RIDA®GENE Bacterial Stool Panel

REF PG2405

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Bacterial Stool Panel 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* in human stool samples.

The RIDA®GENE Bacterial Stool Panel multiplex real-time PCR is intended for use as an aid in diagnosis of gastrointestinal infections caused by bacteria.

2. Summary and explanation of the test

Diarrheal disease is a major health care problem and causes about 2 billion cases worldwide. The World Health Organization (WHO) ranks diarrheal 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 diarrhea each year, more than AIDS, malaria and measles combined.[1,2] Common causes of bacterial diarrheal disease are *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica*.

Campylobacter species are one of the most common causes of bacterial diarrhea worldwide, responsible for 400 million – 500 million cases annually. The disease caused by the genus *Campylobacter* is called campylobacteriosis. More than 80 % of *Campylobacter* infections are caused by *C. jejuni*. The Centers for Disease Control and Prevention (CDC) estimates more than 2 million cases of campylobacteriosis each year in the US. The Foodborne Diseases Active Surveillance Network (FoodNet) reported an incidence rate of 13 cases per 100,000 population in 2008. *C. jejuni* was detected in 5 - 16% of children with diarrhea in developed countries and in 8 - 45% of children with diarrhea in developing countries.[4] Approximately 100 persons with *Campylobacter* infections die each year in the US.[3,4] Infection with *Campylobacter* occurs through contaminated food, especially poultry, water, contact with infected animals or by fecal-oral route, particularly in children. The infectious dose is with 500 bacteria relatively low. After an incubation period of 2 to 5 days people with campylobacteriosis get fever, diarrhea, abdominal cramps, vomiting, abdominal pain and nausea. Potential long-term complications are autoimmune disorders, for example the Guillain-Barré syndrome (GBS).[4]

Salmonella species are also a leading cause of bacterial gastroenteritis worldwide. The genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*. So far, more than 2,500 *Salmonella* serotpyes are described which are pathogenic for humans. *Salmonella* species are causing nontyphoidal salmonellosis or typhoid fever. It is estimated that 93.8 million cases of nontyphoidal salmonellosis infections with 155,000 deaths occuring globally each year.[6] The CDC estimates more than 1.2 million cased of nontyphoidal salmonellosis infections each year in the United States, with more than 23,000 hospitalizations and 450 deaths.[5] Most of the nontyphoidal salmonellosis infections are caused by the *S. typhimurium* and *S. enteritidis*, while typhoid fever is caused by *S. typhi* and *S. paratyphi* A, B or C.

Transmission of *Salmonella* occurs through contaminated food, water or contact with infected animals. The infectious dose of *Salmonella* species is varying from 1 to 1000 bacteria. Nontyphoidal salmonellosis infection occurs after an incubation period of 6 – 72 h with clinical symptoms of nausea, vomiting, abdominal cramps, diarrhea, fever and headache. People with typhoid fever get headache, achiness, high fever (from 39 °C to 41 °C), gastrointestinal symptoms, including abdominal pains and diarrhea within 1 to 3 weeks after exposure to the organism.[3,7]
Yersinia enterocolitica is one of three *Yersinia* species (*Y. pestis*, *Y. pseudotuberculosis*) of the genus *Yersinia* that are pathogenic for humans and cause of the gastrointestinal disease called Yersiniosis. According to FoodNet an incidence rate of 1 *Y. enterocolitica* infection per 100,000 persons occurs each year in the U.S. The European Centre for Disease Prevention and Control reported 8,874 cases in 2007, of which about 5000 cases were from Germany. Infection with Yersiniosis occurs after ingestion of contaminated food or water. The estimated infectious dose is between 10^4 to 10^6 bacteria. After an incubation period of 1 to 11 days people with yersiniosis get diarrhea, vomiting and abdominal pain. *Y. enterocolitica* has also been associated with reactive arthritis.[3,8]
Culture is the classical method and for establishing the laboratory diagnosis of bacterial diarrhea, but requires several days.

3. Test principle

The RIDA®GENE Bacterial Stool Panel assay 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. After DNA-isolation, amplification of the gene fragments specific for *Salmonella* spp. (ttr), *Campylobacter* spp. (16S rDNA) and *Y. enterocolitica* (ystA/ystB) occurs, if present. The amplified targets of *Campylobacter* spp., *Salmonella* spp. und *Y. enterocolitica* are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity.
The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Bacterial Stool Panel assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and / or to determine possible PCR-inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Bacterial Stool Panel multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platforms	
R-Biopharm	RIDA®Xtract
Promega	Maxwell® RSC
Real-time PCR instruments	
R-Biopharm	RIDA®CYCLER
Roche	LightCycler® 480II, LightCycler® 480 z
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).
If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II or LightCycler® 480 z
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (BioScience grade, nuclease-free water)

