Arbeitsanleitung/Manual

MutaPLEX®
GastroSys 3
real time RT-PCR kit

Test für den in-vitro-Nachweis der RNA von Rotavirus, Norovirus GI und GII, Sapovirus und Astrovirus sowie der DNA von Adenovirus in klinischen, Umwelt- und Nahrungsmittelproben

Test for the in vitro detection of RNA of rotavirus, norovirus GI and GII, sapovirus, and astrovirus as well as DNA of adenovirus in clinical specimens, environmental and food samples

Gültig ab / Valid from 2018-05-28

KG198832
KG198896

32/96

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1 INTENDED USE

The MutaPLEX® GastroSys 3 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 MutaPLEX® GastroSys 3 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. Immunosuppressed 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 MutaPLEX® GastroSys 3 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 MutaPLEX® GastroSys 3 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 MutaPLEX® GastroSys 3 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 (KG198832) or 96 (KG198896) reactions, respectively.

Table 1: Components of the MutaPLEX® GastroSys 3 real time RT-PCR kit .

Label	Lid Colour	Content	
		32	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)	violet	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

- Nucleic acid isolation kit (e. g. MutaCLEAN® Universal RNA/DNA, KG1038)
- PCR grade water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortex
- Real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation
- Optional: VLP-RNA (virus-like particles, please see chapter 11 for details).

* Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® GastroSys 3 real time RT-PCR kit is shipped on dry ice or cool packs. All components must be stored at maximum -20 °C in the dark immediately after receipt. Up to 20 freeze and thaw cycles are possible. Do not use reagents after the date of expiry printed on the package.

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 MutaPLEX® GastroSys 3 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 instructions 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 MutaPLEX® GastroSys 3 real time RT-PCR kit components of different lot numbers.

9 SAMPLE MATERIAL

Starting material for the MutaPLEX® GastroSys 3 real time RT-PCR is nucleic acid isolated from clinical specimens (e. g. stool samples, vomit), environmental or food samples.

10 SAMPLE PREPARATION

The MutaPLEX® GastroSys 3 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 MutaCLEAN® Universal RNA/DNA (KG1038) are recommended.

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 samples 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 nucleic acid extraction will be detectable.

Please note chapter 11 "Control RNA".

If the real time RT-PCR is not performed immediately, store extracted nucleic acids according to the instructions given by the 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, KG7015) 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

MutaPLEX® GastroSys 3 control RNA or VLP-RNA is added to the RNA 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 RT-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 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 RT-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.
- We recommend to keep reagents and samples at 2–8 °C (e. g. on ice or a cooling block) at all times.

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 11 "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 (control RNA was added during RNA extraction)

Volume per reaction	Volume master mix
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 11 "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.

Table 3: Preparation of the master mix (control RNA is added directly to the master mix)

Volume per reaction	Volume master mix
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 RT-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	No of cycles
Reverse Transcription	10 min	45 °C	1
Initial Denaturation	5 min	95 °C	1
Amplification of cDNA			45
Denaturation	10 s	95 °C	
Annealing and extension	40 s	60 °C	
	Aquisition at the end of this step		

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 MutaPLEX® GastroSys 3 real time RT-PCR.

<table>
<tr><th rowspan="2">Real time RT-PCR Instrument</th><th colspan="2">Parameter</th><th rowspan="2">Detection Channel</th><th rowspan="2" colspan="3">Notes</th></tr>
<tr><th>Reaction mix 1</th><th>Reaction mix 2</th></tr>
<tr><td rowspan="5">LightCycler 480II</td><td rowspan="5">Norovirus GI
Rotavirus
Control RNA
Norovirus GII</td><td rowspan="5">Sapovirus
Astrovirus
Control RNA
Adenovirus</td><td rowspan="5">465–510
533–610
533–580
618–660</td><td colspan="3">Colour compensation kit required</td></tr>
<tr><td>Melt factor</td><td>Quant factor</td><td>Max integration time (s)</td></tr>
<tr><td>1</td><td>10</td><td>1</td></tr>
<tr><td>1</td><td>10</td><td>2</td></tr>
<tr><td>1</td><td>10</td><td>2</td></tr>
<tr><td>1</td><td>10</td><td>3</td></tr>
<tr><td>Stratagene Mx3000P/ Mx3005P</td><td>Norovirus GI
Rotavirus
Control RNA
Norovirus GII</td><td>Sapovirus
Astrovirus
Control RNA
Adenovirus</td><td>FAM
ROX
HEX
Cy5</td><td colspan="2">Gain 8
Gain 1
Gain 1
Gain 4</td><td>Reference Dye:
None</td></tr>
<tr><td>ABI 7500</td><td>Norovirus GI
Rotavirus
Control RNA
Norovirus GII</td><td>Sapovirus
Astrovirus
Control RNA
Adenovirus</td><td>FAM
ROX
JOE
Cy5</td><td colspan="3">Option Reference Dye ROX: NO</td></tr>
<tr><td>Rotor-Gene Q,
Rotor-Gene 3000
Rotor-Gene 6000</td><td>Norovirus GI
Rotavirus
Control RNA
Norovirus GII</td><td>Sapovirus
Astrovirus
Control RNA
Adenovirus</td><td>Green
Orange
Yellow
Red</td><td colspan="3">Gain 5
Gain 5
Gain 5
Gain 5</td></tr>
</table>

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	**ROX channel**	**Cy5 channel**	**HEX channel**	
Norovirus GI	Rotavirus	Norovirus GII	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, norovirus GII or 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	**ROX channel**	**Cy5 channel**	**HEX channel**	
Sapovirus	Astrovirus	Adenovirus	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 neither sapovirus or astrovirus RNA nor adenovirus DNA.

