RIDA®UNITY Bacterial Stool Panel

REF UN2405

R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany
+49 (0) 61 51 81 02-0 / +49 (0) 61 51 81 02-20 / www.r-biopharm.com

CE 0123

1. Intended use

For *in vitro* diagnostic use. The RIDA®UNITY Bacterial Stool Panel test, performed on the RIDA®UNITY platform, is a multiplex real-time PCR for the direct qualitative detection and differentiation of *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., and *Yersinia enterocolitica* DNA in untreated human stool samples from people with signs and symptoms of acute gastroenteritis.
The RIDA®UNITY Bacterial Stool Panel test is intended to support the differential diagnosis of bacterial infections (*Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., and *Yersinia enterocolitica*) in patients with symptoms of gastroenteritis in connection with other clinical and laboratory findings.
Negative results do not rule out an infection with *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., or *Yersinia enterocolitica* and should not be used as the sole basis for diagnosis.

The product is intended for professional use.

2. Summary and explanation of the test

Diarrheal illnesses are a significant health problem, causing approximately 1.7 billion cases per year in children worldwide.[1] According to the World Health Organization (WHO), at approximately 525,000 deaths per year, they are the second leading cause of death in children under the age of 5, especially in developing countries.[1] *Campylobacter* spp., *Salmonella* spp., and *Yersinia enterocolitica* infections are common causes of cases of bacterial diarrhea.[2]
Worldwide, *Campylobacter* species are one of the four most common causes of bacterial diarrhea.[3] In the USA, these pathogens cause approximately 1.5 million cases (campylobacteriosis) per year.[4] According to the Robert Koch Institute (RKI), the incidence in Germany is 80 to 90 illnesses per 100,000 persons.[5] The overwhelming majority of reported *Campylobacter* infections are caused by *C. jejuni*, whose worldwide incidence even surpasses that of *E. coli*.[2, 3] *C. coli* is also an important species within the genus and is responsible for 1 % to 25 % of all *Campylobacter*-induced cases of diarrhea.[3] *Campylobacter* infections in humans are primarily related to foods. The primary route of transmission is therefore the consumption of contaminated, undercooked meat as well as raw milk and contaminated water. An infectious dose is relatively low at 500 bacteria.[5] The typical incubation time of *Campylobacter* spp. is between 1 and 7 days with various symptoms of illness. Characteristic symptoms of campylobacteriosis are diarrhea with watery to bloody stool, fever, weakness, and stomach pain.[2, 5]

Salmonella species are also one of the main causes of bacterial gastroenteritis worldwide. The genus *Salmonella* is divided into 2 species, *S. enterica* and *S. bongori*, and currently includes approximately 2,500 serotypes. Salmonella can cause three forms of salmonellosis: non-invasive and non-typhoid, invasive and non-typhoid, and typhoid fever.[2] According to the Centers for Disease Control and Prevention (CDC), there are approximately 1.35 million cases of salmonellosis per

year in the USA, with more than 26,500 hospitalizations and 420 deaths.[6] Most cases of salmonellosis are caused by *S. typhimurium* and *S. enteritidis*. In contrast, typhus is caused by *S. typhi* and *S. paratyphi* A, B, or C.[7] Worldwide there are nearly 22 million cases of typhus per year, with 200,000 deaths.[8] Salmonella is transmitted through the consumption of improperly prepared eggs or egg-containing products, raw meat, contaminated water, or contact with infected animals.[7] An infectious dose of salmonella varies from 1 to 1,000 bacteria. Salmonellosis appears after an incubation period of 6 to 72 hours with clinical symptoms such as nausea, vomiting, stomach cramps, diarrhea, fever, and headache.[2, 7] Persons with typhus suffer from headache, limb pain, high fever (from 39 °C to 41 °C), and abdominal complaints within 2 to 3 days.[8]

