# Instructions for Use

*In-vitro*-Diagnostic

IVD

The GastroBac *direct* PCR is a real-time qPCR test for simultaneous *in vitro* detection and differentiation of DNA from ***Campylobacter*, *Salmonella*, *Shigella*/**enteroinvasive ***Escherichia coli* (EIEC), *Yersinia enterocolitica*** and ***Yersinia pseudotuberculosis.***

*Ref.No.:*
FBC115-Ax
FBC115-Bx

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

## Content

## 1. Name of the Device

GastroBac *direct* PCR

## 2. Intended Purpose

The GastroBac *direct* PCR is an assay for in vitro examination of bacterial DNA in stool samples to provide information to aid to diagnose patients under suspicion of gastrointestinal infection with *Campylobacter* spp., *Salmonella* spp., *Shigella* spp./enteroinvasive Escherichia coli (EIEC), *Yersinia enterocolitica* and/or *Yersinia pseudotuberculosis*.

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

## 3. Pathogen Information

***Campylobacter* spp.** are Gram-negative bacteria with a spiral or S-shaped morphology. To date, over 30 species have been identified, with *C. jejuni* and *C. coli* being the most significant human pathogens in the genus. Other species, such as *C. lari*, *C. fetus*, and *C. upsaliensis*, can also cause human diseases.

Pathogenic *Campylobacter* species can lead to acute enteritis. In Germany, *Campylobacter* enteritis is the most common bacterial reportable disease, with 40,000 to 50,000 cases annually (45 to 60 cases per 100,000 population). It primarily affects children under the age of 5 and adults between 20 and 29 years of age [1].

Infections are primarily acquired through contaminated food, particularly raw poultry, though other sources include raw minced meat, raw milk, water, and pets. Direct person-to-person transmission is rare but possible [2]. The typical incubation period is 2 to 5 days, and the median duration of bacterial excretion is 2 to 5 weeks [3].

***Salmonella* spp.** are Gram-negative rods classified according to the Kauffmann-Le Minor scheme and differentiated into over 2,500 known serovars. There are two main species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies (I-VI). Subspecies I (*S. enterica* spp. *enterica*) is responsible for nearly all *Salmonella* infections in warm-blooded animals [4]. Non-typhoidal *Salmonella* causes gastroenteritis (also known as salmonellosis), while *S. Typhi* and *S. Paratyphi* A, B, and C cause systemic infections, typically with secondary intestinal involvement [2].

The most common *Salmonella* serovars in Germany are *S. Enteritidis* and *S. Typhimurium*. Infections tend to peak at the end of summer, and children under 5 years are more likely to acquire Salmonella infections than older children or adults [5]. Salmonellosis is a classic foodborne infection, typically transmitted through the oral ingestion of contaminated eggs, raw meat, or even plant-based foods [2]. The infectious dose for adults is approximately $10^4$ to $10^6$ colony-forming units. The incubation period ranges from 6 to 72 hours, with most cases occurring 12 to 36 hours after exposure, depending on the infectious dose and serovar [6].

***Shigella* spp.** are Gram-negative, nonmotile, rod-shaped bacteria in the *Enterobacteriaceae* family, closely related to *Escherichia coli*. The genus is classified into four serogroups:

- Serogroup A: *S. dysenteriae*
- Serogroup B: *S. flexneri*
- Serogroup C: *S. boydii*
- Serogroup D: *S. sonnei*

Serogroups A to C are further divided into serovars. *Shigella* species produce an endotoxin, contributing to inflammation of the intestinal mucosa, as well as the virulence factor ipaH, which facilitates bacterial invasion into epithelial cells. *S. dysenteriae* (serotypes 1-4) and some *S. flexneri* (serotype 2a) can also produce the exotoxin Shiga toxin.

*Shigella flexneri* is the most frequently isolated species globally [7]. In Germany, shigellosis is relatively rare, mainly caused by *S. sonnei* and *S. flexneri*, both of which typically lead to mild disease but are highly contagious. Most shigellosis cases are imported from travelers. Transmission occurs via fecal-oral contact or through indirect contamination. In recent years, there has been a marked increase in highly resistant *Shigella* strains (3-MRGN and 4-MRGN) [8].

The typical incubation period is between 12 and 96 hours. After ingestion, the bacteria invade the colon mucosa. Symptoms often begin with fever, headache, malaise, and cramp-like abdominal pain. Mild forms may present with watery or soft stools, while more severe cases in immunocompromised individuals (e.g., those with HIV), malnourished individuals, or the elderly may lead to dysentery, with bloody and mucous stools, and inflammatory colitis.

**Enteroinvasive *Escherichia coli* (EIEC)** infections resemble shigellosis due to shared virulence factors like ipaH. EIEC can invade the intestinal wall and are classified as enterovirulent *E. coli*. There are no specific foods frequently associated with EIEC infections, and infected humans are the only known reservoirs. Consequently, any food contaminated with human feces from an infected individual, either directly or via contaminated water, can be infectious [9].

***Yersinia* spp.** are Gram-negative, facultative anaerobic bacteria in the *Enterobacterales* order. The genus *Yersinia* consists of 18 species, with *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* being human pathogens. Only *Y. enterocolitica* and *Y. pseudotuberculosis* cause yersiniosis. *Y. enterocolitica* includes six biovars (1A, 1B, 2, 3, 4, 5), with biovars 1B to 5 carrying a virulence plasmid (pYV) and additional virulence factors (e.g., enterotoxin YstA), making them obligate human pathogens. Strains of biovar 1A lack the virulence plasmid but may produce a different enterotoxin (YstB), which can also cause diarrhea, classifying them as facultative human pathogens. Over 70 *Y. enterocolitica* serovars are known, with O:3, O:9, and O:5,27 being most common in Europe. *Y. pseudotuberculosis* has 21 known serotypes and subtypes, all carrying the virulence plasmid pYV [10].

Different animals serve as natural reservoirs for *Yersinia* species. In Germany, *Y. enterocolitica* bioserovar 4/O:3 primarily resides in pigs. Human infections are most often acquired through contaminated food, especially raw pork, though raw milk, contaminated water, and plant-based foods are also potential sources. The typical incubation period is 3 to 7 days.

