Instructions for Use

In-vitro-Diagnostic

IVD

The GastroVir1 *direct* RT-PCR is a real-time RT-qPCR test for simultaneous *in vitro* detection and differentiation of RNA from **Norovirus GI/GII** and **Rotavirus**.

Ref.No.:
FBC108

The GastroVir1 *direct* RT-PCR test was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g., MIC Cycler, AJ qTOWER, LightCycler® 96 or 480 I).

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1. Name of the Device

GastroVir1 *direct* RT-PCR

2. Intended Purpose

The GastroVir1 *direct* RT-PCR test is an assay for *in vitro* examination of viral RNA in stool samples to provide information to aid to diagnose patients under suspicion of gastrointestinal infection with Norovirus GI/GII and/or Rotavirus.

The IVD medical device detects the RNA of the aforementioned pathogens by qualitative measurements based on RT-qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in RT-qPCR and *in vitro* diagnostic techniques. It has to be used in combination with conventional nucleic acid extraction systems for RNA extraction, as well as qPCR cyclers for detection and analysis.

3. Pathogen Information

Noroviruses are non-enveloped, single-stranded RNA viruses in the family Caliciviridae [1]. Human caliciviruses include the genera Norovirus and Sapovirus. Noroviruses are classified into at least 10 genogroups (GI–GX), of which GI, GII, GIV, GVII, and GIX infect humans [1], [2]. These are further subdivided into multiple genotypes; GII.4 is the most common cause of global outbreaks [2].

Noroviruses infect individuals of all ages and are a leading cause of acute gastroenteritis worldwide. Transmission occurs via the fecal–oral route, contaminated food or water, direct contact, and aerosolized vomitus. The incubation period is 12–48 hours. Viral shedding may begin before symptom onset and persist for weeks after recovery, with peak infectivity during acute illness [3].

Rotaviruses are non-enveloped, double-stranded RNA viruses that belong to the family of Reoviridae. Structurally, they are composed of three layers: an outer capsid, an inner capsid, and a core shell that encloses the viral genome [4], [5]. The genome consists of 11 unique segments of double-stranded RNA, each encoding one or more viral proteins. Genetic variants of rotaviruses can arise through reassortment, a process wherein genome segments are exchanged among strains during co-infection [5].

Rotaviruses are classified into 11 serogroups (A-L), of which Group A is the primary cause of rotavirus-associated gastroenteritis in humans globally [4], [6]. The antigenicity of the virus is determined by two surface proteins (VP4 and VP7), which are also used to classify viruses within a serogroup into different serotypes according to a dual classification system [5]. The majority of rotavirus infections in Germany are caused by rotavirus types G1P[8] and G4P[8], followed by G2P[4] and G9P[8] [6].

Rotaviruses are highly stable in the environment, surviving for hours on hands, days to weeks on surfaces, and prolonged periods in water [7]. Transmission occurs primarily via the fecal–oral route, including contaminated surfaces, food, and water [7]. Children are the primary affected group, while adults are less frequently symptomatic. Infectiousness may begin before symptom onset. The incubation period is typically 1–3 days, and viral shedding can persist for 1–3 weeks following vaccination [8], [9].

4. Testing Principle

The GastroVir1 *direct* RT-PCR is a multiplex RT-qPCR test for the detection and differentiation of Norovirus GI/GII and Rotavirus.

A separate Full Process Run Control (Internal Control; IC) is added to each sample prior to RNA extraction and serves as control for nucleic acid isolation from the biological specimen as well as for amplification.

The nucleic acid eluate is added to the ready-to-use reaction solution that contains all reagents necessary for RT-qPCR. The analysis can be performed on a qPCR cycler.

The GastroVir1 *direct* RT-PCR test contains primers and probes specific for the target pathogens as well as the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

The analysis is performed by determining Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies are present in the sample, the lower the Ct value.

Table 1: Target genes of GastroVir1 direct RT-PCR test

ANALYTE	GENE	REGION (NUCLEOTIDES)
Norovirus GI*	ORF1&2	1 - 6950
Norovirus GII*	ORF1&2	1 - 6750
Rotavirus A	NSP5	1 - 600

*Norovirus GI and GII are detected in the same channel and can therefore not be differentiated.

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 2).

Table 2: Package content of GastroVir1 direct RT-PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT	ACTIVE INGREDIENT(S)
Solution A	green	1x; 1050 µL	96	Reaction mix	Reverse transcriptase (approx. 2-fold); Polymerase (approx. 0.2 U/µl); Primers and probes (approx. 50-400 nM)
Internal Control	blue	1x; 400 µL	96	Artificial nucleic acid target	Mix of RNA (approx. 10^7 copies/µl) and plasmid DNA (approx. 1 pg/µl)
Positive Control	red	1x; 50 µL	4	RNA of target analytes	RNA with target regions of Norovirus GI, Norovirus GII and Rotavirus A (approx. 400 copies /µl)

6. Configurations

The GastroVir1 *direct* RT-PCR is available in one configuration, which is suitable for use on a RT-qPCR instrument with at least 3 detection channels (FAM/HEX/Red610).