Signal/Ct Values				Interpretation
FAM channel	**ROX channel**	**Cy5 channel**	**HEX channel**	
Sapovirus	Astrovirus	Adenovirus	Control 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.

* 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.

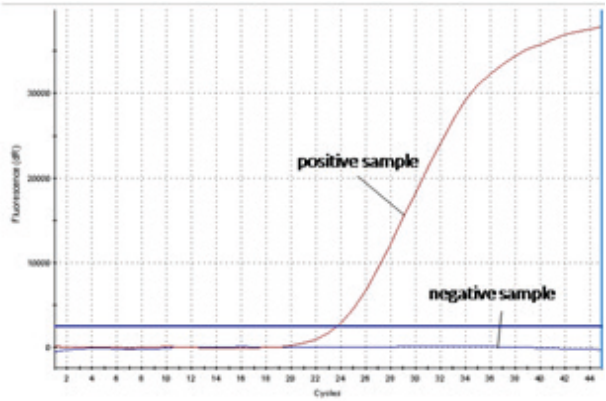

Figure 1: The positive sample shows virus-specific amplification, whereas no fluorescence signal is detected in the negative sample.

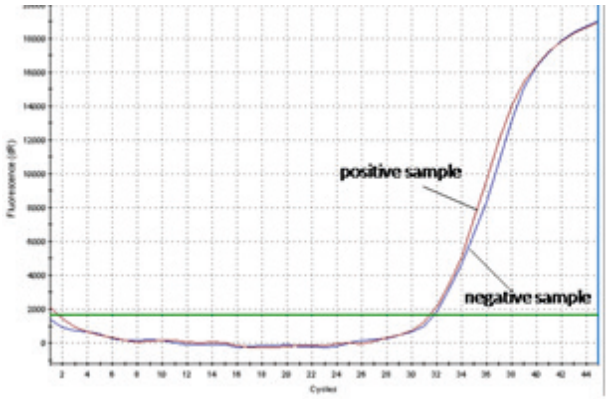

Figure 2: The positive sample as well as the negative sample show a signal in the control RNA-specific VIC®/HEX/JOE/TET channel. The amplification signal of the control RNA in the negative sample shows that the missing signal in the virus-specific channels is not due to RT-PCR inhibition or failure of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

14 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.

15 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time RT-PCR.

No fluorescence signal in the FAM, ROX, or Cy5 channel of the positive controls

The selected channel for analysis does not comply with the protocol

Reaction mix 1: 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 2: 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.

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 chapter "Procedure".

The programming of the thermal profile is incorrect

Compare the thermal profile with the protocol (table 5).

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 chapter "Transport, Storage and Stability".

Weak or no signal of the control RNA and simultaneous absence of a signal in the virus-specific FAM, ROX or Cy5 channel

real time RT-PCR conditions do not comply with the protocol

Check the real time RT-PCR conditions (chapter 12).

real time RT-PCR inhibited

Make sure that you use an appropriate isolation method (see "Sample preparation") 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 chapter "Transport, Storage and Stability".

Detection of a fluorescence signal in the FAM, ROX 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.

16 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 CT – 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 CT of 30.

Internal controls

All internal controls must show a positive (i. e. exponential) amplification curve. The internal control must fall below a CT of 34. If the internal control is above CT 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 CT of 33.

17 KIT PERFORMANCE

17.1 Analytical Sensitivity

The limit of detection (LoD) of MutaPLEX® GastroSys 3 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 MutaPLEX® GastroSys 3 real time RT-PCR.

Virus	Copies per reaction	Expected result	MutaPLEX® GastroSys 3
Norovirus GI	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	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	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

Virus	Copies per reaction	Expected result	MutaPLEX® GastroSys 3
Sapovirus	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	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	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 MutaPLEX® GastroSys 3 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 MutaPLEX® GastroSys 3 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 10.

Table 10: Bacterial and viral pathogens tested for the determination of the analytical specificity of MutaPLEX® GastroSys 3 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

(c)DNA	(complementary) Deoxyribonucleid acid	REF	Catalog number
RNA	Ribonucleid acid	→REF	To be used with
PCR	Polymerase chain reaction	Σ	Contains sufficient for <n> test
RT	Reverse transcription		Upper limit of temperature
VLP	Virus-like particles		Manufacturer

REACTION MIX 1	Reaction mix 1		Use by
REACTION MIX 2	Reaction mix 2	LOT	Lot number
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