7. Precautions for users

For *in-vitro* diagnostic use.
- This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed.
- The instruction manual for the test procedure has to be followed.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage

8.1 Sample preparation from stool samples

For DNA isolation of human stool samples, use a commercially available DNA isolation kit (e.g. RIDA®Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

It is recommend to dilute the stool samples before extraction 1:3 with water. Vortex the diluted stool sample intensely and centrifuge at 1000 x g for 30 sec. From the supernatant use the appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Bacterial Stool Panel real-time PCR assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab. 4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The

Internal Control DNA should always be added to the specimen-lysis buffer mixture and **not** directly to the specimen.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run.

We recommend calculating an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the negative control PCR-Mix.

Sample: Add 5 µl DNA-Extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the positive control PCR-Mix.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab. 5, Tab. 6, Tab. 7, Tab. 8).

9.3 PCR instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: Real-time PCR profile for LightCycler® series, Rotor-Gene Q and RIDA®CYCLER

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 6: Real-time PCR profile for Mx3005P, ABI 7500 und CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RIDA®GENE RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series and RIDA®CYCLER

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI 7500, Rotor-Gene Q and CFX96™

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection Channel	Note
R-Biopharm RIDA®CYCLER	*Salmonella* spp.	Green	-
	ICD	Yellow	
	Yersinia enterocolitica	Orange	
	Campylobacter spp.	Red	
Roche LightCycler® 480II	*Salmonella* spp.	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	533/580	
	Yersinia enterocolitica	533/610	
	Campylobacter spp.	618/660	
Roche LightCycler® 480 z	*Salmonella* spp.	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	540/580	
	Yersinia enterocolitica	540/610	
	Campylobacter spp.	610/670	
Agilent Techn. Mx3005P	*Salmonella* spp.	FAM	**Check that reference dye is none**
	ICD	HEX	
	Yersinia enterocolitica	ROX	
	Campylobacter spp.	Cy5	
ABI 7500	*Salmonella* spp.	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	Yersinia enterocolitica	ROX	
	Campylobacter spp.	Cy5	
Bio-Rad CFX96™	*Salmonella* spp.	FAM	-
	ICD	VIC	
	Yersinia enterocolitica	ROX	
	Campylobacter spp.	Cy5	
Qiagen Rotor-Gene Q	*Salmonella* spp.	Green	**The gain settings have to be set to 5, according to the default settings**
	ICD	Yellow	
	Yersinia enterocolitica	Orange	
	Campylobacter spp.	Red	

10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacture' s instruction. Positive and negative controls have to show correct results (see Table 10, Fig. 1, Fig. 2, Fig. 3) in order to determine a valid run.

The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies, respectively.

Tab. 10: For a valid run, the following conditions must be met

Sample	Assay result	ICD Ct	Target Ct
Positive Control	Positive	NA *1	See Quality Assurance Certificate
Negative Control	Negative	Ct > 20	Not detectable

