Arbeitsanleitung/Manual

MutaPLEX®
GastroSys 4

real time PCR kit

For the qualitative detection and differentiation of the DNA of Salmonella spp., Campylobacter spp., Shigella spp. and Yersinia spp.

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1 INTENDED USE

MutaPLEX® GastroSys 4 is a multiplex real-time PCR for the qualitative detection and differentiation of the DNA of Salmonella spp., Campylobacter spp., Shigellla spp. and Yersinia spp. using Roche LC480II instruments.

2 BACKGROUND INFORMATION

Diarrhoeal disease is a major health care problem and causes about 2 billion cases per year worldwide. The World Health Organization (WHO) ranks diarrhoeal disease as 2nd most common cause of child deaths among children under 5 years globally, particularly in developing countries. About 1.9 million children younger than 5 years of age perish from diarrhoea each year, more than AIDS, malaria and measles combined. Common causes of bacterial diarrhoeal disease are **Campylobacter** spp., **Salmonella** spp., **Y. enterocolitica** and **Shigella** spp..

The burden of foodborne diseases is substantial: every year almost 1 in 10 people fall ill and 33 million of healthy life years are lost. Foodborne diseases can be severe, especially for young children. Diarrhoeal diseases are the most common illnesses resulting from unsafe food, with 550 million people falling ill yearly (including 220 million children under the age of 5 years).

The most common clinical symptoms of **Campylobacter** infections include diarrhoea (frequently bloody), abdominal pain, fever, headache, nausea, and/or vomiting. The symptoms typically last 3 to 6 days. Death from **campylobacteriosis** is rare and is usually confined to very young children or elderly patients, or to those already suffering from another serious disease such as AIDS. Complications such as bacteraemia, hepatitis, pancreatitis, and miscarriage have been reported with various degrees of frequency. Post-infection complications may include reactive arthritis and neurological disorders such as Guillain-Barré syndrome. In 2016, Campylobacter was the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) and has been so since 2005. The number of reported confirmed cases of human campylobacteriosis was 246 307, with an EU notification rate of 66.3 per 100 000 population. This represented an increase of 6.1 % compared with 2015. There was a significantly increasing trend over the period 2008–2016, however, in the last 5 years (2012–2016) the EU/EEA trend has not shown any statistically significant increase or decrease. While the high number of human campylobacteriosiscases, their severity in terms of reported case fatality was low (0.03 %), even though this was the third most common cause of mortality amongst the pathogens considered.

Salmonellosis is a disease caused by the bacteria **Salmonella**. It is usually characterised by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. The onset of disease symptoms occurs 6–72 hours (usually 12–36 hours)

after ingestion of Salmonella, and illness lasts 2–7 days. 60–80 % of all salmonellosis cases are not recognised as part of a known outbreak and are classified as sporadic cases, or are not diagnosed as such at all. Salmonella is a gram negative rods genus belonging to the Enterobacteriaceae family. Within 2 species, Salmonella bongori and Samonella enterica, over 2 500 different serotypes or serovars have been identified to date. Salmonella is a ubiquitous and hardy bacteria that can survive several weeks in a dry environment and several months in water. While all serotypes can cause disease in humans, a few are host-specific and can reside in only one or a few animal species: for example, Salmonella enterica serotype Dublin in cattle and Salmonella enterica serotype Choleraesuis in pigs. When these particular serotypes cause disease in humans, it is often invasive and can be life-threatening. Most serotypes, however, are present in a wide range of hosts. Typically, such serotypes cause gastroenteritis, which is often uncomplicated and does not need treatment, but disease can be severe in the young, the elderly, and patients with weakened immunity. This group features Salmonella enterica serotype Enteritidis and Salmonella enterica serotype Typhimurium, the two most important serotypes of Salmonella transmitted from animals to humans in most parts of the world. The top five most commonly reported serovars in human cases acquired in EU during 2016 were, in decreasing order S. enteritidis, S. typhimurium, monophasic S. typhimurium, S. infantis and S. derby. The proportion of human salmonellosis illnesses due to S. enteritidis continued to increase in 2016. The data reported on food and animals showed that S. enteritidis was markedly associated with laying hens, broilers and broiler meat. A similar evolution during 2012–2016 was noticeable between the proportion of S. enteritidis illnesses in humans acquired in EU and the EU flock prevalence of S. enteritidis in laying hens that significantly increased during 2015 and 2016. S. typhimurium cases in humans decreased.