The majority of yersiniosis cases are caused by *Y. enterocolitica* (>90%), with a smaller proportion caused by *Y. pseudotuberculosis*. *Y. enterocolitica* infections typically present with enterocolitis, characterized by diarrhea, fever, and abdominal pain, and primarily affect children under 5 years. In older children and adolescents, infections can lead to mesenteric lymphadenitis (swelling of the lymph nodes) and terminal ileitis, causing right-sided abdominal pain and fever, often mistaken for appendicitis. In some cases, complications such as pharyngitis, liver abscesses, and inflammation of other organs may occur [10].

## 4. Testing Principle

The GastroBac *direct* PCR is a multiplex qPCR test for the detection and differentiation of the following pathogens:

- *Campylobacter* spp. (*C. coli, C. jejuni, C. lari, C. upsaliensis,* and *C. hyointestinalis*)
- *Salmonella* spp. (*Salmonella enterica* subsp. *enterica* including S. Typhimurium, S. Enteritidis, S. Typhi, S. Paratyphi A/B/C)
- *Shigella* spp. (*S. flexneri, S. boydii, S. dysenteriae, S. sonnei*) and EIEC
- *Yersinia* (*Y. enterocolitica* and *Y. pseudotuberculosis*)

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

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

The GastroBac *direct* PCR contains primer and probes specific for the targets listed in Table 1 as well as the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

*Table 1: Analytes and target gene regions of GastroBac direct PCR*

| ANALYTE | GENE | REGION (NUCLEOTIDES) |
|---|---|---|
| *Campylobacter* spp. | *16S rRNA* | 1180 - 1292 |
| *Salmonella* spp. | *invA* | 527 - 642 |
| *Shigella* spp./EIEC | *ipaH* | 980 - 1043 |
| *Y. enterocolitica** | *ystA* | 84 - 188 |
| *Y. pseudotuberculosis** | *inv* | 388 - 539 |

** Y. enterocolitica and Y. pseudotuberculosis are detected in the same channel and can therefore not be differentiated.*

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

## 5. Package Content

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

*Table 2: Package content of GastroBac direct PCR*

| MATERIAL | LID COLOUR | #VIALS; VOLUME | #RXNS. | COMMENT | ACTIVE INGREDIENT(S) |
|---|---|---|---|---|---|
| Solution A | green | 1x; 1050 µL | 96 | Reaction mix | Polymerase (approx. 0.2 U/µl); Primers and probes (approx. 150-200 nM) |
| Internal Control | blue | 1x; 400 µL | 96 | Artificial nucleic acid target | Mix of RNA (approx. $10^7$ copies/µl) and plasmid DNA (approx. 1 pg/µl) |
| Positive Control | red | 1x; 50 µL | 4 | DNA of target analytes | DNA plasmids with target regions of *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia* (each approx. 0.01 pg/µl) |

## 6. Configurations

The GastroBac *direct* PCR kit is available in the following variants:

*Table 3: Configurations of GastroBac direct PCR*

| REFERENCE NUMBER | CONFIGURATION | IC CHANNEL |
|---|---|---|
| FBC115-Ax | suitable for use on a qPCR instrument with at least 5 detection channels (**Cyan500**/FAM/HEX/Red610/Cy5) | IC on **Cyan500** |
| FBC115-Bx | suitable for use on a qPCR instrument with at least 5 detection channels (FAM/HEX/Red610/Cy5/**Cy5.5**) | IC on **Cy5.5** |

## 7. Additional Equipment and Reagents (not provided)

- qPCR cycler (with at least 5 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- sterile filter-tips for PCR testing (DNA/RNA-free)
- Table centrifuge
- DNA extraction kit (IVD-1049 chemagic™ Pathogen NA gDNA Kit H96 by PerkinElmer chemagen Technologie GmbH, MagNA Pure 96 DNA and Viral NA Small Volume Kit by Roche Life Science, or similar devices)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

## 8. Transport, Storage and Stability

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

## 9. Warnings, Safety Precautions and Additional Information Access

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

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

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases).

- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering a different area.
- The test kits are intended for single use and must not be reused after performing qPCR reaction.
- If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated and a repeat run should be considered.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/
- Pending EUDAMED entry, the Summary of Safety and Performance (SSP) can be downloaded via www.frizbiochem.de

## 10. Specimen Collection, Handling, Transport, and Storage

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

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

## 11. Important Points before Starting

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

In every qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia*. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the qPCR run and the results must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. DNA extraction reagents are not part of the GastroBac *direct* PCR kit. Performance evaluation studies have been conducted using the IVD-1049 chemagic™ Pathogen NA gDNA Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH) and the MagNA Pure 96 DNA and Viral NA Small Volume Kit on a MagNA Pure 96 instrument (Roche Life Science) for DNA isolation.

## 12. Test Procedure

Thaw all reagents completely and keep them cool (+2 °C to +8 °C) before starting the test, use within 8 hours, avoid exposure to light.

*Table 4: Test procedure of GastroBac direct PCR*

| TEST PROCEDURE | |
|---|---|
| **Sample preparation** | |
| **1** | Thaw all reagents completely. |
| **2** | Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer). |
| **3** | Perform nucleic acid extraction according to your laboratory's standard procedure. |
| **qPCR** | |
| **4** | Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes. |
| **5** | Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run. |
| **6** | Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided. |
| **7** | Briefly centrifuge the microtiter plate or reaction tubes. |
| **8** | Place the filled plate/reaction tubes in the qPCR cycler. |
| **9** | Start program. |

## 13. Instrument Settings

The following thermal profile is to be used (Table 5). An alternative thermal profile can be found in the appendix (Table 9). Channel settings depend on the qPCR cycler used and should be checked before starting the analysis (Table 6). Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

