Instruction for Use

gastroplexVirus PLUS 2.0

real time RT-PCR Kit

For the qualitative *in-vitro* detection of RNA from Rotavirus, Norovirus GI and Norovirus GII, Sapovirus and Astrovirus and DNA from Adenovirus in clinical specimens, environmental and food samples.

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Σ	32	96

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1 Intended Use

The gastroplexVirus PLUS 2.0 real time RT-PCR Kit is an assay for the detection of the RNA of Rotavirus, Norovirus GI, Norovirus GII, Sapovirus and Astrovirus and the DNA of Adenovirus in clinical specimens (e.g. stool samples, vomit), environmental and food samples using real time PCR microplate systems.

2 Pathogen Information

Acute gastroenteritis is a worldwide major cause of morbidity and mortality. Gastroenteritis or infectious diarrhoea is an inflammation of the gastrointestinal tract. Both the stomach and the small intestine are involved. Typical symptoms are diarrhoea, vomiting, abdominal pain, and cramps, often followed by dehydration.
The causative agent can be viral or bacterial. Enteric viruses are the major pathogens for gastroenteritis especially in children. Noro-, Rota-, Adeno-, Sapo- and Astroviruses are the most important viral pathogens.

Noroviruses are small non-enveloped RNA viruses belonging to the family of Caliciviridae. They cause approximately 90 % of epidemic non-bacterial outbreaks of gastroenteritis around the world.
The viruses are transmitted by fecally contaminated food or water and by person-to-person contact. For this reason, outbreaks of norovirus infection often occur in closed or semi-closed communities, such as long-term care facilities, hospitals, prisons, dormitories, and cruise ships. Noroviruses are highly contagious and are stable at temperatures between -20°C to +60°C and in acidic environments up to pH 3. Norovirus infections occur throughout the year, however, in Europe, seasonal increases are observed between October and March.
The gastroplexVirus PLUS 2.0 real time RT-PCR detects Norovirus strains of high genetic diversity separately, such as the following:
GI: Norwalk, Desert Shield, Winchester, Queensarms, Southhampton, Chiba
GII: Lordsdale, Bristol, Melksham, Toronto, Hawaii

Infections with **Rotavirus** are the most common cause of severe diarrhoea among children. Worldwide more than 450,000 children under 5 years of age die from rotavirus infections each year. Most of them in developing countries.
The double-stranded RNA virus of the family Reoviridae is transmitted faecal-orally and infects the enterocytes. It causes diarrhoea, vomiting, fever, and dehydration, seldomly abdominal pain. Sometimes infections of the upper

respiratory tract occur in correlation with gastroenteritis. With each infection immunity develops, so subsequent infections are less severe. By the age of 5, nearly every child in the world has at least once gone through a rotavirus infection.
Rotaviruses are classified into the groups A-G, among which A-C are human pathogenic. More than 90% of rotavirus infections are caused by group A viruses.

Adenoviruses mainly cause infections of the respiratory system. However, dependent on the serotype, numerous other diseases can be caused, such as gastroenteritis, keratoconjunctivitis epidemica, cystitis, rhinitis, pharyngitis, and diarrhoea. Respiratory symptoms range from mild flu to acute bronchitis and pneumonia. Immuno-suppressed patients are prone to severe complications, such as acute respiratory distress syndrome. Although the epidemiological characteristics of Adenoviruses vary from type to type, all types are transmitted by direct contact, feacal-orally, and rarely by water. Some types cause persistent, asymptomatic infections of the palatine and pharyngeal tonsils, and the gastrointestinal tract. Spreading of the virus can occur over months or years.

Astroviruses are single stranded RNA (ssRNA) Viruses belonging to the family of Astroviridae. Diarrhoea is the most prevalent symptom of an Astrovirus-associated gastroenteritis, but also concomitant symptoms like vomiting and fever are described. In industrial countries, the incidence is 2-9%, especially in young children of under 2 years. Most relevant are the serotypes 1-5 of 8 serotypes known to date. The infection occurs by contaminated food and water or through the fecal-oral pathway.