**1 No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid, prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

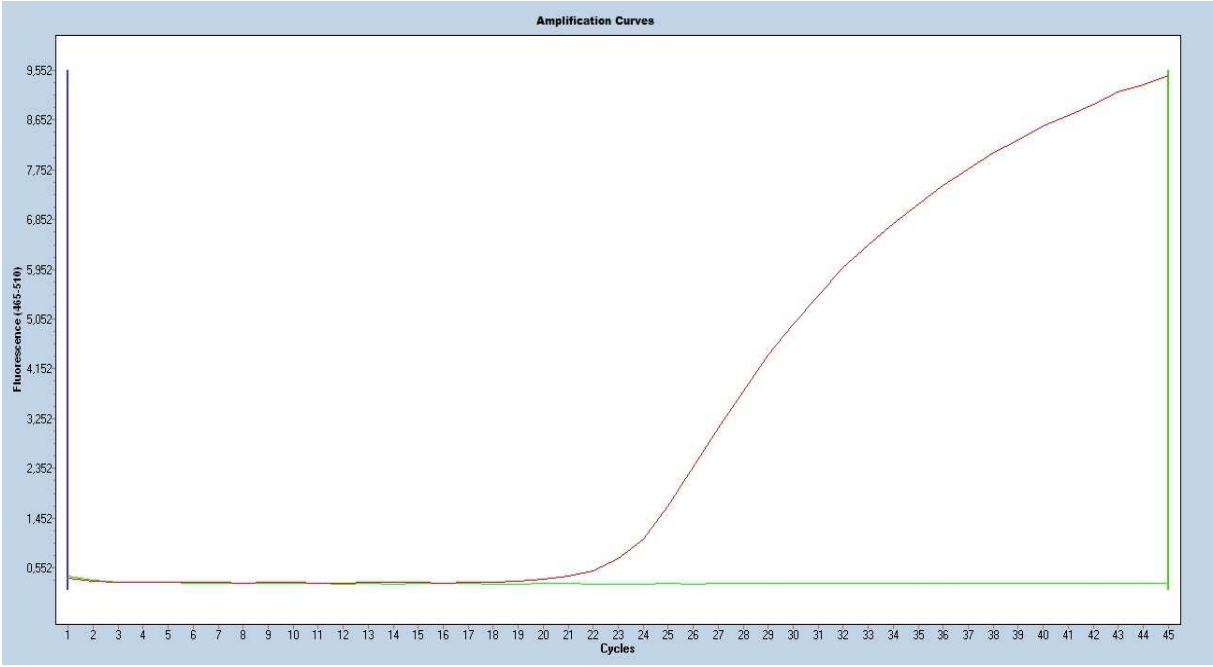

Fig. 1: Correct run of the positive and negative control (*Salmonella* spp.) on the LightCycler® 480II

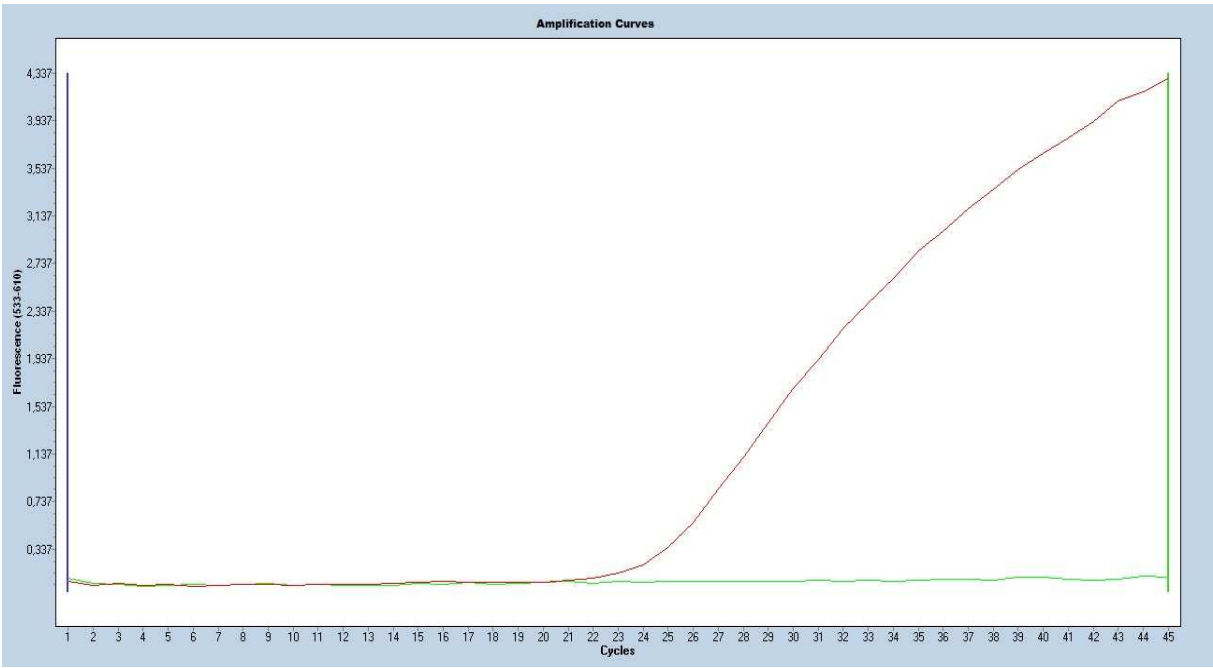

Fig. 2: Correct run of the positive and negative control (*Y*ersinia *enterocolitica*) on the LightCycler® 480II

