RIDA®QUICK Norovirus

REF N1402

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1. Intended use

For *in vitro* diagnostic use. The RIDA®QUICK Norovirus Test is a quick qualitative immunochromatographic test for determining Genogroup 1 (GG I) and Genogroup 2 (GG II) noroviruses in stool samples. It is used as an aid in the diagnosis of gastroenteritis and for the analysis of stool samples from children and adults with the symptoms of suspected gastroenteritis caused by the Norovirus.

2. Summary and explanation of the test

Noroviruses are a major cause of gastroenteritis world-wide with an estimated 23 million cases a year in the USA (1, 2). They are frequently involved in outbreaks in communal facilities, such as nursing homes, hospitals, day nurseries and prisons, and on cruise ships (3, 4, 5). Outbreaks caused by Noroviruses are reported more often than outbreaks caused by bacterial pathogens and they have a considerable impact on public health (6).
The RIDA®QUICK Norovirus Test, which is based on monoclonal antibodies, makes it possible to determine antigens in stool samples quickly and reliably and therefore speeds up patient management. The quick test is a straightforward, sensitive method for determining the antigens of both Genotypes I and II of the Norovirus. It is particularly suitable for use on small sample series.

3. Test principle

This rapid test is a one-step lateral flow immunochromatographic assay employing both biotinylated and gold-labeled anti-norovirus antibodies. When noroviruses are present in the positive specimen, immune complexes form with the gold-labeled anti-norovirus antibodies and migrate through the reaction membrane. Streptavidin captures the migrating immune complexes at the test line (T line) via the biotin coupled to the anti-norovirus antibodies, resulting in red-violet staining of the T line. Migrating gold-labeled antibodies not bound in the complex are bound later at the control line (C line). If norovirus antigens are not present in the specimen, the binding of gold-labeled immunocomplexes will not occur at the T line but only at the C line. The presence of a red C line confirms that the test was valid.

4. Reagents provided

Each kit contains sufficient reagents for 25 tests.

Cassette	25 tests	25 individually packaged test cassettes
Reagent A	13.5 ml	Specific anti-norovirus antibodies (mouse); contains 0.05 % azide, ready for use, blue colored
Reagent B	13.5 ml	Specific anti-norovirus antibodies (mouse); contains 0.05 % azide, ready for use, yellow colored
Pipet	25	1 bag of 25 disposable pipets
Reagent vial	25	1 bag of 25 reaction vessels
Pipet Tip	25	1 bag of 25 pipet tips
Microlit Pipet	1	Microliter pipette for 150 µl volumes

Dangerous substances are indicated according to labelling obligations. For more details, refer to Safety Data Sheets (SDS) at www.r-biopharm.com.

5. Storage instructions

Store the kit at 2 to 25 °C. Kit contents are stable until the expiration date printed on the product label. The quality of the product cannot be guaranteed after the expiration date. Likewise, the usability of the cassettes can no longer be guaranteed if the cassette packaging is damaged.

6. Reagents required but not provided

6.1 Necessary reagents

No additional reagents are needed to perform this test.

6.2 Necessary laboratory equipment

The following equipment is needed to perform this test:

Equipment
Vortex mixer (optional)
Waste receptacle containing 0.5 % sodium hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for carrying out this test. Do not pipette samples or reagents by mouth. Avoid contact with broken skin and mucous membranes. Wear personal protective equipment (suitable gloves, apron, protective glasses) when handling reagents and samples and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are being processed.
For more details, refer to the Safety Data Sheets (SDS) www.r-biopharm.com.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

The reagents contain sodium azide as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

All reagents and materials that come into contact with potentially infectious specimens must be treated with suitable disinfectants (e.g., sodium hypochlorite) or autoclaved at 121 °C for at least one hour.

8. Collection and storage of specimens

Stool samples should be collected in clean containers without preservatives and stored at 2 to 8 °C until processed for testing. If the samples cannot be processed within 3 days, they should be stored at - 20 °C or colder (Tab. 1). Frozen specimens must be thawed completely and equilibrated to room temperature before testing. Repeated freezing and thawing of samples should be avoided.
When rectal swabs are to be used, ensure that sufficient fecal matter (ca. 50 mg) is available for performance of the assay.