*Table 5: Instrument settings of GastroBac direct PCR*

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

**only for mixed RNA-/DNA runs*

*Table 6: Channel settings of GastroBac direct PCR (Ref. No.: FBC115-Ax and FBC115-Bx)*

| | ***Campylobacter*** | ***Salmonella*** | ***Shigella*** | ***Yersinia pseudotuberculosis*** | ***Yersinia enterocolitica*** | **Internal Control** | |
|---|---|---|---|---|---|---|---|
| | *16S rRNA* | *invA* | *ipaH* | *inv* | *ystA* | **FBC115-Ax** | **FBC115-Bx** |
| Reporter dye | FAM | HEX | Red 610 | Cy5 | Cy5 | Cyan500 | Cy5.5 |
| Colour | green | yellow-green | orange | red | red | blue | far-red |
| Emission [nm] | 520 | 560 | 610 | 670 | 670 | 480 | 700 |
| Quencher | Black Hole Quencher | | | | | | |

The GastroBac *direct* PCR was validated with the Roche LightCycler® 480 II and BioRad CFX Opus 96™. In general, FRIZ Lab *direct* PCR tests are compatible with many qPCR cyclers. FBC115-Ax is suitable for qPCR cyclers with at least the five channels Cyan 500, FAM, HEX, Red 610, and Cy5 (e.g., LightCycler® 480 II) and FBC115-Bx is suitable for qPCR cyclers with at least the five channels FAM, HEX, Red 610, Cy5, and Cy5.5 (e.g., BioRad CFX Opus 96™ or analytik jena qTOWERiris).

## 14. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1).

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

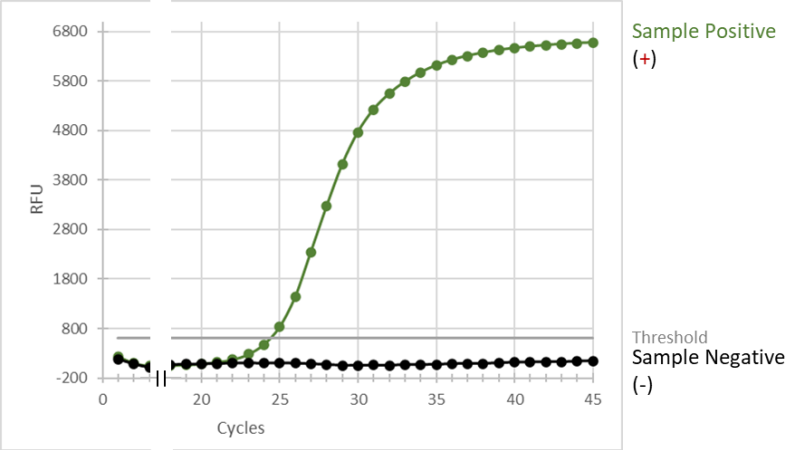


*Figure 1: Exemplary amplification curves*

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all channels. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC should show a positive amplification curve in both positive and negative samples.

For distribution of results within the channels see Table 7.

*Table 7: Results of GastroBac direct PCR (Ref. No.: FBC115-Ax and FBC115-Bx)*

| ***Campylobacter*** | ***Salmonella*** | ***Shigella*** | ***Yersinia*** | **Internal Control** | | | |
|---|---|---|---|---|---|---|---|
| | | | | **FBC115-Ax** | **FBC115-Bx** | | |
| FAM | HEX | Red 610 | Cy5 | Cyan500 | Cy5.5 | **Result** | **Interpretation** |
| + | – | – | – | + | | Valid | *Campylobacter* detected. |
| – | + | – | – | + | | Valid | *Salmonella* detected. |
| – | – | + | – | + | | Valid | *Shigella* detected. |
| – | – | – | + | + | | Valid | *Yersinia* detected. |
| – | – | – | – | + | | Valid | No *Campylobacter*, *Salmonella*, *Shigella,* or *Yersinia* detected. |
| +/– | +/– | +/– | +/– | – | | Invalid | The test result cannot be evaluated. |

## 15. Limitations of the Method

The results support the differential diagnosis of infections with *Campylobacter*, *Salmonella*, *Shigella*/EIEC, *Yersinia enterocolitica* and/or *Yersinia pseudotuberculosis*. The bacterial DNA is generally detectable in stool samples during the acute phase of the infection.

Positive results indicate the presence of the respective pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of *Campylobacter*, *Salmonella*, *Shigella*/EIEC, *Yersinia enterocolitica* or *Yersinia pseudotuberculosis*, as results depend on correct sampling, the absence of inhibitors and sufficient DNA to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, amplification or detection of the target nucleic acids. For information on tested interfering substances, please refer to the Appendix (page 18).

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

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

The GastroBac *direct* PCR detects the presence of the respective nucleic acid but not the presence of a vital pathogen, which may lead to false positive results.

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

The detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur. In particular, there is potential for competitive interference of *Salmonella enterica* at low concentration (3x LoD) when *Yersinia pseudotuberculosis* concentration is ≥ $10^6$ DNA copies/ml (see Competitive Interference in the Appendix, page 19).

## 16. Analytical Performance

### 16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of genomic DNA. The analytes were tested in 24 replicates per concentration on a LightCycler® 480 II instrument. The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 10.0.2 software (see Table 8).

*Table 8: Limit of Detection of GastroBac direct PCR*

| ANALYTES | LOD (COPIES/REACTION) | UPPER LIMIT | LOWER LIMIT |
|---|---|---|---|
| *Campylobacter jejuni* subsp. *jejuni* | 0.38 | 1.40 | 0.09 |
| *Salmonella enterica* | 1.20 | 1.77 | 0.74 |
| *Shigella flexneri* | 1.67 | 5.06 | 0.41 |
| Enteroinvasive *Escherichia coli* | 4.16 | 9.64 | 1.43 |
| *Yersinia pseudotuberculosis* | 6.88 | 15.49 | 2.52 |
| *Yersinia enterocolitica* subsp. *enterocolitica* | 3.55 | 6.50 | 1.64 |

**16.2 Other Analytical Performance Parameters**

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) are shown in the Appendix.

## 17. Diagnostic Performance

In total, 170 stool samples were tested. Thereof, 144 were clinical samples pre-analyzed by culture and 26 were spiked samples due to the limited number of clinical samples that could be acquired in the study period. Diluted stool samples were extracted on a MagNA Pure 96 Instrument using the DNA and viral NA small volume kit (Roche). Eluates were used for parallel analysis with the GastroBac *direct* PCR and a CE certified test from another manufacturer in direct comparison on a LightCycler® 480 II (Roche). In the event of a discrepancy between the results, the culture-based result was used to resolve the discrepancy.