Shigellosis is a diarrhoeal disease caused by a group of bacteria called **Shigella**. Shigella causes about 500 000 cases of diarrhoea in the United States annually. There are four different species of Shigella: Shigella sonnei (the most common species in the United States), Shigella flexneri, Shigella boydii, Shigella dysenteriae. S. dysenteriae and S. boydii continue to be important causes of disease in the developing world. Shigella dysenteriae type 1 can be deadly. Symptoms of shigellosis typically start 1–2 days after exposure to the germ and include diarrhoea (sometimes bloody), fever and stomach pain. For most people, symptoms usually last about 5 to 7 days. In some cases, it may take several months before bowel habits (for example, how often someone passes stool and the consistency of their stool) are entirely normal. Shigellosis is a relatively uncommon disease in the EU/EEA, but remains of concern in some countries and for some population groups. In 2016, 29 EU/EEA countries reported 5 631 confirmed shigellosis cases. The overall notification rate was 1.5 cases per 100 000 population in 2016, slightly below the rates observed for the period 2012–2015. The

highest notification rate was noted in children under five years of age, followed by adults aged 24–44 years. Sexual transmission of shigellosis among men who have sex with men increased in several European countries in recent years.

Besides Yersinia pestis (the plague) the **Yersinia** group of bacteria also includes two species frequently causing **yersiniosis** (mainly enteritis) in humans; Yersinia enterocolitica and Yersinia pseudotuberculosis. Both are zoonoses, with a large number of animals, but mainly pigs, acting as reservoirs. Raw/undercooked meat consumption is often the cause of infection in humans. Direct transmission from other animals (e.g. pets) or through contaminated food or drink is also possible. After an incubation period of 3–7 days, the clinical presentation includes fever, diarrhoea and abdominal pain in the right lower part of the abdomen, mimicking appendicitis. Both infections respond well to antibiotics, but untreated symptoms of abdominal pain may last for a long while. Children and adolescents are most affected. Other manifestations such as joint inflammation, erythema nodosum and Reiter's syndrome (inflammation of eyes and joints) can also appear. Outbreaks are sometimes detected as a sudden increase in appendectomies due to mistaken diagnoses of appendicitis. Prophylactic measures include adequate hygiene in meat processing (especially of pork), hand hygiene and protection of water supplies. In 2016, 28 countries reported 6 918 confirmed yersiniosis cases in the EU/EEA. The overall notification rate was 1.8 per 100 000 population and remained stable from 2012–2016. The highest rate was detected in 0–4 year-old children (7.5 per 100 000 population). The highest rates were reported by Finland, the Czech Republic and Lithuania.

3 PRINCIPLE OF THE TEST

The MutaPLEX® GastroSys 4 real time PCR Kit contains specific primers and dual-labelled probes for the amplification and detection of the DNA of Salmonella spp., Campylobacter spp., Shigellla spp. and Yersinia spp. extracted from biological specimens. The presence of DNA is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the Salmonella spp. specific probes is measured in the ROX (533-610 nm) channel. The fluorescence of the Campylobacter spp. specific probes is measured in the FAM (465-510 nm) channel. The fluorescence of the Shigellla spp. specific probes is measured in the Cyan500 channel (440-488 nm). The fluorescence of the Yersinia spp. specific probes is measured in the Cy5 channel (618-660 nm). Furthermore, the MutaPLEX® GastroSys 4 real time PCR Kit contains a Control DNA, which is added during DNA extraction and detected in the same reaction by a differently labelled probe. The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acid was extracted from the biological specimen. The fluorescence of the Control DNA is measured in the HEX (533-580 nm) channel.

4 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 or 384 reactions respectively.

Table 1: Components of the MutaPLEX® GastroSys 4 real time PCR Kit.

Label	Lid Colour	Content	
		96	384
Reaction Mix	yellow	1 x 1536 µl	4 x 1536 µl
Positive Control	red	1 x 100 µl	1 x 100 µl
Negative Control	green	1 x 200 µl	1 x 200 µl
Control DNA	colourless	1 x 480 µl	4 x 480 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA extraction kit (e. g. MutaCLEAN® Universal RNA/DNA, KG1038, or MutaCLEAN® Mag RNA/DNA, KG1023 / KG1024)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Roche LC480 II real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® GastroSys 4 real time 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 WARNINGS AND PRECAUTIONS

- Read the Instructions for use carefully before using the product.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- 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.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations

8 SAMPLE MATERIAL

Starting material for the MutaPLEX® GastroSys 4 real time PCR Kit is DNA extracted from biological specimens (e.g. stool samples, vomit, environmental or food samples).