Tab. 1: Specimen storage

Undiluted stool specimens	
2 to 8 °C	≤ - 20 °C
≤ 3 days	> 3 days

9. Test procedure

9.1 General information

Bring all test specimens, reagents and test cassettes to room temperature (20 to 25 °C) before use. The test cassettes should not be removed from the packaging until immediately before use. Each test cassette is for single use only and cannot be reused. Do not perform the test in direct sunlight. Excess reagent must not be put back in the vial because this could cause contamination.

9.2 Preparing the specimens

Add **0.5 ml** each of Reagent A Reagent | A and **0.5 ml** of Reagent B Reagent | B to a labeled reaction vessel Reagent vial. Strictly adhere to the 0.5 ml and 1.0 ml graduation marks on the reaction vessel. Reagents A and B must be present at a ratio of **1:1**!

9.2.1 Preparation of stool samples

If liquid, 50 µl of stool specimen is drawn into a disposable pipette Pipet (to the second bulge) and added to the reagents in the reaction vessel to yield a suspension.
If solid, ca. 50 mg of stool specimen is analogously processed to yield a suspension. Next, the reaction vessel is sealed carefully and the sample is homogenized by thorough mixing (and vortexing: optional). Subsequently, the homogenized stool suspension is allowed to stand for **5 minutes** to yield a largely particle-free supernatant. The reaction vessel can be placed in one of the three central openings of the reagent holder for sedimentation.

9.3 Specimen testing

Remove the test cassette Cassette from the packaging and place on a flat surface. After placing an unused tip Pipette Tip on the microliter pipette Microlit Pipette, remove 150 µl of the supernatant from the respective reaction vessel and dispense into the sample well of the test cassette. Ensure that the liquid flows freely through the membrane. If the test was performed correctly, the color band at control line C should appear within about 3 minutes. If no control line appears within 3 minutes, the test is invalid and must be repeated. In repeat testing, the test specimen should be better sedimented (optionally, by centrifuging it at 2000 g for 2 minutes), and the supernatant pipetted into the sample well of a new test cassette.
Always read the test result within **15 minutes** of applying the specimen to the sample well. Color development of the lines can intensify during the entire development time, and the color of the lines may change from red-violet to bluish/grayish violet as the strip dries.

10. Quality control – indication of instability or deterioration of reagents

The test is invalid if the test cassette is damaged before application of the sample suspension into the sample well or if it any exhibits discoloration or color lines prior to use. The test is invalid if no red-violet control line appears within the 15-minute incubation period. If no control line appears, the following checks should be performed before repeating the assay:

- Check the expiration date of the test cassette and of the reagents used.
- Check to determine whether the test has been performed correctly.
- Check for contamination of the reagents.

If the control line still is not visible after repeating the test with a new cassette, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

No more than two lines should appear, and they should appear in the following order relative to the sample well: One red-violet test line (T), or reaction band, and one red-violet control line (C), or control band. **If no control line appears, the test is invalid and must be repeated.**

Test results are interpreted as follows:

- **Norovirus-positive:** Both the test line and the control line appear.
- **Norovirus-negative:** Only the control line appears.
- **Invalid:** If no control line C appears, even if a test line is visible, the test is invalid. Likewise, if no significant color line develops until significantly later than 15 minutes after sample application, the test is also invalid and must be repeated.

12. Limitations of the method

The RIDA®QUICK Norovirus rapid test specifically detects noroviruses of the GGI and GGII subtypes in human stool samples. No correlation between the intensity of the specific color lines and the occurrence or clinical severity of clinical symptoms can be derived from the test result. **The test result must always be interpreted in conjunction with the clinical signs and symptoms.**

A **positive** test does not rule out the possibility of other infectious agents or causes.

A **negative** test does not rule out the possibility of infection with noroviruses. The test may be negative due to intermittent pathogen excretion or to concentrations of noroviruses below the limit of detection. If there is justified suspicion of norovirus infection based on the case history, another stool sample should be tested.

Excessive amounts of stool sample can result in the test strip developing brown stains, which may cover the red-violet color of the specific test and control lines. In the event of such staining, the test should be repeated with a smaller quantity of stool sample; alternatively, stronger centrifugation of the stool suspension can be performed to determine whether the noroviruses are indeed present in the sample but the specific test line is masked by an excess of stool matrix.

13. Performance characteristics

13.1 Clinical sensitivity and specificity

In a validation study, a total of 75 stool specimens (fresh and frozen specimens) were measured using the RIDA®QUICK Norovirus rapid test versus real-time RT-PCR for noroviruses of the genogroups 1 and 2. The results are summarized in the following table.