Yersinia enterocolitica is one of three *Yersinia* species (*Y. pestis*, *Y. pseudotuberculosis*) of the genus *Yersinia*. *Y. enterocolitica* and *Y. pseudotuberculosis* can cause intestinal yersiniosis. According to the CDC, in the USA nearly 117,000 *Y. enterocolitica* cases occur each year, with 640 hospitalizations and 35 deaths.[9] An infection with *Y. enterocolitica* comes from contaminated food, especially raw or undercooked pork, or contaminated water.[10] After an incubation time of 3 to 7 days, persons with yersiniosis suffer from diarrhea, vomiting, and stomach pain. The symptoms can last for up to 3 weeks.[10, 11]

3. Test principle

The RIDA®UNITY Bacterial Stool Panel is a multiplex real-time PCR for the direct, qualitative detection of *Campylobacter* spp. (*C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., and *Yersinia enterocolitica* in human stool samples.
Processing is fully automated with the RIDA®UNITY system. First, the nucleic acids are extracted using the RIDA®UNITY Universal Extraction Kit and the Internal Control Kit.
The target sequence is detected in a one-step real-time RT-PCR format, that is, reverse transcription (RT) and subsequent PCR are performed in one reaction vial. In the process, the isolated RNA is transcribed into cDNA with the help of reverse transcriptase. The specific gene fragments for *Campylobacter* spp. (16S rDNA), *Salmonella* spp. (ttr), and *Yersinia enterocolitica* (ystA/ystB) are then amplified using real-time PCR.
The amplified target sequences of *Campylobacter* spp., *Salmonella* spp., and *Y. enterocolitica* are detected using hydrolysis probes that are labeled with a quencher on one end and a fluorescent reporter dye (fluorophore) on the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, Taq polymerase separates the reporter from the quencher. The reporter emits a fluorescent signal that is detected by the optical unit of a real-time PCR instrument. The fluorescent signal increases with the quantity of formed amplicons.
The RIDA®UNITY Internal Control Kit must be used at the same time to be able to check sample preparation and/or potential PCR inhibition.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.*

Tab. 1: Reagents provided

Reagent	Amount		Lid color
Reaction Mix	1 ×	1935 µL	yellow, ready for use
Enzyme Mix	1 ×	350 µL	red, ready for use
Positive Control	1 ×	200 µL	blue, ready for use
Negative Control	1 ×	450 µL	white, ready for use

*With repeated use and in smaller series, the number of reactions may be reduced.

5. Storage instructions

- Please follow the handling guidelines in Table 2 and store the kit directly after use according to the information specified.
- All reagents must be stored away from light at -16 °C to -28 °C and, if unopened, can be used until the expiration date printed on the label. The quality guarantee is no longer valid after the expiration date.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 - 8 °C).
- Repeated freezing and thawing up to 8 times does not affect the test properties.

- **Tab. 2:** Storage conditions and information

	Storage temperature	Maximum storage time
unopened	-16 °C to -28 °C	Can be used until the printed expiration date
opened	-16 °C to -28 °C	8 freeze-thaw cycles

6. Reagents required but not provided

The RIDA®UNITY Bacterial Stool Panel multiplex real-time RT-PCR test is intended exclusively for use with the RIDA®UNITY system. The following products are absolutely required for correct use:

6.1 Reagents

The following reagents are needed for performing the RIDA®UNITY Bacterial Stool Panel test:

Reagents	Item number
RIDA®UNITY Universal Extraction Kit (R-Biopharm AG)	UN0001
RIDA®UNITY Internal Control Kit (R-Biopharm AG)	UN0010

6.2 Laboratory equipment

The following equipment is needed for performing the RIDA®UNITY Bacterial Stool Panel test:

Equipment
RIDA®UNITY system (R-Biopharm)
RIDA®UNITY consumables (tips, plates, reaction vials, films). See the instructions for use for the RIDA®UNITY system, ordering information for consumables.
Vortexer
Tabletop centrifuge
Powder-free disposable gloves

The RIDA®UNITY Bacterial Stool Panel kit can be used in conjunction with other compatible cyclers. Alternative real-time PCR instruments must be verified/validated by the user. Please contact R-Biopharm AG at pcr@r-biopharm.de to check the compatibility.