Diagnostic sensitivity and specificity are summarized in the following:

**Campylobacter samples**

| | | positive | negative | |
|---|---|---|---|---|
| **GastroBac** | positive | 48 | 1 | Sensitivity: 100.0% (92.6 - 100.0) |
| | negative | 0 | 49 | Specificity: 98.0% (89.4 - 100.0) |

**Salmonella samples**

| | | positive | negative | |
|---|---|---|---|---|
| **GastroBac** | positive | 21 | 0 | Sensitivity: 100.0% (83.9 - 100.0) |
| | negative | 0 | 50 | Specificity: 100.0% (92.9 - 100.0) |

**Yersinia samples**

| | | positive | negative | |
|---|---|---|---|---|
| **GastroBac** | positive | 19 | 0 | Sensitivity: 100.0% (82.4 - 100.0) |
| | negative | 0 | 50 | Specificity: 100.0% (92.9 - 100.0) |

**Shigella samples**

| | | positive | negative | |
|---|---|---|---|---|
| **GastroBac** | positive | 20 | 0 | Sensitivity: 100.0% (83.2 - 100.0) |
| | negative | 0 | 50 | Specificity: 100.0% (92.9 - 100.0) |

The diagnostic sensitivity of the GastroBac *direct* PCR is dependent on the DNA extraction method used to isolate DNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used.

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

## 18. Symbols

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

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

Document Version:

| FBC115_IFU_EN | |
|---|---|
| Version 1.0<br>Date: 2025-07-15 | First version |
| Version 1.1<br>Date: 2025-11-18 | • Chapter 5 Package Content: removal of "*One Positive Control/kit is included. If more Positive Control is needed, it can be purchased separately: Reference number FBC115-PC."<br>• Chapter 8 Transport, Storage and Stability: "Reagents should be handled at +2 °C to +8 °C and used within 8 hours." was changed to "After thawing, reagents should be kept at +2 °C to +8 °C before starting the test and used within 8 hours."<br>• Chapter 9: Addition of "In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated and a repeat run should be considered."<br>• Insertion of chapter 19 "Technical Support and Contact Information"<br>• Rephrasing of paragraph in Chapter 15 "Limitations of the Method": "Test results should always be seen in the context of the clinical findings and should not be used as the only basis for diagnosis, treatment, or other patient management decisions. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings by an appropriate healthcare professional."<br>• Addition of sentence to chapter 15 "Limitations of the Method": " In particular, there is potential for competitive interference of *Salmonella enterica* at low concentration (3x LoD) when *Yersinia pseudotuberculosis* concentration is ≥ $10^6$ DNA copies/ml"<br>• Insertion of "Active ingredient(s)" to table 2 (package content) |

## 19. Technical Assistance and Contact Information

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

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

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

FRIZ Biochem GmbH ▫ Floriansbogen 2-4 ▫ 82061 Neuried ▫ Germany

Tel +49 (0) 89 72 44 09 0 ▫ Fax +49 (0) 89 72 44 09 10

info@frizbiochem.de ▫ www.frizbiochem.de

# 20. Appendix

## 20.1 Alternative Thermal Profile

*Table 9: Alternative instrument settings of GastroBac direct PCR*

| STEPS | TEMPERATURE [°C] | TIME | ACQUISITION | #CYCLES |
|---|---|---|---|---|
| Initial step (optional)* | 55 | 10 min | - | 1 |
| Denaturation | 95 | 2 min | - | 1 |
| Amplification | 95 | 10 sec | - | 45 |
| | 60 | 30 sec | on | |

**only for mixed RNA-/DNA runs*

## 20.2 Cross-reactivity

Cross-reactivity was tested with *in silico* analysis as well as with wet-lab testing.

For the *in silico* analysis, all primer and probe sequences of the GastroBac *direct* PCR were aligned to the organisms in Table 10 using nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

*Table 10: In silico analysis for cross-reactivity of the used primers and probes. Potentially cross-reactive species are highlighted in yellow.*

| **Viruses** | | |
|---|---|---|
| Astrovirus | Norovirus | Rotavirus |
| Adenovirus | Sapovirus | Cytomegalovirus |
| Enterovirus | Hepatitis A virus | Aichivirus A |
| Coxsackie virus | Echovirus | |
| **Bacteria** | | |
| *Campylobacter* spp. | *Salmonella* spp. | *Fusobacterium* |
| *Campylobacter hyointestinalis* | *Salmonella subterranea* | *Ruminococcus* |
| *Campylobacter concisus* | *Shigella* spp. | *Roseburia* |
| *Campylobacter curvus* | *Yersinia* spp. | *Faecalibacterium* spp. |
| *Campylobacter gracilis* | *Yersinia bercovieri* | *Faecalibacterium prausnitzii* |
| *Campylobacter helveticus* | *Yersinia rohdei* | *Peptococcus* |
| *Campylobacter hominis* | *Helicobacter* spp. | *Peptostreptococcus* |
| *Campylobacter rectus* | *Helicobacter felis* | *Xanthomonas* |
| *Campylobacter showae* | *Helicobacter fennelliae* | *Pseudomonas* spp. |
| *Campylobacter sputorum* | *Helicobacter pylori* | *Pseudomonas putida* |
| *Campylobacter fetus* | *Vibrio* spp. | *Pseudomonas aeruginosa* |
| *Hafnia* | *Vibrio parahaemolyticus* | *Klebsiella* spp. |
| *Proteus* spp. | *Vibrio cholerae* | *Klebsiella oxytoca* |
| *Proteus mirabilis* | *Clostridium* | *Klebsiella ozaenae (K. pneumonia* subsp. *ozaenae)* |
| *Proteus penneri* | *Escherichia* | *Klebsiella pneumoniae* |
| *Proteus vulgaris* | *Bacteroides* | *Citrobacter* spp. |
| *Providencia* spp. | *Actinobacteria* | *Citrobacter amalonaticus* |
| *Providencia alcalifaciens* | *Actinomyces naeslundii* | *Citrobacter freundii* |
| *Providencia stuartii* | *Eubacterium* spp. | *Citrobacter koseri* |
| *Providencia rettgeri* | *Eubacterium rectale* | *Citrobacter sedlakii* |
| *Morganella* spp. | *Enterococcus* spp. | *Enterobacter* spp. |
| *Morganella morganii* subsp. *morganii* | *Enterococcus casseliflavus* | *Enterobacter aerogenes* |
| *Staphylococcus* spp. | *Enterococcus cecorum* | *Enterobacter cloacae* |
| *Staphylococcus epidermidis* | *Enterococcus dispar* | *Arcobacter* |
| *Staphylococcus aureus* | *Enterococcus faecalis* | *Bacillus* spp. |
| *Lactobacillus* spp. | *Enterococcus faecium* | *Bacillus subtilis* |
| *Lactobacillus acidophilus* | *Enterococcus gallinarum* | *Bacillus cereus* |
| *Lactobacillus casei* | *Enterococcus hirae* | *Serratia* spp. |