Tab.2: Sensitivity and specificity of RIDA®QUICK Norovirus versus real-time RT-PCR

		RIDA®QUICK Norovirus	
		+	-
RT-PCR	+	**23**	**2**
	-	**1**	**49**

Sensitivity : 92.0 %
Specificity : 98.0 %
Positive predictive value: 95.8 %
Negative predictive value: 96.0 %

13.2 Precision

The precision of the RIDA®QUICK Norovirus test was measured on the basis of the intra-assay reproducibility (10 replicates / 1 day / 1 operator / 1 lot), the inter-day reproducibility (3 replicates / 10 days / 1 operator / 1 lot), the inter-operator reproducibility (3 replicates / 1 day / 3 operators / 1 lot), and the inter-lot reproducibility (3 replicates / 1 day / 1 operator / 3 lots). Five references were measured for each test: one negative, two weakly positive, and two moderately positive references. The RIDA®QUICK Norovirus test yielded the anticipated result in all measurements.

13.3 Cross-reactivity

Various pathogenic bacteria of the intestinal tract were tested with the RIDA®QUICK Norovirus test and exhibited no cross-reactivity. The tests were performed using bacteria suspensions (10^7 to 10^9 CFU/ml), parasite cultures (10^7 to 10^9 organisms/ml), cell-culture supernatants from virus-infected cells, and a stool specimen.

The results are summarized in the following table:

Test organism	Origin / Source	Result
Adenovirus	Cell-culture supernatant	Negative
Aeromonas hydrophila	Culture	Negative
Astrovirus	Cell-culture supernatant	Negative
Bacillus cereus	Culture	Negative
Bacteroides fragilis	Culture	Negative
Campylobacter coli	Culture	Negative
Campylobacter fetus	Culture	Negative
Campylobacter jejuni	Culture	Negative
Campylobacter lari	Culture	Negative
Campylobacter upsaliensis	Culture	Negative
Candida albicans	Culture	Negative
Citrobacter freundii	Culture	Negative
Clostridium difficile	Culture	Negative
Clostridium perfringens	Culture	Negative
Clostridium sordellii	Culture	Negative
Clostridium sporogenes	Culture	Negative
Cryptosporidium parvum	Culture	Negative
E. coli (O26:H-)	Culture	Negative
E. coli (O6)	Culture	Negative
E. coli (O157:H7)	Culture	Negative
Entamoeba histolytica	Culture	Negative
Enterobacter cloacae	Culture	Negative
Enterococcus faecalis	Culture	Negative
Giardia lamblia	Stool specimen	Negative
Klebsiella oxytoca	Culture	Negative
Proteus vulgaris	Culture	Negative
Pseudomonas aeruginosa	Culture	Negative
Rotavirus	Cell-culture supernatant	Negative
Salmonella enteritidis	Culture	Negative
Salmonella typhimurium	Culture	Negative
Serratia liquefaciens	Culture	Negative
Shigella flexneri	Culture	Negative
Staphylococcus aureus	Culture	Negative
Staphylococcus epidermidis	Culture	Negative
Vibrio parahaemolyticus	Culture	Negative
Yersinia enterocolitica	Culture	Negative

13.4 Interfering substances

The following substances exhibited no significant effect on the test results when mixed into norovirus-positive and -negative stool samples at the specified concentrations:

Loperamide	5 % w/w	Barium sulfate	5 % w/w
Pepto-Bismol	5 % v/w	Cyclamate	5 % v/w
Human blood	5 % v/w		
Stearic acid/palmitic acid (1:1 ratio)	40 % w/w	Metronidazole 0.5	5 % v/w
Mucin	5 % w/w	Diclofenac	0.00263 % v/w

14. Version history

Version number	Chapter and designation
2012-10-26	Previous version
2019-07-08	General revision 4. Reagents provided 7. Warnings and precautions for the users 8. Collection and storage of specimens 9.2 Preparing the specimens

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Cassette	Test cassette
Reagent | A	Reagent A
Reagent | B	Reagent B
Pipet	Disposable pipet
Reagent vial	Reaction vessel
Pipet Tip	Pipet tip
Microlit Pipet	Microliter pipette

16. References

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5. Corwin AL, et al. Shipboard impact of a probable Norwalk virus outbreak from coastal Japan. Am J Trop Med Hyg 1999; 61(6)898-903.
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