7. Warnings and precautions for the users

For *in vitro* diagnostic use.
This test must be carried out only by qualified laboratory personnel. The guidelines for working in medical laboratories must be observed.
Always adhere strictly to the operating manual when carrying out this test.
Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes.
Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test.
Do not smoke, eat, or drink in areas where samples are handled.
Avoid contaminating the samples and components of the kit with microbes and nucleases (DNase/RNase).
Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
Do not exchange or mix the components (Reaction Mix, Enzyme Mix, Positive Control, Negative Control) of one kit lot with the components of another lot.
The test kit can be used within 4 weeks after the first opening and should not be used after the expiration date. These specifications are also checked by the RIDA®UNITY system.
Users are responsible for the proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

Further details on the Safety Data Sheet (SDS) can be found under the item number at https://clinical.r-biopharm.com/search/.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

The summary of safety and performance (SSP) for this product will be available at https://ec.europa.eu/tools/eudamed once the European Database on Medical Devices (EUDAMED) gets underway. In the database, search for the device using the UDI-DI located on the outer packaging of the device.

8. Collection and storage of samples

It is recommended to use fresh sample material to achieve the best possible performance of the RIDA®UNITY Bacterial Stool Panel assay.

Avoid repeated freezing and thawing of the sample.

Do not collect the stool samples in transport containers that contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since such substances can interfere with the RIDA®UNITY tests.

It is recommended to produce aliquots of the specimens to avoid repeated thawing and freezing. Frozen specimens should be thawed immediately prior to extraction to prevent degradation of the nucleic acids.

Please follow the sample storage specifications in Table 3, Table 4, and Table 5.

Tab. 3: Sample storage - detection of *Campylobacter* spp. *(C. coli, C. lari, C. jejuni)*

Native samples - stool		
20 - 25 °C	2 - 8 °C	-20 °C / -80 °C
≤ 7 days	≤ 7 days	≤ 3 months / ≤ 6 months

In eluate		
+30 °C	+4 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

At a storage temperature of -20 °C / -80 °C, repeated freezing/thawing of the eluate for up to 3 times does not affect the test properties.

Tab. 4: Sample storage - detection of *Salmonella* spp.

Native samples - stool		
20 - 25 °C	2 - 8 °C	-20 °C / -80 °C
< 1 day	≤ 7 days	≤ 6 months

In eluate		
+30 °C	+4 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

At a storage temperature of -20 °C / -80 °C, repeated freezing/thawing of the samples/eluate for up to 3 times does not affect the test properties.

Tab. 5: Sample storage - detection of *Yersinia enterocolitica*

Native samples - stool		
20 - 25 °C	2 - 8 °C	-20 °C / -80 °C
-	≤ 7 days	≤ 6 months

In eluate		
+30 °C	+4 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

At a storage temperature of -20 °C / -80 °C, repeated freezing/thawing of the samples/eluate for up to 3 times does not affect the test properties.

8.1 DNA preparation from stool samples

To isolate DNA from stool samples, use the RIDA®UNITY Universal Extraction Kit (UN0001). Follow the direct procedures in the instructions for use for the RIDA®UNITY Universal Extraction Kit (Section: Nucleic acid preparation from stool samples).

9. Test procedure

Place both the samples and the reagents of the RIDA®UNITY Bacterial Stool Panel kit on the RIDA®UNITY system at the beginning of use.

Beforehand, adequately mix the Reaction Mix, Negative Control, and Positive Control using a vortexer. Do not vortex the Enzyme Mix. Afterward, briefly centrifuge all components.

The PCR tubes for the samples to be examined must be positioned beforehand in the integrated PCR cycler.

Carriers are available for correctly loading the system with reagents and consumables. For the loading process, follow the instructions of the RIDA®UNITY system. Observe the relevant sections in the manual of the RIDA®UNITY system (Section: Performing a run).

The RIDA®UNITY Bacterial Stool Panel test may be used only in combination with the RIDA®UNITY Internal Control Kit. This allows for early recognition of potential PCR inhibition, verification of reagent integrity, and confirmation of successful nucleic acid extraction. The procedure is described in the instructions for use of the RIDA®UNITY Internal Control Kit (Section: Test procedure).

Automated processing is described in the RIDA®UNITY system manual (Section: Performing a run).