Sapoviruses belong to the family of **Caliciviridae**. Along with Noroviruses, Sapoviruses are the most common pathogens causing gastroenteritis worldwide. Although the highest incidence of Sapovirus infections is in young children under 5 years old, Sapovirus-associated gastroenteritis also occurs in adults. Clinical symptoms are similar to Norovirus infections like diarrhoea, vomiting, and fever, but the symptoms are milder.
To date less epidemiological studies are available and due to less sensitive diagnostic methods Sapoviruses were seldomly diagnosed.

3 Principle of the Test

The gastroplexVirus PLUS 2.0 real time RT-PCR contains one vial (Reaction Mix 1) with specific primers and hydrolysis probes for the detection of the nucleic acids of Rotavirus and Norovirus GI and GII in clinical specimens (e.g. stool samples, vomit), environmental and food samples. The reverse transcription (RT) of viral RNA to cDNA and the subsequent amplification of virus specific fragments are performed in a one-step RT-PCR. The amplification can be detected when specific probes are hydrolysed by the Polymerase. The emitted fluorescence is measured in the FAM (*Norovirus GI*), Cy5 (*Norovirus GII*) and ROX (*Rotavirus*) channel.

The gastroplexVirus PLUS 2.0 real time RT-PCR contains a second vial (Reaction Mix 2) with specific primers and hydrolysis probes for the detection of the RNA of *Sapovirus and Astrovirus* and the DNA of *Adenovirus* in clinical specimens (e.g. stool samples, vomit), environmental and food samples after the extraction of RNA and DNA from the sample material. The reverse transcription (RT) of viral RNA to cDNA and the subsequent amplification of virus specific fragments are performed in a one-step RT-PCR. The amplification can be detected when specific probes are hydrolysed by the Polymerase. The emitted fluorescence is measured in the FAM (*Sapovirus*), ROX (*Astrovirus*) and Cy5 (Adenovirus) channel.

Furthermore, the gastroplexVirus PLUS 2.0 real time RT-PCR contains a Control RNA, which is detected in a second amplification system. Added during the extraction, the Control RNA allows not only for the detection of RT-PCR inhibition but also detects possible mistakes during extraction. This greatly reduces the risk of false-negative results. The Control RNA can also be used solely as Internal Control by adding it directly to the Mastermix. The fluorescence of the Control RNA is measured in the VIC®/HEX/JOE™/TET channel.

4 Package Contents

The reagents supplied are sufficient for 32 or 96 reactions respectively.

Table 1: Components of the gastroplexVirus PLUS 2.0 real time RT-PCR Kit.

Label	Lid Colour	Content 32	Content 96
Reaction Mix 1 (Rotavirus, Norovirus)	yellow	1 x 506 µl	2 x 759 µl
Reaction Mix 2 (Sapovirus, Astrovirus, Adenovirus)	orange	1 x 506 µl	2 x 759 µl
Enzyme	blue	1 x 12.8 µl	2 x 19.2 µl
Positive Control 1 (Rotavirus, Norovirus)	red	1 x 50 µl	1 x 100 µl
Positive Control 2 (Sapovirus, Astrovirus, Adenovirus)	violett	1 x 50 µl	1 x 100 µl
Negative Control	green	1 x 100 µl	1 x 200 µl
Control RNA	colourless	1 x 320 µl	2 x 480 µl

5 Equipment and Reagents to be Supplied by User

- DNA/RNA isolation kit (e.g. NukEx Pure RNA/DNA, gerbion Cat. No. G05004 or NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation
- Optional: VLP-RNA (gerbion Cat. No. G07008, please look at page 8 for details)

6 Transport, Storage and Stability

The gastroplexVirus PLUS 2.0 real time RT-PCR Kit is shipped on dry ice or cool packs.

All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible.

For convenience, opened reagents can be stored at +2-8°C for up to 6 months.

Protect kit components from direct sunlight during the complete test run.

