NOROVIRUS

For *in Vitro* diagnostic use only

Rapid immunochromatographic test for the simultaneous detection and differentiation of *Norovirus* genogroups I and II antigens in human feces.

I. INTRODUCTION AND INTENDED USE

Noroviruses, members of the Caliciviridae family, are a group of more than 40 extremely heterogeneous viruses. Infection is typically characterized by self-limited vomiting and diarrhea, with symptoms lasting 12–60 h.
Noroviruses are divided into five distinguishable genogroups (GI–GV) based on genome sequence similarity; however, only virus strains from genogroups I–II are known to widely infect humans. Additional strains in the newly identified genogroup IV have also been detected in human stools. Noroviruses within a genogroup can differ by up to 40% in capsid aminoacid sequence and >50% between genogroups.
The *Norovirus* **genogroups I and II (**GI-GII) Cardtest is a rapid chromatographic immunoassay for the qualitative detection of *Norovirus* genogroups I and II antigens in human faeces specimens to aid in the diagnosis of *Norovirus* infection.

II. PRINCIPLE OF THE TEST

The *NOROVIRUS* is a qualitative immunoassay for the detection of *Norovirus* GI and GII antigen in human faeces samples. The membrane is pre-coated in the pad area with antibodies against *Norovirus* GI and GII and the same antibodies are adsorbed on the test line region. During testing, the sample reacts with the particle coated with anti-*Norovirus* GI and anti-*Norovirus* GII 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 conjugates and generate one or two coloured lines. A green coloured band always appears in the control line (third 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 25 determinations:
Norovirus card (N.25) cassettes packaged with a desiccant in individual aluminum pouches
Diluent –Extraction buffer (25x1.0 mL tubes LIQ.EXTR) Sample Diluent of buffer containing proteins and preservative
Instruction for use

Required materials (not supplied)

Specimen collection container, Disposable gloves and container, Plastic pipettes and Timer

IV. PRECAUTIONS

- For professional and *in Vitro* diagnostic use only.
- Do not use any of the kit contents after the expiration date and if not properly stored. Do not mix kit components from different lots.
- Follow the instructions carefully
- The test is intended for single use only. Do not reuse the card for other tests
- The test must remain in the sealed pouch until use
- Follow the Good Laboratory Practices, wear protective clothing and disposable gloves, do not eat drink or smoke in the work area
- All specimens should be considered potentially hazardous and handled in the same way as infectious agents.
- The test should be eliminated in a special container in accordance with regulations in force

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. SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 7 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/-4ºF. In this case, the sample needs to be totally thawed, and brought to room temperature before testing.

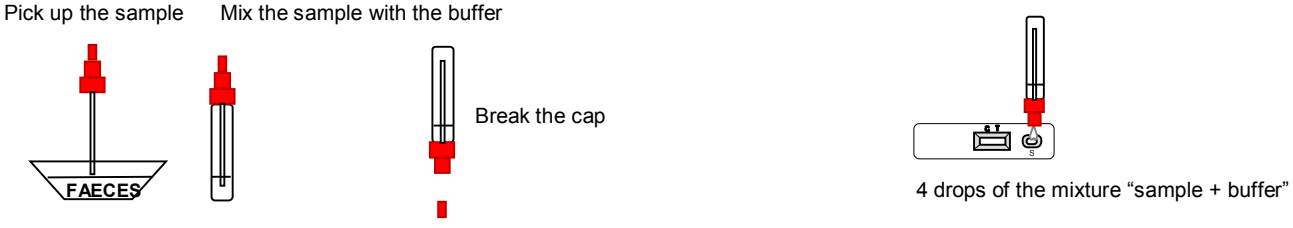
VII. ASSAY PROCEDURE

Review “specimen collection” instructions. Do not open pouches until ready to perform the assay. Make sure that all reagents are at room temperature before beginning the assay.