9.1 Device settings

9.1.1 Universal real-time PCR profile

To harmonize the RIDA®UNITY assays, the RIDA®UNITY Bacterial Stool Panel assay is verified exclusively in the universal profile. This makes it possible to combine DNA and RNA assays with each other. Generally speaking, reverse transcription therefore comes first in the universal profile.

Tab. 6: Universal real-time PCR profile for the RIDA®UNITY

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation	10 sec, 95 °C
Annealing/extension	15 sec, 60 °C
Temperature transition rate/ ramp rate	Maximum

Note: Annealing and extension take place in the same step.

9.2 Detection channel setting

Tab. 7: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
R-Biopharm RIDA®UNITY	*Salmonella* spp.	FAM	SEEK channel Sal
	Internal Control	HEX	SEEK channel ICD
	Yersinia enterocolitica	ROX	SEEK channel Yers
	Campylobacter spp.	Cy5	SEEK channel Campy

10. Quality control - indication of instability or expiration of reagents

The samples are evaluated using the RIDA®SEEK analytical software of the RIDA®UNITY system. The Negative Control and Positive Control must show the correct results (see Tab. 8).

The Positive Control is present in a concentration of 10^3 copies/µL. It is used in a total quantity of 5×10^3 copies in every PCR run.

The Negative Control already contains the RIDA®UNITY Internal Control. Since the controls do not contain a template, no signals are to be anticipated in the target channels. Positive signals in the IC channel with which the internal control is detected are essential (see Tab. 8).

Tab. 8: A valid PCR run must meet the following conditions:

Sample	Result	IC Ct	Target gene Ct
Positive control	+	n/a *	See Certificate of Analysis
Negative control	-	Ct > 20	0

** A Ct value for the IC is not needed to obtain a positive result of the positive control.*

If the positive control is not within the specified Ct range, but the negative control is valid, all reactions, including the controls, need to be reanalyzed in the PCR.

If the negative control is not negative, but the positive control is valid, all reactions, including the controls, need to be reanalyzed in the PCR.

If the specified values are not met, check the following items before repeating the test:

- Expiration date of the reagents used
- Functionality of the equipment being used
- Correct test procedure

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Result interpretation

Sample evaluation and interpretation are done using the RIDA®UNITY system analytical software, the RIDA®SEEK.

At this time, there is no internationally recognized reference method or reference material for standardization. The control materials are metrologically traceable to internal R-Biopharm AG standards based on specific DNA amplicons.

For further information on metrological traceability, please contact R-Biopharm AG.

The specified values, ranges, and further details can be found in the Certificate of Analysis (CoA).

Tab. 9: Result interpretation*

Detection				
***Salmonella* spp.**	***Yersinia enterocolitica***	***Campylobacter* spp.**	**IC**	**Result**
+	-	-	**+/-**	***Salmonella* spp. detectable**
-	**+**	-	**+/-**	***Yersinia enterocolitica* detectable**
-	-	**+**	**+/-**	***Campylobacter* spp. detectable**
+	**+**	-	**+/-**	***Salmonella* spp. and *Yersinia enterocolitica* detectable**
+	-	**+**	**+/-**	***Salmonella* spp. and *Campylobacter* spp. detectable**
-	**+**	**+**	**+/-**	***Yersinia enterocolitica* and *Campylobacter* spp. detectable**
+	**+**	**+**	**+/-**	***Salmonella* spp., *Yersinia enterocolitica*, and *Campylobacter* spp. detectable**
-	-	-	**+**	**Target genes not detectable**
-	-	-	**-**	**Invalid**

* + = positive
  - = negative

A sample is positive if the sample DNA and the Internal Control show an amplification signal in the detection system.

A sample is also positive if the sample DNA shows an amplification signal, but no amplification signal can be seen for the Internal Control in the detection system. Detecting the Internal Control is not necessary in this case because high amplicon concentrations can result in a weak or absent signal of the Internal Control.

A sample is negative if the sample DNA does not show an amplification signal, but an amplification signal is visible for the Internal Control in the detection system.

Inhibition of the PCR reaction can be ruled out by the detection of the Internal Control.

