E.coli Verotoxin 1 e 2 CARD

For professional *in Vitro* diagnostic use only.

Card rapid test for the detection of VT1/SLT-I and VT2/SLT-II antigens in stool specimen

I. INTRODUCTION AND INTENDED USE

The E.coli Verotoxin 1 e 2 CARD is a rapid chromatographic immunoassay for the simultaneous qualitative detection of VT1 and VT2 in human faeces specimens to aid in the diagnosis of enterohaemorrhagic *E. coli* (EHEC) infection.
Enterohemorrhagic *Escherichia Coli* (EHEC) is a subset of pathogenic *E. coli* that cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults. The organism produces two Verotoxins classified into two principal categories: Verotoxin 1 (VT1) and Verotoxin 2 (VT2).
EHEC are transmitted by the fecal–oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Its capacity to cause epidemic outbreaks together with the seriousness of the complications caused by enteritis make this microorganism of great importance to Public Health.
Humans can be infected asymptomatically or they may develop watery diarrhea, hemorrhagic colitis and/ or hemolytic uremic syndrome. This syndrome is most common in children, the elderly and those who are immunocompromised.

II. PRINCIPLE OF THE TEST

The E.coli Verotoxin 1 e 2 CARD is a qualitative lateral flow immunoassay for the detection of Verotoxins 1 and 2 in human faeces samples. The membrane is pre-coated with monoclonal antibodies against VT1 and VT2 on the test line regions. During testing, the sample reacts with the particle coated with anti-VT1 and anti-VT2 antibodies, which were 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 one or two coloured lines. A green coloured line 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 material for 10 determinations:
E.coli Verotoxin 1 e 2 CARD (N.10) cassettes packaged with a desiccant in individual aluminum pouches
Diluent –Extraction buffer (10x1.0 mL tubes) Sample Diluent of buffer containing proteins and preservative
Positive Control: N.1 vial with dropper containing non-infectious components, sodium azide (NaN_3) as preservative (1 x 0,5 mL).
Negative Control: use Extraction buffer-Diluent
Instruction for use (1)
Required materials (not supplied)
Specimen collection container, Disposable gloves and container, Plastic pipettes and Timer.
For sample enrichment: GN broth (Gram Negative broth) or MacConkey broth or mTSB (modified Tryptic Soy Broth).

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 professional use and for in vitro diagnosis only.
- Avoid touching the nitrocellulose strip.
- Use disposable gloves during sample manipulation.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- **Always perform the enrichment of the sample. Do not use directly on stool samples. A lack of enrichment could produce unreliable results**.
- Do not use reagents from other kits
- Discard the diluent if contaminated
- Do not use after expiration date. Do not use the test if pouch is damaged.

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

Stool samples should be collected in clean and dry containers. The samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 24 hours prior to culture. 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. PROCEDURES FOR SAMPLE ENRICHMENT

Mix well the faecal sample with a pipette or a stick, transfer 50-100 µL or an equivalent quantity of solid stool (50-100 mg) in a test tube containing the broth chosen for enrichment. Incubate not completely closed at 37±2°C for 18-24 hours. In case of the presence of bacterial growth proceeding with the test.

VIII. PROCEDURES FOR TEST

Allow the tests, samples and buffers to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open the pack 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.
3. Dispense 4-5 drops of enriched sample (about 150 µL) into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

Procedure for the controls
Allow the reagents to reach to room temperature. Remove the Card from its sealed pouch.

Add 2-3 drops (100 µL) of **Positive/Negative control** into the specimen well (S) and read the result at 10 minutes.

IX. INTERPRETING THE RESULTS

POSITIVE:
VT1 positive: Two lines appear a **red** test line marked with "T1" and a **green** control line marked with "C".
VT2 positive: Two lines appear, a **red** test line marked with "T2" and a **green** control line marked with "C".
VT1-VT2 positive: Three lines appear, the two **red** test lines (T1 and T2) and the **green** control line marked with "C".
NEGATIVE: Only one **green** line appears in the region marked with the letter C (control line).
INVALID: Total absence of the green control coloured line regardless the appearance or not of the red test lines. Review the procedure and repeat the test with a new test.

X. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test: a green line appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

XI. PERFORMANCE

A. Expected values
EHEC infections can occur as sporadic cases or in outbreaks. The annual incidence varies greatly depending on the geographical area. For example in Scotland, the U.S., Germany, Australia, Japan and the Republic of Korea ranged from 0.08 to 4.1 per 100,000 population.
B. Sensitivity and Specificity
The results showed using E.coli Verotoxin 1e 2 CARD in comparison with another commercial immunoassay test (IC test: Shiga Toxin Quick Chek, TechLab®), were: Sensitivity >99% and Specificity >99%.
C. Cross-reactivity and interference
It was performed an evaluation to determine the cross reactivity of E.coli Verotoxin 1 e 2 CARD. There is not cross reactivity with common gastrointestinal parasites occasionally present in faeces.

- *Campylobacter*
- *Citobacter freundii*
- *Clostridium difficile*
- *Helicobacter Pylori*
- *Klebsiella pneumoniae*
- *Listeria monocytogenes*
- *Morganelle morganii*
- *Proteus mirabilis*
- *Salmonella*
- *Staphylococcus aureus*
- *Yersinia enterolitica*

XII. 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 cause wrong results (brown lines appear). Dilute the sample with the buffer and repeat the test.
3. Some stool samples can decrease the intensity of the control line.
4. Avoid repeated freezing and thawing, which may give erroneous results.
5. 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 infection caused by EHEC.
6. The E.coli Verotoxin 1 e 2 CARD will only indicate the presence of Verotoxin 1 and/or 2 in the specimen (qualitative detection) and should be used for the detection of Verotoxin 1 and/or 2 in feces specimens only. Neither the quantitative value nor the rate of increase in antigens concentration can be determined by this test.This test provides a presumptive diagnosis of infection caused by EHEC. All results must be interpreted together with other clinical information and laboratory findings available to the physician.
7. The EHEC VT-VT2 Device may give a positive result with Shiga toxin produced by Shigella dysenteriae type 1, due to their similar structure. Determination and differentiation of Shigella dysenteriae type 1 and EHEC should be carried out on selective culture and with biochemical analysis.

XIII. REFERENCES

- A.C. MANIA, T. WILLIAMS, C.M ANAND and G.W. HAMMOND "Detection of Verotoxin in Stool Specimens" Journal of Clinical Microbiology, 1990, P. 134-135.
-Boldtsetseg Tserenpuntsag, Hwa-Gan Chang,Perry F. Smith and Dale L. Morse Hemolytic Uremic Syndrome Risk and Escherichia coli O157:H7

	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) **COD. VC1031**

E.coli Verotoxin 1 e 2 CARD	10 Devices (Card)
Extraction Buffer-Diluent	10 x 1mL
Positive Control	1 x 0.5 mL
Instruction for use	1item

EDMA CODE 15 01 15 11 00 CE IVD