7. Additional Equipment and Reagents (not provided)

- RT-qPCR cycler (with at least 3 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Sterile filter-tips for PCR testing (DNA/RNA-free)
- Table centrifuge
- RNA isolation kit (IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 by PerkinElmer chemagen Technologie GmbH, MagNA Pure 96 DNA and Viral NA Small Volume Kit by Roche Diagnostics International AG, or similar device)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

8. Transport, Storage and Stability

The GastroVir1 *direct* RT-PCR test is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. All reagents must be kept at +2 °C to +8 °C during procedure steps. Exposure to light should be avoided. You may thaw and refreeze the kit components up to 6 times. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

The GastroVir1 *direct* RT-PCR test is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases and RNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering a different area.

- The test kits are intended for single use and must not be reused after performing RT-qPCR reaction.If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated, and a repeat run should be considered.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for RT-PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- The instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- The sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

11. Important Points before Starting

This test is intended for use with samples derived from stool. Other specimen types may cause invalid results.

In every RT-qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of Norovirus GI, Norovirus GII and Rotavirus A. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the RT-qPCR run and the results must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. RNA extraction reagents are not part of the GastroVir1 *direct* RT-PCR test. Performance evaluation studies have been conducted using the IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH) and the MagNA Pure 96 DNA and Viral NA Small Volume Kit on a MagNA Pure 96 instrument (Roche Diagnostics International AG) for RNA isolation.

12. Test Procedure

All components are stored at -25 °C to -18 °C in the dark. All reagents must be kept at +2 °C to +8 °C during procedure steps.

Table 3: Test procedure of GastroVir1 direct RT-PCR

TEST PROCEDURE	
	Sample preparation
1	Thaw Internal Control only.
2	Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer; add one additional water sample and use as Negative Control).
3	Put the Internal Control back in the kit box in the deep freezer.
4	Perform RNA preparation according to your laboratory's standard procedure.
	RT-qPCR
5	Thaw Solution A and Positive Control only.
6	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes. (If IC is not added to sample during extraction, 0.1 µl IC/sample could be added to Solution A).
7	Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
8	Put Solution A and the Positive Control back in the kit box in the deep freezer.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the qPCR cycler.
12	Start program.

13. Instrument Settings

The following thermal profile is to be used (see Table 4). Channel settings depend on the RT-qPCR cycler used and should be checked before starting the analysis (see Table 5). FBC108 is suitable for RT-qPCR cyclers with at least the three channels: FAM, HEX, and Red 610. Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

*Table 4: Instrument Settings of GastroVir1 direct RT-PCR**

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	15 sec	on	
	72	15 sec	-	

* If required, the amplification step can be adapted to a two-step cycling protocol by combining the two 60°C/15sec and 72°C/15sec steps into a single 60°C/30sec step.

Table 5: Channel settings of GastroVir1 direct RT-PCR (Ref.No.: FBC108)

	Norovirus GI/GII	**Rotavirus**	**Internal Control (IC)**
	ORF1&2	NSP5	artificial NA
Reporter dye	FAM	HEX	Red 610
Colour	green	yellow-green	orange
Emission [nm]	520	560	610
Quencher	Black Hole Quencher		

The GastroVir1 *direct* RT-PCR test was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g., MIC Cycler, AJ qTOWER, LightCycler® 96 or 480 I).

14. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1). A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, are considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

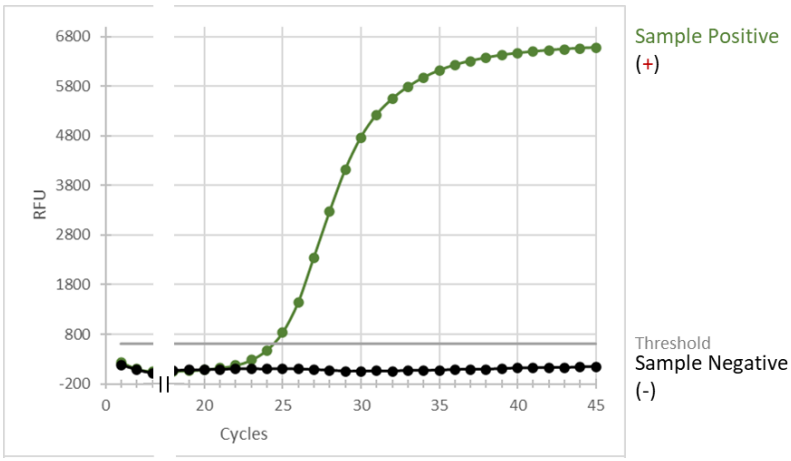

Figure 1: Exemplary amplification curves

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all target channels. If the IC is directly included in Solution A, an amplification curve must also be observed in the IC channel of the Positive Control. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC should show a positive amplification curve in both positive and negative samples.