A sample is invalid if the sample DNA and the Internal Control do not show an amplification signal in the detection system. PCR inhibitors are present in the sample or an error occurred during the extraction process.

12. Limitations of the method

1. The RIDA®UNITY Bacterial Stool Panel test detects *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., and *Yersinia enterocolitica* DNA in untreated human stool samples. A connection between the level of the determined Ct value and the occurrence of severe clinical symptoms cannot be derived from this. The results obtained must always be interpreted in combination with the complete clinical symptoms.
2. The diagnosis should not be based on the result of the molecular biological analysis alone, but should always take the patient's medical history and symptoms into account.
3. This test is approved only for automated processing using the RIDA®UNITY system.
4. This test is verified only for stool samples.
5. Improper sampling, transport, storage, and handling, or a pathogen load below the test's analytical sensitivity can lead to false-negative results.
6. The presence of PCR inhibitors can lead to false-negative or invalid results.
7. As with all PCR-based *in vitro* diagnostic tests, extremely low concentrations of the target sequences, which are under the limit of detection (LoD 95 %), can be detected. The results obtained are not always reproducible.
8. Mutations or polymorphisms in the primer or probe binding sites can interfere with the detection of new or unknown variants and can lead to false-negative results using the RIDA®UNITY Bacterial Stool Panel.
9. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (*Salmonella* spp. (ttr), *Yersinia enterocolitica* (ystA/ystB)*, Campylobacter* spp. (16S rDNA, only *C. coli, C. lari, C. jejuni*)) are present.
10. This assay should be performed in compliance with the regulation on good laboratory practice (GLP). Users must precisely follow the manufacturer's instructions when performing the test.

13. Performance characteristics

13.1 Clinical performance characteristics

The RIDA®UNITY Bacterial Stool Panel multiplex real-time PCR was compared in an external laboratory with a CE-marked reference test.
A total of 405 stool samples from patients with clinical symptoms of a gastrointestinal infection, which were pretested positive or negative for *Campylobacter* spp., *Salmonella* spp., and *Yersinia enterocolitica*, were analyzed using the RIDA®UNITY Bacterial Stool Panel and the CE-marked reference test.
The results of the individual pathogens are shown in Tables 10 to 12:

Tab. 10: Detection of *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*)

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Bacterial Stool Panel - *Campylobacter* spp. *(C. coli, C. lari, C. jejuni)*	Positive	118	0	118
	Negative	11	276	287
	Total	129	276	405

Relative sensitivity (95 % CI)	91.5 % (85.3 % - 95.7 %)
Relative specificity (95 % CI)	100 % (98.7 % - 100 %)

Tab. 11: Detection of *Salmonella* spp.

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Bacterial Stool Panel - *Salmonella* spp.	Positive	118	1	119
	Negative	18	268	286
	Total	136	269	405

Relative sensitivity (95 % CI)	86.8 % (79.9 % - 92.0 %)
Relative specificity (95 % CI)	99.6 % (97.9 % - 100 %)

Tab. 12: Detection of *Yersinia enterocolitica*

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Bacterial Stool Panel - *Yersinia enterocolitica*	Positive	94	0	94
	Negative	8	303	311
	Total	102	303	405

Relative sensitivity (95 % CI)	92.2 % (85.1 % - 96.6 %)
Relative specificity (95 % CI)	100 % (98.8 % - 100 %)

13.2 Analytical performance characteristics

13.2.1 Detection limit (LoD 95 %)

A positive control specimen (negative stool samples, spiked) was measured in five dilution steps (in 0.25-log steps) for each target and matrix with 20 replicates per step in one lot to determine the LoD. This was followed by a probit analysis. Next, the calculated LoD was confirmed with 20 replicates per target and matrix for the calculated dilution step/concentration.
The following strains were used for testing:

Salmonella typhimurium: ATCC® 14028™
Yersinia enterocolitica: DSM 13030
Campylobacter jejuni: ATCC® 33291™
Campylobacter coli: ATCC® 43478™
Campylobacter lari: DSM 11375

To detect *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni), Salmonella* spp., and *Yersinia enterocolitica* DNA using the RIDA®UNITY Bacterial Stool Panel assay, the following limits of detection (LoD) were identified on the RIDA®UNITY system.
The results of these measurements are shown in Table 13.