7 Important Notes

- The gastroplexVirus PLUS 2.0 real time RT-PCR must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Clinical samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

8 General Precautions

- Stick to the protocol described in the Instruction for Use.
- Set up different laboratory areas for the preparation of samples and for the set up of the RT-PCR in order to avoid contaminations.
- Pipettes, tubes and other materials must not circulate between those different laboratory areas.
- Always use filter tips.
- Regulary decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine gastroplexVirus PLUS 2.0 real time RT-PCR Kit components of different lot numbers.

9 Sample Material

Starting material for the gastroplexVirus PLUS 2.0 real time RT-PCR is the nucleic acid isolated from clinical specimens (e.g. stool samples, vomit), environmental or food samples.

10 Sample Preparation

The gastroplexVirus PLUS 2.0 real time RT-PCR is suitable for the detection of *Rotavirus, Adenovirus, Norovirus GI and Norovirus GII, Sapovirus, and Astrovirus* in clinical specimens (e.g. stool samples, vomit), environmental and food samples isolated with suitable isolation methods.

Commercial kits for RNA isolation such as the following are recommended:

- NukEx Pure RNA/DNA, gerbion Cat. No. G05004
- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Important: In addition to the samples always run a "water control" in your extraction. Treat this water control analogous to a sample.
Comparing the amplification of the Control RNA in the sample to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time RT-PCR. Furthermore, possible contaminations during RNA extraction will be detectable.

Please note the chapter ‚Control RNA'.

If the real time RT-PCR is not performed immediately, store extracted RNA according to the instructions given by the RNA extraction kit's manufacturer.

11 Control RNA

A Control RNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the RNA isolation procedure and to check for possible real time RT-PCR inhibition.
The Virus-Like Particles (VLP-RNA) are not supplied, but must be added to the clinical or environmental samples directly. VLP-RNA can be used as patient-side extraction control and in automated extraction systems, when pipetting of the Control RNA to the first buffer of (e.g. binding buffer) of the respective extraction kit is not possible due to extraction instrument specifications.

RNA isolation from clinical specimens (e.g. stool samples, vomit), environmental and food samples

a) Control RNA or VLP-RNA used as Extraction Control:

gastroplexVirus PLUS 2.0 real time RT-PCR Control RNA or VLP-RNA is added to the extraction.
Add 5 µl Control RNA or VLP-RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions. Please follow protocol A.

The Control RNA must be added to the Lysis Buffer of the extraction kit.

b) Control RNA used as Internal Control of the real time PCR:
If only inhibition will be checked please follow protocol B.

12 Real time RT-PCR

12.1 Important Points Before Starting:

- Please pay attention to the to the chapter 7 ‚Important Notes'.
- Before setting up the real time RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every RT-PCR run one Positive Control (1 or 2) for the respective Reaction Mix 1 or 2 and one Negative Control should be included.
- Before each use, all reagents - except the Enzyme - should be thawed completely at room temperature, thouroughly mixed, and centrifuged very briefly.

12.2 Procedure

If the Control RNA or VLP-RNA is used to control both, the real time RT-PCR and the RNA isolation procedure, please follow protocol A. If the Control RNA is solely used to detect possible inhibition of the real time RT-PCR, please follow protocol B.

Protocol A

The Control RNA or VLP-RNA was added during RNA extraction (see chapter ‚Control RNA'). In this case, prepare the Master Mix according to Table 2.
The Master Mix contains all of the components needed for RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix 1 or 2
15.8 µl Reaction Mix 1 or 2	15.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Protocol B

The Control RNA is used for the control of the real time RT-PCR only (see chapter ‚Control RNA'). In this case, prepare the Master Mix according to Table 3.

The Master Mix contains all of the components needed for real RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Important: Dilute the Control RNA 1:10 in PCR-grade dH_2O (e.g. 1 µl Control RNA + 9 µl PCR grade Water before adding it to the Master Mix.