For distribution of results within the channels see Table 6.

Table 6: Results of GastroVir1 direct RT-PCR (Ref. No.: FBC108)

Norovirus GI/GII	Rotavirus	Internal Control (IC)		
ORF1&2	NSP5	artificial NA		
FAM	HEX	Red 610	**Result**	**Interpretation**
+	–	+	Valid	Norovirus GI/GII detected.
–	+	+	Valid	Rotavirus detected.
+	+	+	Valid	Norovirus GI/GII and Rotavirus detected.
–	–	+	Valid	No Norovirus GI/GII or Rotavirus detected.
+/–	+/–	–	Invalid	The test result cannot be evaluated.

15. Limitations of the Method

The results support the differential diagnosis of infections with Norovirus GI/GII and/or Rotavirus. The viral RNA is generally detectable in stool samples during the acute phase of the infection. Positive results indicate the presence of the pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of Norovirus GI/GII or Rotavirus as results depend on correct sampling, the absence of inhibitors and sufficient RNA to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, transcription and/or amplification or detection of the target nucleic acids.

Test results should always be seen in the context of the clinical findings and should not be used as the only basis for diagnosis, treatment, or other patient management decisions. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings by an appropriate healthcare professional.

The detection of analyte target does not mean that they are the causative agents of clinical symptoms.

The GastroVir1 *direct* RT-PCR detects the presence of the respective nucleic acids but does not differentiate between viable and non-viable pathogens. Therefore, positive results may not necessarily indicate the presence of an active infection and should be interpreted in the context of all available clinical information.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

16. Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of target-specific RNA. The analytes were tested in 20 replicates per concentration on a LightCycler® 480 II (Roche Diagnostics International AG). The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 9.3.1 software (see Table 7).

Table 7: Limit of Detection

ANALYTE	LOD (COPIES/REACTION)	UPPER LIMIT	LOWER LIMIT
Norovirus GI	368.2	753.2	151.4
Norovirus GII	28.9	81.9	9.2
Rotavirus A	16.5	38.8	5.5

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) were performed and demonstrated no impact on assay performance, with all test acceptance criteria met.

17. Diagnostic Performance

In total, 175 stool samples were analysed in an independent medical laboratory in January 2026. Thereof, 152 were clinical samples pre-analyzed by RT-qPCR and 23 were spiked samples due to the limited number of clinical samples that could be acquired in the study period. Diluted stool samples were extracted on a MagNA Pure 96 Instrument using the DNA and viral NA small volume kit (Roche Diagnostics International AG). Subsequently, samples were analysed in parallel with the GastroVir1 *direct* RT-PCR test and a CE certified test from another manufacturer in direct comparison on a LightCycler® 480 II (Roche Diagnostics International AG). In the event of a discrepancy between the results, another CE certified test was used to resolve the discrepancy

Diagnostic sensitivity and specificity are summarized in the following:

Norovirus samples

		positive	negative	
GastroVir1	positive	49	0	Sensitivity: 98.0% (89.4 - 100.0)
	negative	1	75	Specificity: 100.0% (95.2 - 100.0)

Rotavirus samples

		positive	negative	
GastroVir1	positive	49	0	Sensitivity: 100.0% (92.8 - 100.0)
	negative	0	75	Specificity: 100.0% (95.2 - 100.0)

The diagnostic sensitivity of the GastroVir1 *direct* RT-PCR test is dependent on the RNA extraction method used to isolate RNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used for RNA isolation from biological samples.

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for in vitro diagnostic medical devices.

18. Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	--
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

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19. Technical Assistance and Contact Information

For technical assistance, product support, or questions related to the performance or interpretation of this test, users may contact the manufacturer directly.

Manufacturer:	FRIZ Biochem GmbH
Address:	Floriansbogen 2-4 / 82061 Neuried / Germany
Telephone:	+49 (0) 89 72 44 09 0
Email (Technical Assistance):	support@frizbiochem.de
Email (General Enquiry):	info@frizbiochem.de
Website:	www.frizbiochem.de

Technical support is available during regular business hours, and all inquiries will be handled by qualified personnel. Users are encouraged to contact the manufacturer immediately in the event of suspected malfunction, unexpected results, or any issue requiring expert clarification.

20. References

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