Tab. 13: Limit of detection results of the RIDA®UNITY Bacterial Stool Panel test for the parameters *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni), Salmonella* spp., and *Yersinia enterocolitica.*

	***Campylobacter* spp.**	***Salmonella* spp.**	***Yersinia enterocolitica***
LoD	• *Campylobacter coli*: 14,028 CFU*/mL • *Campylobacter jejuni*: 8,128 CFU*/mL • *Campylobacter lari*: 2,762 CFU*/mL	144,212 CFU/mL	102,565 CFU/mL

*CFU: Colony Forming Units

The LoD for the parameter *Campylobacter coli* in stool samples was determined at 14,028 CFU/mL.

The LoD for the parameter *Campylobacter jejuni* in stool samples was determined at 8,128 CFU/mL.

The LoD for the parameter *Campylobacter lari* in stool samples was determined at 2,762 CFU/mL.

The LoD for the parameter *Salmonella* spp. in stool samples was determined at 144,212 CFU/mL.

The LoD for the parameter *Yersinia enterocolitica* in stool samples was determined at 102,565 CFU/mL.

13.2.2 Analytical specificity

Interfering substances

The presence of RT-PCR inhibitors and interfering substances can lead to false-negative or invalid results. Therefore, the effects of various substances that may exist given their widespread use for gastrointestinal infections or widespread occurrence in the corresponding specimens were investigated.
Substances that could possibly significantly influence the test results were first examined in an interference screen. Various substances that could be present as residue from the extraction either due to widespread use in gastrointestinal infections (various pharmaceutical or prescription drugs) or due to widespread occurrence in the corresponding control samples (e.g., mucins on the surface of mucous membranes or blood) were initially examined in high concentrations (three times the daily dose or simulation of the worst case).
No interference was found for the substances listed in Table 14.

Tab. 14: Potentially interfering substances

Potentially interfering substance	Concentration
Mucins	5 % (v/v)
Human blood/hemoglobin	5 % (v/v)
Stearic/palmitic acid	40 % (v/v)
Azithromycin-ratiopharm® 500 mg film-coated tablets (azithromycin)	84 mg/mL (w/v)
Charcoal tablets	6.0 % (w/v)
Ethanol	5 % based on the eluate
Guanidinium hydrochloride	5 % based on the eluate

Cross-reactivity

Various organisms (bacteria, parasites, fungi, and viruses) that are commonly found in the stool matrix were investigated. The microorganisms to be investigated for this assay were chosen because either they naturally occur in stool samples, or they cause corresponding symptoms as gastrointestinal pathogens. Bacterial cultures (between 10^6 and 10^9 CFU/mL) or fungal cultures, supernatants of viral cultures, isolates, or LGS standards for the given organisms were used for the analyses.

The RIDA®UNITY Bacterial Stool Panel multiplex real-time PCR is specific for *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni*), *Salmonella* spp., and *Yersinia enterocolitica*. No cross-reactivities with the following species were detected (see Tab. 15):

Tab. 15: Potentially cross-reactive organisms

Organism	Test result*		
	Campylobacter* spp. *(C. coli, C. lari, C. jejuni)	***Salmonella* spp.**	***Yersinia enterocolitica***
Adenovirus 40, human, strain Dugan	-	-	-
Adenovirus 41, human, strain Tak	-	-	-
Aeromonas hydrophila	-	-	-
Arcobacter butzleri	-	-	-
Astrovirus Type 2	-	-	-
Bacillus cereus	-	-	-
Bacteroides fragilis	-	-	-
Campylobacter fetus subsp. *fetus*	-	-	-
Campylobacter upsaliensis	-	-	-
Candida albicans	-	-	-
Citrobacter freundii	-	-	-
Clostridium bifermentans	-	-	-
Clostridium difficile	-	-	-
Clostridium novyi	-	-	-
Clostridium perfringens	-	-	-
Clostridium septicum	-	-	-
Clostridium sordellii	-	-	-
Clostridium sporogenes	-	-	-
Cryptosporidium muris	-	-	-
Cryptosporidium parvum	-	-	-
E. coli (O157:H7)	-	-	-