Table 3: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix 1 or 2
15.8 µl Reaction Mix 1 or 2	15.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)
0.2 µl Control RNA* **(diluted 1:10)**	0.2 µl x (N+1)*

*The increase in volume caused by adding the Control RNA is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Protocol A and B: real time RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument.
- Pipet **16 µl** of each Master Mix (1 and 2) into two separate optical PCR reaction tubes.
- Add **4 µl** of the eluates from the RNA/DNA isolation (including the eluate of the water control), the respective Positive Control, and the Negative Control to the corresponding optical PCR reaction tubes (Table 4).

- Close the optical PCR reaction tubes immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time PCR

Component	Volume
Master Mix	16.0 µl
Sample	4.0 µl
Total Volume	20.0 µl

12.3 Instrument Settings

For the real time RT-PCR use the thermal profile shown in Table 5.

Table 5: real time RT-PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of cDNA			45
Denaturation	10 sec	95°C	
Annealing and Extension	40 sec Aquisition at the end of this step	60°C	

Dependent on the real time instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the gastroplexVirus PLUS 2.0 real time RT-PCR Kit.

Real time PCR Instrument	Parameter Reaction Mix 1	Parameter Reaction Mix 2	Detection Channel	Notes		
				Colour Compensation required		
				Melt Factor	Quant Factor	Max Integration Time (sec)
LightCycler 480II	Norovirus GI	Sapovirus	465-510	1	10	1
	Rotavirus	Astrovirus	533-610	1	10	2
	Control RNA	Control RNA	533-580	1	10	2
	Norovirus GII	Adenovirus	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	Norovirus GI	Sapovirus	FAM	Gain 8		
	Rotavirus	Astrovirus	ROX	Gain 1		Reference Dye: None
	Control RNA	Control RNA	HEX	Gain 1		
	Norovirus GII	Adenovirus	Cy5	Gain 4		
ABI 7500	Norovirus GI	Sapovirus	FAM	Option Reference Dye ROX: NO		
	Rotavirus	Astrovirus	ROX			
	Control RNA	Control RNA	JOE			
	Norovirus GII	Adenovirus	Cy5			
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	Norovirus GI	Sapovirus	Green	Gain 5		
	Rotavirus	Astrovirus	Orange	Gain 5		
	Control RNA	Control RNA	Yellow	Gain 5		
	Norovirus GII	Adenovirus	Red	Gain 5		

13 Data Analysis

The virus specific amplifications are measured in the FAM, ROX, Cy5 channels. The amplification of the Control RNA is measured in the VIC®/HEX/JOE™/TET channel. The Positive Control contains in vitro transcripts of the respective nucleic acid sequences of Norovirus GI, Norovirus GII, Rotavirus, Sapovirus and Astrovirus and DNA of Adenovirus. For the Positive Control, signals in the FAM, ROX, Cy5 channels must be detected. The interpretation of the test results is described in table 7 and table 8.

Table 7: Interpretation **Reaction Mix 1**

Signal/Ct Values				Interpretation
FAM Channel Norovirus GI	**ROX Channel** Rotavirus	**Cy5 Channel** Norovirus GII	**HEX Channel** Control RNA	
positive	negative	negative	positive or negative*	**Positive result, the sample contains Norovirus GI-RNA.**
negative	**positive**	negative	positive or negative*	**Positive result, the sample contains Rotavirus-RNA.**
negative	negative	**positive**	positive or negative*	**Positive result, the sample contains Norovirus GII-RNA.**
negative	negative	negative	≤ 34**	**Negative result, the sample contains no Norovirus GI-RNA, Norovirus GII-RNA and Rotavirus-RNA.**
negative	negative	negative	negative or > 34**	**No diagnostic statement can be made.** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.

Table 8: Interpretation **Reaction Mix 2**

Signal/Ct Values				Interpretation
FAM Channel Sapovirus	**ROX Channel** Astrovirus	**Cy5 Channel** Adenovirus	**HEX Channel** Control RNA	
positive	negative	negative	positive or negative*	**Positive result, the sample contains Sapovirus-RNA.**
negative	**positive**	negative	positive or negative*	**Positive result, the sample contains Astrovirus-RNA.**
negative	negative	**positive**	positive or negative*	**Positive result, the sample contains Adenovirus-DNA.**
negative	negative	negative	≤ 34**	**Negative result, the sample contains no Sapovirus-RNA, Astrovirus-RNA and Adenovirus-DNA.**
negative	negative	negative	negative or > 34**	**No diagnostic statement can be made.** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.