| | | |
|---|---|---|
| *Lactobacillus reuteri* | *Enterococcus raffinosus* | *Serratia liquefaciens* |
| *Plesiomonas shigelloides* | *Listeria* spp. | *Serratia marcescens* |
| *Porphyromonas asaccharolytica* | *Listeria grayi* | *Acinetobacter* spp. |
| *Chlamydia trachomatis* | *Listeria innocua* | *Acinetobacter haemolyticus* |
| *Cronobacter sakazakii* | *Listeria monocytogenes* | *Acinetobacter lwoffii* |
| *Edwardsiella tarda* | *Aeromonas* spp. | *Acinetobacter baumannii* |
| *Stenotrophomonas maltophilia* | *Aeromonas hydrophila* | *Corynebacterium* spp. |
| *Streptococcus* spp. | *Abiotrophia defectiva* | *Corynebacterium genitalium* |
| *Streptococcus dysgalactiae* subsp. *dysgalactiae* | *Akkermansia muciniphila* | *Corynebacterium glutamicum* |
| *Streptococcus pyogenes* | *Alcaligenes faecalis* subsp. *faecalis* | *Eggerthella lenta* |
| *Streptococcus salivarius* | *Anaerococcus tetradius* | *Veillonella parvula* |
| *Streptococcus uberis* | *Atopobium vaginae* | *Lactococcus lactis* |
| *Capnocytophaga gingivalis* | *Bifidobacterium* | *Mycoplasma fermentans* |
| *Cedecea davisae* | *Blastocystis hominis* | *Peptoniphilus asaccharolyticus* |
| *Chryseobacterium gleum* | *Desulfovibrio piger* | *Peptostreptococcus anaerobius* |
| *Trabulsiella guamensis* | *Gemella morbillorum* | *Porphyromonas levii* |
| *Veillonella atypica* | *Leminorella grimontii* | *Prevotella melaninogenica* |
| **Parasites** | | |
| *Giardia* | *Cryptosporidium* | *Cyclospora* |
| *Dientamoeba* | *Entamoeba* | |
| **Fungi** | | |
| *Candida albicans* | *Saccharomyces* | *Penicillium* |
| *Rhodotorula* | *Candida catenulata* | *Sclerotinia* |
| *Trametes* | *Galactomyces* | *Bullera* |
| *Pleospora* | | |
| **Human** | | |
| human DNA | | |

According to the *in silico* analysis, analytical specificity of the GastroBac *direct* PCR was evaluated by testing a panel of 40 bacterial strains representing common gastrointestinal organisms that could potentially cross-react with oligonucleotides from the test. Three replicates of each strain at $10^6$ copies/ml were tested as spiked and non-spiked groups (Table 11). Spiked groups contained target analytes at concentrations of 3x LoD.

In the spiked group, positive signals were observed for all four targets in each set of triplicate samples. In contrast, unspiked samples displayed positive signals only for the Internal Control, while the targets showed negative signals.

*Table 11: Wet-lab analysis for cross-reactivity of GastroBac direct PCR. D: detected; ND: not detected*

| Nr. | Organisms | Final Concentration (copies/ml) | Spiked Sample | | Unspiked Sample | |
|---|---|---|---|---|---|---|
| | | | Targets | Internal Control | Targets | Internal Control |
| 1 | *Acinetobacter baumannii* | 1.00E+06 | D | D | ND | D |
| 2 | *Aeromonas hydrophila* | 1.00E+06 | D | D | ND | D |
| 3 | *Bacillus cereus* | 1.00E+06 | D | D | ND | D |
| 4 | *Campylobacter fetus* subsp. *fetus* | 1.00E+06 | D | D | ND | D |
| 5 | *Enterobacter cloacae* subsp. *cloaecae* | 1.00E+06 | D | D | ND | D |
| 6 | *Enterococcus faecalis* | 1.00E+06 | D | D | ND | D |
| 7 | *Enterococcus faecium* | 1.00E+06 | D | D | ND | D |
| 8 | *Eubacterium limosum* | 1.00E+06 | D | D | ND | D |
| 9 | *Helicobacter pylori* | 1.00E+06 | D | D | ND | D |
| 10 | *Klebsiella aerogenes* | 1.00E+06 | D | D | ND | D |
| 11 | *Klebsiella pneumoniae* | 1.00E+06 | D | D | ND | D |
| 12 | *Listeria monocytogenes* | 1.00E+06 | D | D | ND | D |