9 SAMPLE PREPARATION

Commercial kits for DNA extraction 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 DNA in the sample to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

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

10 CONTROL DNA

A Control DNA can be used as extraction control or only as inhibition control. This allows the user to control the DNA extraction procedure and to check for possible real time PCR inhibition.

DNA isolation from clinical specimens

(e.g. stool samples, vomit, environmental and food samples

a) *Control DNA used as Extraction Control:*

Add 5 µl Control DNA per extraction (5 µl x (N+1)). Mix well. Perform the DNA isolation according to the manufacturer's instructions. Please follow protocol A.

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

b) *Control DNA used as Internal Control of the real time PCR:*

If only inhibition will be checked please follow protocol B.

11 REAL TIME PCR

11.1 Important Points Before Starting

- Please pay attention to the to the chapter **„**Warnings and Precautions**"**.
- Before setting up the real time 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 PCR run a Positive Control and a 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.

11.2 Procedure

Protocol A

The Control DNA was added during DNA extraction (chapter „Control DNA"). In this case, prepare the Master Mix according to Table 2.

The Master Mix contains all of the components needed for 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 DNA was added during DNA extraction)

Volume per Reaction	Volume Master Mix
16.0 µl Reaction Mix	16.0 µl x (N+1)

Protocol B

The Control DNA is used for the control of the real time PCR only (see chapter „Control DNA"). In this case, prepare the Master Mix according to Table 3.

The Master Mix contains all of the components needed for real time 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 DNA is added directly to the Master Mix)

Volume per Reaction	Volume Master Mix
16.0 µl Reaction Mix	16.0 µl x (N+1)
0.5 µl Control DNA*	0.5 µl x (N+1)*

*The increase in volume caused by adding the Control DNA is not taken into account when preparing the PCR assay.

Protocol A and B: real time 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 the Master Mix into each optical PCR reaction tube.
- Add **4 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube (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

11.3 Instrument Settings

For the real time PCR use the thermal profiles shown in Table 5 or Table 6, alternatively.

Table 5: real time PCR thermal profile 1

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		

For convenience purposes, the Reverse Transcription step can be omitted.

Table 6: real time PCR thermal profile 2

Description	Time	Temperature	Number of Cycles
Reverse Transcription	5 min	55 °C	1
Initial Denaturation	3 min	95 °C	1
Amplification of cDNA			45
Denaturation	5 s	95 °C	
Annealing	15 s	60 °C	
	Aquisition at the end of this step		
Extension	15 s	72 °C	

For convenience purposes, the Reverse Transcription step can be omitted.

Table 7: Instrument settings of LC480 II required for the MutaPLEX® GastroSys 4 real time PCR.

Real time PCR Instrument	Parameter Reaction Mix	Detection Channel	Notes		
			Colour Compensation Kit **KG19-5-CC MutaPLEX® CC-1** required		
			Melt Factor	**Quant Factor**	**Max Integration Time (s)**
LightCycler 480II	Shigella	Cyan 500 440-488	1	5	1
	Campylobacter	FAM 465-510	1	10	1
	Control DNA	HEX 533-580	1	10	2
	Salmonella	ROX 533-610	1	10	2
	Yersinia	Cy5 618-660	1	10	3

12 DATA ANALYSIS

The interpretation of the test results is described in table 8.

Table 8: Interpretation of Results.

Signal/C_T Values					Interpretation
440-488	**465-510**	**533-610**	**618-660**	**533-580**	
Shigella spp.	Campylo-bacter spp.	Salmo-nella spp.	Yersinia spp.	Control DNA	
positive	negative	negative	negative	positive or negative*	**Positive result, the sample contains Shigella spp. DNA.**
negative	**positive**	negative	negative	positive or negative*	**Positive result**, the sample contains **Campylobacter spp. DNA**.
negative	negative	**positive**	negative	positive or negative*	**Positive result**, the sample contains **Salmonella spp. DNA**.
negative	negative	negative	**positive**	positive or negative*	**Positive result**, the sample contains **Yersinia spp. DNA**.
negative	negative	negative	negative	≤ 34	**Negative result**, the sample contains no Shigella spp. DNA, Campylobacter spp. DNA, Salmonella spp. DNA and Yersinia spp. DNA
negative	negative	negative	negative	negative or > 34	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred while DNA extraction.

* A strong positive signal in the pathogen specific channel can inhibit the IC. In such cases the result for the Control DNA can be neglegted.

Figure 1 and figure 2 show examples for positive and negative real time PCR results.