* A strong positive signal in the FAM, Cy5 and/or ROX can inhibit the IC. In such cases the result for the Control RNA can be neglegted.

** Depending on the PCR instrument and/or the chosen extraction method, the Ct values might be shifted. The water control can be used as reference. If the HEX Ct value of a sample differs a lot from the water control, partial inhibition has occurred, leading to false negative results in case of weak positive samples.

Figure 1 and **Figure 2** show examples for positive and negative real time RT-PCR results.

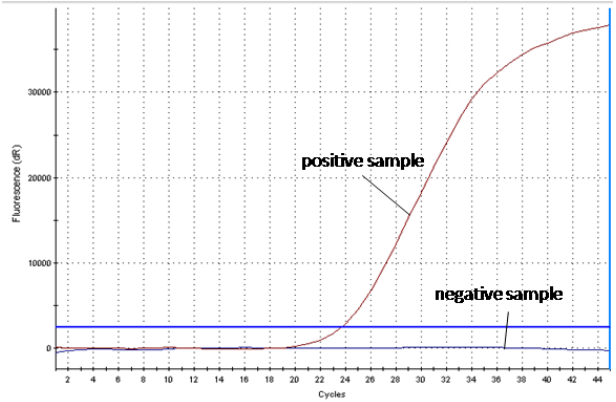

Figure 1: The positive sample shows virus-specific amplification, whereas no fluorescence signal is detected in the negative sample.

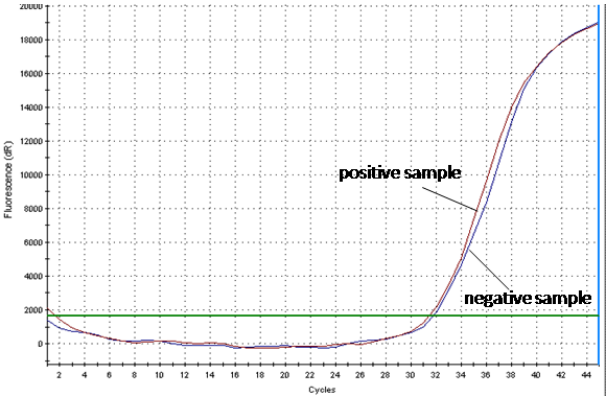

Figure 2: The positive sample as well as the negative sample show a signal in the Control RNA specific VIC®/HEX/JOE/TET channel. The amplification signal of the Control RNA in the negative sample shows, that the missing signal in the virus specific channels is not due to RT-PCR inhibition or failure of RNA isolation, but that the sample is a true negative.

14 Assay Validation

Set a threshold as follows:

Negative Controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high C_T – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

Positive Controls

All the positive controls must show a positive (i.e. exponential) amplification curve. The positive controls must fall below a C_T of 30.

Internal Controls

All internal controls must show a positive (i.e. exponential) amplification curve. The internal control must fall below a C_T of 34. If the internal control is above C_T 34, this points to a purification problem or a strong positive sample that can inhibit the IC. In the latter case, the assay is valid. If a water control run is performed, the IC must fall below a C_T of 33.

15 Limitations of the Method

The results must always be considered in relation to the clinical symptoms. Therapeutical consequences should be made in consideration of clinical data.
A negative test result does not exclude a gastroenteric virus infection.