| Nr. | Organisms | Final Concentration (copies/ml) | Spiked Sample | | Unspiked Sample | |
|---|---|---|---|---|---|---|
| | | | Targets | Internal Control | Targets | Internal Control |
| 13 | *Proteus mirabilis* | 1.00E+06 | D | D | ND | D |
| 14 | *Proteus vulgaris* | 1.00E+06 | D | D | ND | D |
| 15 | *Providencia rettgeri* | 1.00E+06 | D | D | ND | D |
| 16 | *Pseudomonas aeruginosa* | 1.00E+06 | D | D | ND | D |
| 17 | *Serratia marcescens* | 1.00E+06 | D | D | ND | D |
| 18 | *Vibrio cholerae* | 1.00E+06 | D | D | ND | D |
| 19 | *Actinomyces israelii* | 1.00E+06 | D | D | ND | D |
| 20 | *Morganella morganii* subsp. *morganii* | 1.00E+06 | D | D | ND | D |
| 21 | Homo sapiens (HELA) | 1.00E+06 | D | D | ND | D |
| 22 | *Bacillus subtilis* | 1.00E+06 | D | D | ND | D |
| 23 | *Campylobacter concisus* | 1.00E+06 | D | D | ND | D |
| 24 | *Campylobacter curvus* | 1.00E+06 | D | D | ND | D |
| 25 | *Campylobacter gracilis* | 1.00E+06 | D | D | ND | D |
| 26 | *Campylobacter hominis* | 1.00E+06 | D | D | ND | D |
| 27 | *Campylobacter rectus* | 1.00E+06 | D | D | ND | D |
| 28 | *Campylobacter showae* | 1.00E+06 | D | D | ND | D |
| 29 | *Campylobacter sputorum* | 1.00E+06 | D | D | ND | D |
| 30 | *Corynebacterium glutamicum* | 1.00E+06 | D | D | ND | D |
| 31 | *Edwardsiella tarda* | 1.00E+06 | D | D | ND | D |
| 32 | *Klebsiella oxytoca* | 1.00E+06 | D | D | ND | D |
| 33 | *Klebsiella pneumonia* subsp. *ozaenae* | 1.00E+06 | D | D | ND | D |
| 34 | *Lacticaseibacillus casei* | 1.00E+06 | D | D | ND | D |
| 35 | *Lactococcus lactis* | 1.00E+06 | D | D | ND | D |
| 36 | *Limosilactobacillus reuteri* | 1.00E+06 | D | D | ND | D |
| 37 | *Listeria innocua* | 1.00E+06 | D | D | ND | D |
| 38 | *Pseudomonas putida* | 1.00E+06 | D | D | ND | D |
| 39 | *Stenotrophomonas maltophilia* | 1.00E+06 | D | D | ND | D |
| 40 | *Staphylococcus aureus* | 1.00E+06 | D | D | ND | D |

Moreover, three replicates of all target species were analyzed at $10^6$ copies/ml for cross-reactivity with the other target analytes (Table 12). Only the Internal Control and the respective organism that was present were detected in all three replicates.

*Table 12: Wet-lab analysis of potential cross-reactivity of target organisms. D: detected; ND: not detected*

| Nr. | Organisms | Final Concentration (copies/ml) | Channel (Target) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Cyan500 (IC) | FAM (Camp.) | HEX (Salm.) | Red610 (Shig.) | Cy5 (Yers.) |
| 41 | *Campylobacter jejuni* subsp. *jejuni* | 1.00E+06 | D | D | ND | ND | ND |
| 42 | *Campylobacter hyointestinalis* | 1.00E+06 | D | D | ND | ND | ND |
| 43 | *Campylobacter coli* | 1.00E+06 | D | D | ND | ND | ND |
| 44 | *Campylobacter upsaliensis* | 1.00E+06 | D | D | ND | ND | ND |
| 45 | *Campylobacter lari* | 1.00E+06 | D | D | ND | ND | ND |
| 46 | *Salmonella enterica* subsp. *enterica* | 1.00E+06 | D | ND | D | ND | ND |
| 47 | *Salmonella enterica* subsp. *enterica, Typhimurium* | 1.00E+06 | D | ND | D | ND | ND |
| 48 | *Salmonella bongori* | 1.00E+06 | D | ND | D | ND | ND |
| 49 | *Shigella boydii* | 1.00E+06 | D | ND | ND | D | ND |
| 50 | *Shigella dysenteriae* | 1.00E+06 | D | ND | ND | D | ND |
| 51 | *Shigella flexneri* | 1.00E+06 | D | ND | ND | D | ND |
| 52 | *Shigella sonnei* | 1.00E+06 | D | ND | ND | D | ND |
| 53 | *Yersinia enterocolitica* subsp. *enterocolitica* | 1.00E+06 | D | ND | ND | ND | D |
| 54 | *Yersinia pseudotuberculosis* | 1.00E+06 | D | ND | ND | ND | D |

### 20.3 Endogenous and Exogenous Interfering Substances

In total, 20 endogenous and exogenous substances potentially present in clinical samples and possibly affecting the performance of the GastroBac *direct* PCR were tested in the highest concentration that could occur in a clinical setting and that is technically possible concerning the sample composition. The substances were spiked into 0.9 % NaCl solution and extracted via the IVD-1049 chemagic™ Pathogen NA gDNA Kit H96 on a chemagic™ 360 instrument in triplicates. The eluates were tested with the GastroBac *direct* PCR in spiked (containing target analytes at 3x LoD) and non-spiked groups (Table 13). Within the spiked groups, all five targets were detected for each set of replicates whereas for the non-spiked groups, no target signals were obtained. The IC signal was detected for all samples.

*Table 13: Tested potentially interfering substances (ND: not detected; D: detected)*