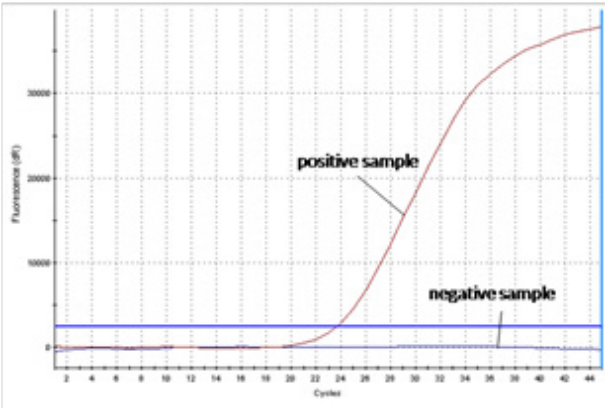

Figure 1: The positive sample shows specific amplification, whereas no fluorescence signal is detected in the negative sample.

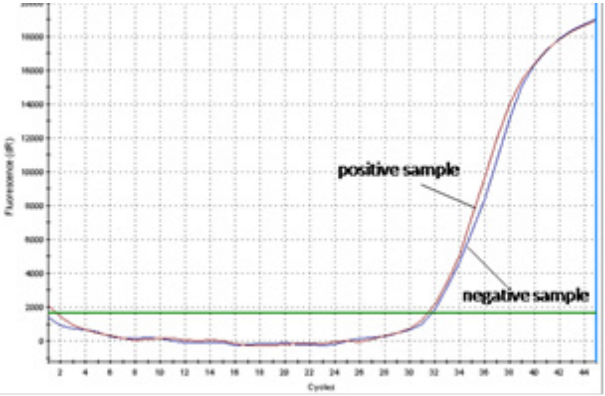

Figure 2: The positive sample as well as the negative sample show a signal in the Control DNA specific channel. The amplification signal of the Control DNA in the negative sample shows, that the missing signal in the pathogen specific channels is not due to PCR inhibition or failure of DNA isolation, but that the sample is a true negative.

13 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 in DNA-extraction or a strong positive eluate 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 < 34.

14 LIMITATIONS

- Strict compliance with the Instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the pathogen genomes covered by the primers and/or probes used in the kit may result in failure to detect the respective DNA.

- As with any diagnostic test, results of the MutaPLEX® GastroSys 4 real time PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 TROUBLESHOOTING

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

No fluorescence signal in the bacteria specific channels of the Positive Control

The selected channel for analysis does not comply with the protocol

Select the channel according to Table 7.

Incorrect configuration of the real time 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 and Table 6).

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 6 „Transport, Storage and Stability".

Weak or no signal of the Control DNA and simultaneous absence of a signal in the bacteria specific channels.

Real time PCR conditions do not comply with the protocol

Check the real time PCR conditions (see chapter „Real time PCR").

Real time PCR inhibited

Make sure that you use an appropriate isolation method (see chapter „Sample Preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed. An additional centrifugation step at high speed is recommended before elution of the DNA.

DNA loss during isolation process

In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the 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".

Detection of a fluorescence signal in the bacteria specific channel of the Negative Control

Contamination during preparation of the PCR

Repeat the real time 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 PCR.

16 ASSAY VALIDATION

16.1 Analytical Sensitivity and Linearity

The limit of detection (LoD) of MutaPLEX® GastroSys 4 real time PCR was determined using serial dilutions of synthetic DNA sequences (gBlocks) of the respective target sequences for the detection of Shigella spp., Campylobacter spp., Salmonella spp. and Yersinia spp. using a Roche LC480 II real time PCR instrument.

Table 9: Analytical sensitivity of MutaPLEX® GastroSys 4 real time PCR.

Sample	copies per reaction	C_P value 440-488	Mean C_P 440-488
gBlock_Shigella	1 000 000	19.48	19.49
		19.47	
		19.51	

Sample	copies per reaction	C_P value 440-488	Mean C_P 440-488
gBlock_Shigella	100 000	22.85	22.91
		22.93	
		22.96	
gBlock_Shigella	10 000	26.33	26.36
		26.50	
		26.26	
gBlock_Shigella	1 000	29.79	29.73
		29.67	
		29.73	
gBlock_Shigella	100	32.65	32.68
		32.80	
		32.60	
gBlock_Shigella	10	35.29	35.81
		35.62	
		36.52	
gBlock_Shigella	1	45.00	45.00
		45.00	
		45.00	