E. coli (O26:H-)	-	-	-
E. coli (O6)	-	-	-
Entamoeba histolytica HK-9	-	-	-
Enterobacter cloacae	-	-	-
Enterococcus faecalis	-	-	-
Giardia lamblia	-	-	-
Klebsiella oxytoca	-	-	-
Norovirus GI	-	-	-
Norovirus GII	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas aeruginosa	-	-	-
Rotavirus strain Wa	-	-	-
Serratia liquefaciens	-	-	-
Shigella flexneri	-	-	-
Staphylococcus aureus	-	-	-
Staphylococcus epidermidis	-	-	-
Vibrio parahaemolyticus	-	-	-

* - = negative

13.2.4 Precision

The precision of the RIDA®UNITY Bacterial Stool Panel real-time PCR test was determined for the following levels of consideration.

Intra-assay precision: Determination of 5 control samples using 20 replicates each on the RIDA®UNITY under identical conditions.

Inter-assay precision: Determination of 5 control samples in 20 runs in duplicate on 10 workdays (2 runs per day) performed by different technicians under reproducible conditions.

Testing for *intra*- and *inter*-assay precision was carried out using three different lots.

The precision data were obtained using five control samples, as well as the PTC and NTC belonging to the assay.

The obtained coefficients of variation of each measurement using the RIDA®UNITY Bacterial Stool Panel real-time PCR test on the RIDA®UNITY were less than 4.19 %.

Tab. 16: Precision results for the RIDA®UNITY Bacterial Stool Panel test for *Campylobacter* spp. *(C. coli*, *C. lari*, *C. jejuni)*.

Ct Mean value / CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	24.31	25.0	24.3	23.4	23.4	24.3	23.4
	CV (%)	0.95	0.43	0.35	1.86	1.81	1.90	1.86
2	Ct	31.3	31.2	31.4	27.9	27.9	27.9	27.9
	CV (%)	0.77	0.59	0.74	4.19	3.82	3.84	3.96
3	Ct	30.8	30.6	30.5	30.4	30.1	30.2	30.3
	CV (%)	0.49	0.67	0.84	1.73	2.44	1.73	2.00
4	Ct	29.0	28.9	29.3	29.1	28.9	29.0	29.0
	CV (%)	0.41	0.30	0.80	3.08	2.99	3.01	3.03
5	Ct	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Tab. 17: Results for precision of the RIDA®UNITY Bacterial Stool Panel test for *Salmonella* spp.

Ct Mean value / CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	23.6	23.7	23.7	23.6	23.7	23.6	23.7
	CV (%)	0.86	0.41	0.39	1.34	1.32	1.44	1.37
2	Ct	30.4	30.4	30.4	27.0	26.9	27.0	27.0
	CV (%)	0.71	0.58	0.74	1.31	1.24	1.29	1.27
3	Ct	30.3	30.3	30.1	30.2	30.2	30.2	30.2
	CV (%)	0.38	0.65	0.74	1.02	1.03	1.20	1.09
4	Ct	27.3	27.2	27.3	27.9	27.8	27.8	27.9
	CV (%)	0.54	0.54	0.60	1.30	1.22	1.21	1.24
5	Ct	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Tab. 18: Results for precision of the RIDA®UNITY Bacterial Stool Panel test for *Yersinia enterocolitica*.

Ct Mean value / CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	25.1	26.1	25.2	24.4	24.4	24.4	24.4
	CV (%)	0.84	0.36	0.58	1.61	1.61	1.70	1.64
2	Ct	32.6	32.5	32.4	28.6	28.5	28.4	28.5
	CV (%)	1.63	1.15	1.31	1.51	1.45	1.78	1.58
3	Ct	31.9	31.8	31.8	31.5	31.4	31.4	31.4
	CV (%)	0.81	0.91	1.08	1.60	1.58	1.45	1.54
4	Ct	29.1	29.0	29.2	29.7	29.6	29.7	29.7
	CV (%)	0.63	0.54	0.59	2.22	2.22	2.19	2.21
5	Ct	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

13.2.5 Analytical reactivity

The reactivity of the RIDA®UNITY Bacterial Stool Panel multiplex real-time PCR was examined using various *Salmonella* serovars, *Campylobacter* species, and *Yersinia enterocolitica* subspecies (see Tab. 19).