Process the collected stool samples

Use a separate swab or stick, dropper and testing tube or vial for each sample. Unscrew the top of the extraction buffer tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen. Verify to transfer a small portion (150-250 mg) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools, withdraw the sample using a separate pipette. Dispense 250 µl of each stool into an extraction tube. Mix carefully, then vortex 15 seconds.

Illustration 1 *Illustration 2*



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 Norovirus card from its sealed pouch and use it as soon as possible. Use a separate device for each sample.
2. Break the cap of the tube
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. INTERPRETING THE RESULTS

POSITIVE: *Norovirus* GI positive: Two lines appear across the central window, the **red** test line marked with the letter T1 and the **green** control line marked with the letter C.
Norovirus GII positive: Two lines appear across the central window, the **red** test line marked with the letter T2 and the **green** control line marked with the letter C.
Norovirus GI-GII positive: Three lines appear across the central window, the two **red** test lines (T1 and T2) and the **green** control line marked with the letter C. See illustration 3.
NEGATIVE: Only one **green** band appears in the region marked with the letter C (control line). See illustration 3.
INVALID: A total absence of the green control coloured line regardless the appearance or not of the red test lines. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local distributor. See illustration 3.

IX. CHARACTERISTICS

A) Expected values

Noroviruses are recognized as the most common cause of viral gastroenteritis among adults in the United States. It is estimated that more than 40% of foodborne outbreaks of gastroenteritis are attributable to Noroviruses. These highly contagious viruses can be transmitted by contaminated food, water, or direct person-to-person contact. Norovirus outbreaks have been documented on cruise ships, at daycare centers and schools, and among members of the army. Severe illness is rare, but unusual complications can occur in the elderly, in children, and in immunocompromised individuals.

B)Sensitivity and Specificity

Some stool samples from patients of different hospitals in Europe were tested. The *Norovirus* GI-GII Card (Mascia Brunelli) was compared with other immunochromatographic test and the results were confirmed by a PCR technique:
Sensitivity: >99% of sensitivity for *Norovirus* GI and GII
Specificity: >99% of specificity for *Norovirus* GI and GII

C) Cross reactivity

An evaluation was performed to determine the cross reactivity of *Norovirus* GI-GII Card (Mascia Brunelli) There is no cross reactivity with common gastrointestinal parasites occasionally present in feces: *Adenovirus, Astrovirus, Enterovirus, Rotavirus, Salmonella, Shigella*.

X. QUALITY CONTROL

Internal procedural controls are included in the test:
A green line (C) appearing in the results window confirms sufficient specimen volume and correct procedural technique.
Good laboratory practices include the use of an external control to ensure proper kit performance. Each day of testing (or according to the laboratory quality control procedures), a negative and a positive control containing a low level of *Norovirus* antigens should be tested.

XI. LIMITS OF THE TEST

- ***Norovirus*** GI-GII Card will only indicate the presence of virus in the specimen (qualitative detection) and should be used for the detection of *Norovirus* GI and GII antigens in faeces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- 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 *Norovirus* infection.
- After one week of infection, the number of parasites in faeces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.
- This test provides a presumptive diagnosis of infection caused by *Norovirus*. All results must be interpreted together with other clinical information and laboratory findings available to the physician.

XII. BIBLIOGRAPHY

1. KISSMANN J., et al. "Physical stabilization of Norwalk Virus-like Particles". *Journal of Pharmaceutical Sciences,* VOL.97, NO. 10, OCTOBER 2008.
2. LOBUEA A., ET AL. "Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains". *Vaccine* 24 (2006) 5220–5234.

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

CONTENTS **Ref. VC1027(25 test)**

Norovirus Card	25 items
Liq.Extr tubes	25 x 1.0 mL
Instruction for use	1 item

EDMA CODE 15 04 80 00 CE IVD

Mascia Brunelli S.p.A. Viale Monza 272, 20128 Milano. Tel. n° 02-25209.1, Fax n° 02-2576428,
E-mail: mktg@masciabrunelli.it ; sito Web: www.masciabrunelli.it