16 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time RT-PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM, ROX, Cy 5 channel of the Positive Controls	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of the Norovirus GI specific amplification, the ROX channel for analysis of the Rotavirus specific amplification and the Cy5 channel for analysis of the Norovirus GII specific amplification (Reaction Mix 1). Select the FAM channel for analysis of the Sapovirus specific amplification, the ROX channel for analysis of the Astrovirus

	specific amplification and the Cy5 channel for analysis of the Adenovirus specific amplification (Reaction Mix 2). Select the VIC®/HEX/JOE™/TET channel for the amplification of the Control RNA. Due to amplification in the specific channels, amplification of the Internal Control can be inhibited in the Positive Controls 1 and 2.
Incorrect configuration of the real time RT-PCR	Check your work steps and compare with ‚Procedure' on page 9.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol (Table 5, page 11).
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability', page 7.
Weak or no signal of the Control RNA and simultaneous absence of a signal in the virus specific FAM channel, ROX channel or Cy5 channel.	
real time RT-PCR conditions do not comply with the protocol	Check the real time RT-PCR conditions (page 8).
real time RT-PCR inhibited	Make sure that you use an appropriate isolation method (see ‚Sample Preparation', page 8) and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed. An additional centrifugation step at high speed is recommended before elution of the RNA/DNA.
RNA/DNA loss during isolation process	In case the Control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA/DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability', page 7.
Detection of a fluorescence signal in the FAM channel, ROX channel or Cy5 channel of the Negative Control.	
Contamination during preparation of the RT-PCR	Repeat the real time RT-PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time RT-PCR.

17 Kit Performance

17.1 Analytical Sensitivity

The limit of detection (LoD) of gastroplexVirus PLUS 2.0 real time RT-PCR was determined using serial dilutions of in vitro transcripts (Norovirus GI, Norovirus GII, Rotavirus, Astrovirus, Sapovirus) and synthetic target sequences for Adenovirus in a Stratagene Mx3000 real time PCR instrument.

Table 9: Samples tested for the validation of the sensitivity of gastroplexVirus PLUS 2.0 real time RT-PCR.

Norovirus GI	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

Norovirus GII	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

Rotavirus	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

Sapovirus	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

Astrovirus	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

Adenovirus	Copies per Reaction	Expected Result	gastroplexVirus PLUS 2.0
	1.000.000	positive	positive
	100.000	positive	positive
	10.000	positive	positive
	1.000	positive	positive
	100	positive	positive
	10	positive	positive
	1	positive	positive/negative

17.2 Analytical Specificity

The specificity of the gastroplexVirus PLUS 2.0 real time RT-PCR was evaluated by in silico analysis and by amplification of RNA and DNA of other relevant viruses and bacteria found in clinical samples.

The gastroplexVirus PLUS 2.0 real time RT-PCR showed positive results for the samples containing Norovirus GI, Norovirus GII, Rotavirus, Sapovirus, Astrovirus and Adenovirus, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 9.

Table 10: Bacterial and viral pathogens tested for the determination of the analytical specificity of gastroplexVirus PLUS 2.0 real time RT-PCR.

Pathogen	Expected Result	Result
Enterovirus 68	negative	negative
Coxsackievirus B3	negative	negative
Coxsackievirus A16	negative	negative
Coxsackievirus B5	negative	negative
Salmonella	negative	negative
Listeria monocytogenes	negative	negative
Escherichia coli	negative	negative
Campylobacter	negative	negative
Shigella	negative	negative
Yersinia	negative	negative

Norovirus GI	positive	positive
Norovirus GII	positive	positive
Rotavirus	positive	positive
Adenovirus	positive	positive
Sapovirus	positive	positive
Astrovirus	positive	positive

18 Abbreviations and Symbols

cDNA	complementary Deoxyribonucleid Acid	REF	Catalog number
RNA	Ribonucleid Acid	Σ	Contains sufficient for <n> test
PCR	Polymerase Chain Reaction	-18°C	Upper limit of temperature
RT	Reverse Transcription		Manufacturer
REACTION MIX 1	Reaction Mix 1		Use by YYYY-MM
REACTION MIX 2	Reaction Mix 2	LOT	Batch code
ENZYME	Enzyme	CONT	Content
CONTROL 1 +	Positive Control 1		Consult instructions for use
CONTROL 2 +	Positive Control 2	IVD	*In vitro* diagnostic medical device
CONTROL −	Negative Control		
CONTROL RNA IC	Control RNA		

19 Literature

[1] Lothar Thomas, Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik, 8. Auflage, 2012, TH-Books, ISBN-10: 3980521583