Tab. 19: Analytical reactivity testing

Strain	Concentration	Result*		
		Campylobacter* spp. *(C. coli, C. lari, C. jejuni)	***Salmonella* spp.**	***Yersinia enterocolitica***
Campylobacter coli	6.5×10^2 CFU/mL	+	-	-
Campylobacter jejuni	1.5×10^3 CFU/mL	+	-	-
Campylobacter lari ssp. *lari*	1.04×10^2 CFU/mL	+	-	-
S. enterica ssp. enterica serovar Typhi	1.49×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Typhimurium	7.7×10^3 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Enteritidis	3.3×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Paratyphi A	1.75×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Paratyphi B	2.08×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Paratyphi C	1.1×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Newport	3.1×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Heidelberg	5.9×10^3 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Javiana	1.79×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Dublin	2.88×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Agona	1.91×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Choleraesuis	9.6×10^3 CFU/mL	-	+	-
S. enterica ssp. *enterica serovar Infantis*	1.3×10^4 CFU/mL	-	+	-
S. enterica ssp. enterica serovar Montevideo	8.4×10^3 CFU/mL	-	+	-

S. enterica ssp. enterica serovar Anatum	2.38 × 10^4 CFU/mL	-	**+**	-
S. enterica ssp. enterica serovar Mississippi	2.27 × 10^4 CFU/mL	-	**+**	-
S. enterica ssp. enterica serovar Bareily	1.43 × 10^4 CFU/mL	-	**+**	-
Yersinia enterocolitica ssp. *enterocolitica*	4.2 × 10^3 CFU/mL	-	-	**+**
Yersinia enterocolitica ssp. *palearctica*	4.9 × 10^3 CFU/mL	-	-	**+**

*+ = positive (at least 2 of 3 replicates positive)
- = negative

14. Version history

Version number	Section and designation
2022-04-28	Release version

15. Explanation of symbols

General symbols

	For *in vitro* diagnostic use
	Follow instructions for use
	Batch number
	Use before
	Storage temperature
	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix	Reaction Mix
Enzyme Mix	Enzyme Mix
Negative Control	Negative control
Positive Control	Positive control

16. References

1. World Health Organisation. Diarrhoeal disease 2017, May 2 [Available from: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease#:~:text=Infection%3A%20Diarrhoea%20is%20a%20symptom%20of%20infections%20caused,and%20safe%20water%20for%20drinking%2C%20cooking%20and%20cleaning.] 2021-09-07

2. Chlebicz A, Śliżewska K. Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A Review. Int J Environ Res Public Health. 2018;15(5).

3. Costa D, Iraola G. Pathogenomics of Emerging Campylobacter Species. Clin Microbiol Rev. 2019;32(4).

4. Centers for Disease Control and Prevention. Campylobacter (Campylobacteriosis) 2021 [Available from: https://www.cdc.gov/campylobacter/index.html.] 2021-09-07

5. Institut RK. Campylobacter-Enteritis 2018 [Available from: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Campylobacter.html.] 2021-09-07

6. Centers for Disease Control and Prevention. Salmonella 2019 [Available from: https://www.cdc.gov/salmonella/index.html.

7. Robert Koch Institut. Salmonellose 2016 [Available from: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Salmonellose.html.] 2021-09-07

8. Robert Koch Institut. Typhus abdominalis, Paratyphus. 2015.

9. Centers for Disease Control and Prevention. Yersinia enterocolitica (Yersiniosis) 2016 [Available from: https://www.cdc.gov/yersinia/index.html.] 2021-09-07

10. Rosner BM, Stark K, Werber D. Epidemiology of reported Yersinia enterocolitica infections in Germany, 2001-2008. BMC Public Health. 2010;10(1):337.

11. Robert Koch Institut. Yersiniose 2019 [Available from: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Yersiniose.html.] 2021-09-07