**Arbeitsanleitung/Manual**

Distribuito in ITALIA da
**Li StarFish S.r.l.**
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

# 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.***

Valid from 2019-05-16

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**Immundiagnostik AG**, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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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.

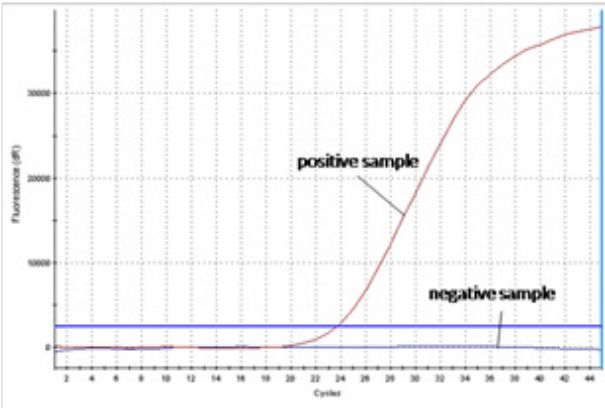


Figure 1: The positive sample shows specific amplification, whereas no fluorescence signal is detected in the negative sample.

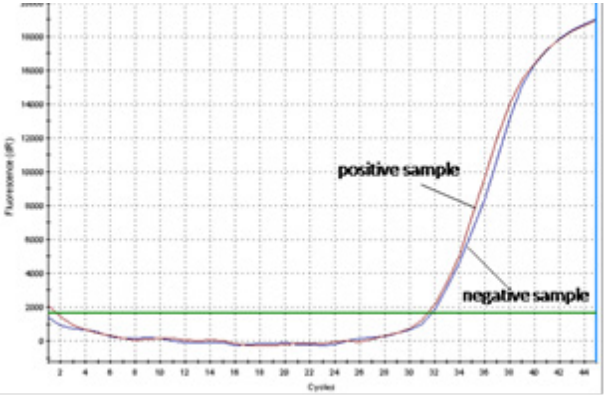


Figure 2: The positive sample as well as the negative sample show a signal in the Control DNA specific channel. The amplification signal of the Control DNA in the negative sample shows, that the missing signal in the pathogen specific channels is not due to PCR inhibition or failure of DNA isolation,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