| # | Substance | Concentration | Unit | Spiked Sample | | Unspiked Sample | |
|---|---|---|---|---|---|---|---|
| | | | | Targets | Internal Control | Targets | Internal Control |
| 1 | Trigylcerides | 5 | %(v/v) | D | D | ND | D |
| 2 | Cholesterol | 4 | mg/mL | D | D | ND | D |
| 3 | Blood clot/blood | 2 | %(v/v) | D | D | ND | D |
| 4 | Mucin | 1 | mg/mL | D | D | ND | D |
| 5 | Hemoglobin | 2 | mg/mL | D | D | ND | D |
| 6 | Calcium carbonate | 5 | mg/mL | D | D | ND | D |
| 7 | Magnesium hydroxide | 1 | mg/mL | D | D | ND | D |
| 8 | Sennoside A | 0.2 | mg/mL | D | D | ND | D |
| 9 | Bismuth(III) subsalicylate | 3.5 | mg/mL | D | D | ND | D |
| 10 | Loperamide | 5 | mM | D | D | ND | D |
| 11 | Naproxen | 10 | mg/mL | D | D | ND | D |
| 12 | Hydrocortisone | 1 | mg/mL | D | D | ND | D |
| 13 | Azithromycin | 0.0111 | mg/mL | D | D | ND | D |
| 14 | Ceftriaxone | 0.84 | mg/mL | D | D | ND | D |
| 15 | Bacitracin | 250 | U/mL | D | D | ND | D |
| 16 | Ampicillin | 0.152 | mM | D | D | ND | D |
| 17 | Nystatin | 10000 | U/mL | D | D | ND | D |
| 18 | Mineral oil | 2 | %(v/v) | D | D | ND | D |
| 19 | Ethanol | 1 | %(v/v) | D | D | ND | D |
| 20 | Cary-Blair medium | 100 | %(v/v) | D | D | ND | D |
| 21 | $H_2O$* | 10 | %(v/v) | D | D | ND | D |
| 22 | DMSO* | 10 | %(v/v) | D | D | ND | D |
| 23 | $CHCl_3$:$C_2H_6O$ (1:1)* | 10 | %(v/v) | D | D | ND | D |
| 24 | HCl (5 M)* | 10 | %(v/v) | D | D | ND | D |

**Served as control to exclude potential solvent effects*

### 20.4 Inclusivity

For inclusivity analysis, an *in silico* analysis was performed. In total, 1113 *Campylobacter*, 2247 *Salmonella*, 374 *Shigella*/EIEC, and 66 *Yersinia* sequences available from NCBI Genbank were analyzed. At least 95% identical sequences without mismatches to the primer and probe sequences of the assay were found for *Campylobacter* spp., *Shigella* spp./EIEC, *Y. enterocolitica*, and *Y. pseudotuberculosis*. *Salmonella* sequences showed a higher variability in the target gene region than the other analytes. More than 70% of all *Salmonella* sequences analyzed and more than 99% of the most common serovars *S.* Typhimurium and *S.* Enteritidis were identical to the primer and probe sequences. The typhoidal serovars *S.* Typhi and *S.* Paratyphi A/B/C showed an identity of 100% and 30.95%, respectively. The most frequent mutations in the analyzed *Salmonella* sequences were single mismatches in the forward or reverse primer but not in the 3' region of the primers. Therefore, these mutations are unlikely to affect the performance of the assay. The wet-lab detection of some selected species and serotypes of the underlying analytes was evaluated (Table 14) and show detection of all species at 10 copies/reaction.

*Table 14: Number of positive signals of additional targets with 100 copies/reaction or 10 copies/reaction in 5 replicates each.*

| Analyte | 100 cp/rxn | 10 cp/rxn |
|---|---|---|
| *Campylobacter hyointestinalis* | 5/5 | 5/5 |
| *Campylobacter coli* | 5/5 | 5/5 |
| *Campylobacter upsaliensis* | 5/5 | 5/5 |
| *Campylobacter lari* subsp. *lari* | 5/5 | 5/5 |
| *Salmonella enterica* subsp. *enterica*, Typhimurium | 5/5 | 5/5 |
| *Salmonella bongori* | 5/5 | 5/5 |
| *Shigella boydii* | 5/5 | 5/5 |
| *Shigella dysenteriae* | 5/5 | 5/5 |
| *Shigella sonnei* | 5/5 | 5/5 |

### 20.5 Competitive Interference

To analyse competitive interference, one test analyte was applied at the highest possible concentration ($10^6$ cp/ml), whereas the other test analytes were applied at a low concentration (3x LoD). If analytes at 3x LoD produced negative results, lower concentrations of each spiking analyte ($10^5$ cp/ml) were tested. All possible combinations of analytes were tested in triplicates.

In most cases, a high concentration of spiking analyte up to $10^6$ DNA copies/ml did not affect the detection of other analytes present at a low concentration (3x LoD). However, there was one exception. In the presence of $10^6$ DNA copies/ml *Yersinia pseudotuberculosis*, one out of three *Salmonella enterica* samples was not detectable. Therefore, we conducted a retest with $10^5$ DNA copies/ml *Yersinia pseudotuberculosis* with 3x LoD *Salmonella enterica*, resulting in the successful detection of all 3 replicates of *Salmonella enterica* at 3x LoD (Table 15).

In conclusion, these data show that there is potential for competitive interference of *Salmonella enterica* at low concentration (3x LoD) when *Yersinia pseudotuberculosis* concentration is ≥ $10^6$ DNA copies/ml. As a result of these findings, it is recommended that the detection of *Yersinia pseudotuberculosis* should not exclude the possibility of a co-infection with *Salmonella enterica*.

*Table 15: Competitive interference. Positivity lower than 100% is highlighted in red.*

| Sample | Analyte | Positive Wells | Total | % Positivity |
|---|---|---|---|---|
| ***Campylobacter jejuni* subsp. jejuni** at $10^6$ DNA copies/ml with 3x LoD *Shigella flexneri* | *Shigella* | 3 | 3 | 100.0 |
| *Campylobacter jejuni* subsp. jejuni at $10^6$ DNA copies/ml with 3x LoD *Salmonella enterica* | *Salmonella* | 3 | 3 | 100.0 |
| *Campylobacter jejuni* subsp. jejuni at $10^6$ DNA copies/ml with 3x LoD *Yersinia enterocolitica* subsp. *enterocolitica* | *Y. enterocolitica* | 3 | 3 | 100.0 |
| *Campylobacter jejuni* subsp. jejuni at $10^6$ DNA copies/ml with 3x LoD *Yersinia pseudotuberculosis* | *Y. pseudotuberculosis* | 3 | 3 | 100.0 |
| ***Shigella flexneri*** at $10^6$ DNA copies/ml with 3x LoD *Campylobacter jejuni* subsp. *jejuni* | *Campylobacter* | 3 | 3 | 100.0 |
| *Shigella flexneri* at $10^6$ DNA copies/ml with 3x LoD *Salmonella enterica* | *Salmonella* | 3 | 3 | 100.0 |
| *Shigella flexneri* at $10^6$ DNA copies/ml with 3x LoD *Yersinia enterocolitica* subsp. *enterocolitica* | *Y. enterocolitica* | 3 | 3 | 100.0 |
| *Shigella flexneri* at $10^6$ DNA copies/ml with 3x LoD *Yersinia pseudotuberculosis* | *Y. pseudotuberculosis* | 3 | 3 | 100.0 |
| ***Salmonella enterica*** at $10^6$ DNA copies/ml with 3x LoD *Campylobacter jejuni* subsp. *jejuni* | *Campylobacter* | 3 | 3 | 100.0 |
| *Salmonella enterica* at $10^6$ DNA copies/ml with 3x LoD *Shigella flexneri* | *Shigella* | 3 | 3 | 100.0 |
| *Salmonella enterica* at $10^6$ DNA copies/ml with 3x LoD *Yersinia enterocolitica* subsp. *enterocolitica* | *Y. enterocolitica* | 3 | 3 | 100.0 |
| *Salmonella enterica* at $10^6$ DNA copies/ml with 3x LoD *Yersinia pseudotuberculosis* | *Y. pseudotuberculosis* | 3 | 3 | 100.0 |
| ***Yersinia enterocolitica* subsp. enterocolitica** at $10^6$ DNA copies/ml with 3x LoD *Campylobacter jejuni* subsp. *jejuni* | *Campylobacter* | 3 | 3 | 100.0 |