Sample	copies per reaction	C_P value 465-510	Mean C_P 465-510
gBlock_Campylobacter	1 000 000	19.78	19.79
		19.78	
		19.80	
gBlock_Campylobacter	100 000	22.86	23.06
		23.11	
		23.20	
gBlock_Campylobacter	10 000	26.29	26.34
		26.41	
		26.33	

Sample	copies per reaction	C_P value 465-510	Mean C_P 465-510
gBlock_Campylobacter	1 000	29.47	29.34
		29.16	
		29.40	
gBlock_Campylobacter	100	31.89	31.53
		31.31	
		31.38	
gBlock_Campylobacter	10	33.03	33.08
		33.07	
		33.13	
gBlock_Campylobacter	1	45.00	41.74
		45.00	
		35.22	

Sample	copies per reaction	C_P value 533-610	Mean C_P 533-610
gBlock_Salmonella	1 000 000	21.07	21.07
		21.04	
		21.10	
gBlock_Salmonella	100 000	24.26	24.28
		24.25	
		24.33	
gBlock_Salmonella	10 000	27.33	27.33
		27.33	
		27.32	
gBlock_Salmonella	1 000	30.26	30.18
		30.14	
		30.14	
gBlock_Salmonella	100	33.05	32.72
		32.54	
		32.57	

Sample	copies per reaction	C_P value 533-610	Mean C_P 533-610
gBlock_Salmonella	10	34.48	35.06
		34.86	
		35.85	
gBlock_Salmonella	1	45.00	45.00
		45.00	
		45.00	

Sample	copies per reaction	C_P value 618-660	Mean C_P 618-660
gBlock_Yersinia	1 000 000	21.15	21.17
		21.15	
		21.21	
gBlock_Yersinia	100 000	24.53	24.57
		24.56	
		24.61	
gBlock_Yersinia	10 000	27.83	27.89
		27.88	
		27.96	
gBlock_Yersinia	1 000	31.15	31.08
		31.01	
		31.07	
gBlock_Yersinia	100	33.98	33.89
		34.09	
		33.60	
gBlock_Yersinia	10	35.68	35.37
		34.90	
		35.52	
gBlock_Yersinia	1	37.13	39.94
		37.68	
		45.00	

The MutaPLEX® GastroSys 4 real time PCR shows a consistent sensitivity of at least 10 target copies per reaction for each of the respective pathogens. A linearity of consistently higher than R2 = 0.99 in a range of 5 log10 for the amplification of all target sequences could be shown (see Table 9).

16.2 Analytical Specificity

The specificity of the MutaPLEX® GastroSys 4 real time PCR was evaluated by in silico analysis and by amplification of RNA and DNA of other relevant viruses and bacteria found in biological samples.

The MutaPLEX® GastroSys 4 real time PCR showed positive results for the samples containing Shigella spp., Campylobacter spp., Salmonella spp. and Yersinia spp., 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 4 real time PCR.

pathogen	species/strain	440-488 Shigella	465-510 Campylo-bacter	533-610 Salmonella	618-660 Yersinia
Escherichia	E. coli	negative	negative	negative	negative
Adenovirus	Serogroup 4	negative	negative	negative	negative
Entero coccus	Enterococcus faecalis	negative	negative	negative	negative
Listeria	Listeria mono-cytogenes	negative	negative	negative	negative
Citrobacter	Citrobacter freundii	negative	negative	negative	negative
Klebsiella	Klebsiella spp.	negative	negative	negative	negative
Pseudo-monas	Pseudomonas aeruginosa	negative	negative	negative	negative
Enterobacter	Enterobacter cloacae	negative	negative	negative	negative
Shigella	Shigella sonnei	positive	negative	negative	negative
Salmonella	Salmonella enterica	negative	negative	positive	negative
Campylo-bacter	Campylo-bacter jejunii	negative	positive	negative	negative

pathogen	species/strain	440-488 Shigella	465-510 Campylo-bacter	533-610 Salmonella	618-660 Yersinia
Yersinia	Yersinia enterocolitica	negative	negative	negative	positive

17 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleid acid	REF	Catalog number
PCR	Polymerase chain reaction	→REF	To be used with
REACTION MIX	Reaction mix	CE	European Conformity
CONTROL 1 +	Positive control 1	Σ	Contains sufficient for <n> test
CONTROL –	Negative control		Upper limit of temperature
CONTROL DNA IC	Control DNA		Manufacturer
CONTENT	Content		Use by YYYY-MM-DD
	Consult instructions for use	LOT	Batch
IVD	*In vitro* diagnostic medical device		

18 LITERATURE

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