouch which has been damaged during storage.

The Positive and Negative Controls contain 0.09% of sodium azide. Avoid swallowing and contact with the skin.

References

1. Konowalchuk, J., Dickie, S., Stavric, C., Speirs, J.I. (1978) Properties of an *Escherichia coli* cytotoxin. *Infect. Immun.* 20:575-577
2. Szabo, R.A., Todd, E.C.D., Jean, A. (1986) Method to isolate *E.coli* O157:H7 from food. *J. Food Prot.* 49(10):768-772
3. Vernozy-Rozand C. (1997) Detection of *Escherichia coli* O157:H7 and other verocytotoxin-producing *E. coli* (VTEC) in food. *J. Appl. Microbiology* 82:537-551
4. Bennett, A.R., MacPhee, S., Betts, R.P. (1995) Evaluation of methods for the isolation and detection of *Escherichia coli* O157 in minced beef. *Lett. Appl. Microbiol.* 20(6):375-379

Manufacturer:

Ani Biotech Oy

Tiilitie 3

01720 VANTAA, FINLAND

<http://www.anibiotech.fi>

e-mail: info@anibiotech.fi

tel. +358-20 155 7510

fax. +358-20 155 7517