| | | | | |
|---|---|---|---|---|
| *Yersinia enterocolitica* subsp. enterocolitica at $10^6$ DNA copies/ml with 3x LoD *Shigella flexneri* | *Shigella* | 3 | 3 | 100.0 |
| *Yersinia enterocolitica* subsp. enterocolitica at $10^6$ DNA copies/ml with 3x LoD *Salmonella enterica* | *Salmonella* | 3 | 3 | 100.0 |
| ***Yersinia pseudotuberculosis*** at $10^6$ DNA copies/ml with 3x LoD *Campylobacter jejuni* subsp. *jejuni* | *Campylobacter* | 3 | 3 | 100.0 |
| *Yersinia pseudotuberculosis* at $10^6$ DNA copies/ml with 3x LoD *Shigella flexneri* | *Shigella* | 3 | 3 | 100.0 |
| *Yersinia pseudotuberculosis* at $10^6$ DNA copies/ml with 3x LoD *Salmonella enterica* | *Salmonella* | 2 | 3 | 66.7 |
| *Yersinia pseudotuberculosis* at $10^5$ DNA copies/ml with 3x LoD *Salmonella enterica* | *Salmonella* | 3 | 3 | 100.0 |

### 20.6 Precision

The analytical performance parameter precision is derived from repeatability and reproducibility.

Reproducibility was determined through repeated analyses conducted by two operators on five different days with three different kits and cyclers (one cycler per kit). For repeatability, the analyses were repeated by two operators with one kit on one cycler on seven more days additional to the first five days. The agreement between different variation parameters (lot, instruments, operator) was evaluated (Table 16).

*Table 16: Precision variation parameters*

| Experiment | Number of days | Kit (lot) | Instrument | Number of operators | # positive samples | # negative samples |
|---|---|---|---|---|---|---|
| reproducibility | 5 | #1 | Instrument 1 (LC480 II) | 2 | 90 | 60 |
| | | #2 | Instrument 2 (CFX Opus) | | | |
| | | #3 | Instrument 3 (CFX Opus) | | | |
| repeatability | 12 | #3 | Instrument 3 (CFX Opus) | 2 | 72 | 48 |

The coefficient of variation (CV) was calculated using the following formula:

$$CV = \frac{standard\ deviation}{mean} * 100\%$$

CV values of the reproducibility study were ≤ 5.00% and CV values of the repeatability study were ≤ 3.00% (Table 17). Moreover, all detected targets were shown in the expected channel, and no false positive signals were observed.

*Table 17: Reproducibility and repeatability of CV values.*

| Analyte (3x LoD) | Reproducibility CV (%) | Repeatability CV (%) |
|---|---|---|
| Internal Control | 3.61 | 2.88 |
| *Campylobacter jejuni* subsp. *jejuni* | 1.45 | 0.81 |
| *Salmonella enterica* | 2.92 | 2.38 |
| *Shigella flexneri* | 0.98 | 0.48 |
| *Yersinia enterocolitica* subsp. *enterocolitica* | 2.03 | 1.73 |
| *Yersinia pseudotuberculosis* | 2.03 | 1.30 |

### 20.7 Carryover/Cross-contamination

To analyze potential carryover/cross-contamination, the columns of one 96-well plate were filled in an alternating pattern with negative samples ($H_2O$) and high positive samples ($10^6$ copies/mL for each test analyte). There were no false negative and no false positive test results.

**20.8 References**


[1] RKI, “Ratgeber Campylobacter,” 11 April 2024. [Online]. Available: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Campylobacter.html.

[2] DGVS, “S2k-Leitlinie: Gastrointestinale Infektionen der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten,” *AWMF online,* Version 2.1 - November 2023.

[3] G. Kapperud, J. Lassen, S. Ostroff and S. Aasen, “Clinical features of sporadic Campylobacter infections in Norway,” *Scand J Infect Dis.,* pp. 24(6):741-749, 1992.

[4] S. Porwollik, E. F. Boyd, C. Choy, P. Cheng, L. Florea, E. Proctor and M. McClelland, “Characterization of Salmonella enterica subspecies I genovars by use of microarrays.,” vol. 186, no. 17, 2004.

[5] RKI, “Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2020,” 2021.

[6] RKI, “Ratgeber Salmonellose,” 15 April 2024. [Online]. Available: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Salmonellose.html.

[7] WHO, “State of the art of new vaccine research and development,” 2006.

[8] RKI, “Ratgeber Shigellose,” 17 April 2024. [Online]. Available: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Shigellose.html.

[9] FDA, “Bad Bug Book - Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins,” [Online]. Available: https://www.fda.gov/media/83271/download. [Accessed 25 April 2024].

[10] RKI, “Ratgeber Yersiniose,” 18 April 2024. [Online]. Available: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Yersiniose.html.