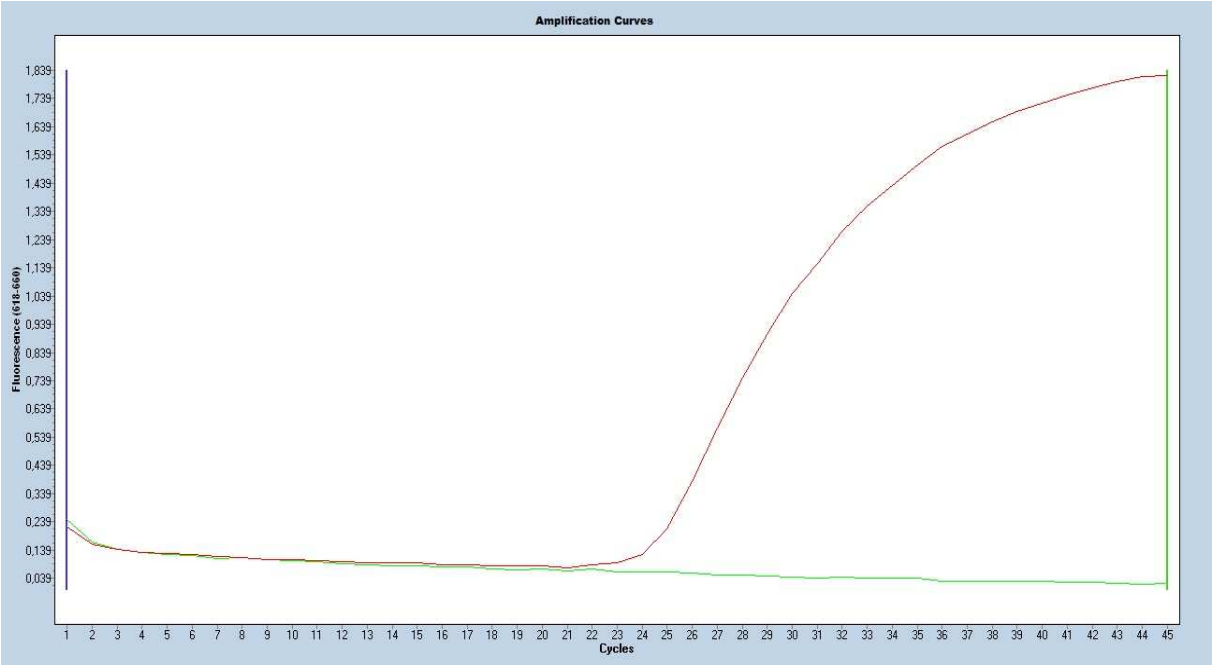

Fig. 3: Correct run of the positive and negative control (*Campylobacter* spp.) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab.11: Sample interpretation

Target genes				
***Salmonella* spp.**	***Yersinia enterocolitica***	***Campylobacter* spp.**	**ICD**	**Result**
positive	negative	negative	**positive/ negative**	***Salmonella* spp. detected**
negative	**positive**	negative	**positive/ negative**	***Yersinia enterocolitica* detected**
negative	negative	**positive**	**positive/ negative**	***Campylobacter* spp. detected**
positive	**positive**	negative	**positive/ negative**	***Salmonella* spp. and *Yersinia enterocolitica* detected**
positive	negative	**positive**	**positive/ negative**	***Salmonella* spp. and *Campylobacter* spp. detected**
negative	**positive**	**positive**	**positive/ negative**	***Yersinia enterocolitica* and *Campylobacter* spp. detected**
positive	**positive**	**positive**	**positive/ negative**	***Salmonella* spp., *Yersinia enterocolitica* and *Campylobacter* spp detected**
negative	negative	negative	**positive**	**Target genes not detected**
negative	negative	negative	**negative**	**Invalid**

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

A sample is also evaluated positive, if the sample shows an amplification signal in the detection system, but the Internal Control DNA is negative. The detection of the Internal Control DNA is not necessary, because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA.

A sample is evaluated negative, if the sample shows no amplification signal in the detection system, but the Internal Control DNA is positive. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control DNA.

A sample is evaluated invalid, if both, the sample and the Internal Control DNA show no amplification signal in the detection system. The sample contained a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for stool samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Bacterial Stool Panel assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the respective target genes (*Salmonella* spp. (ttr), *Y. enterocolitica* (ystA/ystB), *Campylobacter* spp. (16S rDNA only *C. coli, C. lari, C. jejuni*)).
8. Mucin, azithromycin and stearic/palmitic acid may show interfering characteristics even in small quantities.

13. Performance characteristics

13.1 Clinical performance

In a retrospective clinical validation study 282 extracted stool samples were analyzed with the RIDA®GENE Bacterial Stool Panel assay and an in-house real-time PCR assay in a laboratory in in the Netherlands.

Tab. 12: Correlation of the *Salmonella* spp. results with the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR and reference in-house real-time PCR.

		In-house real-time PCR			
		Positive	Negative	Total	Comments
RIDA®GENE Bacterial Stool Panel	Positive	50	0	50	Pos. agreement: 100 %
	Negative	0	232	232	Neg. agreement: 100 %
	Total	50	232	282	

Tab. 13: Correlation of the *Yersinia enterocolitica* results with the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR and reference in-house real-time PCR.

		In-house real-time PCR			
		Positive	Negative	Total	Comments
RIDA®GENE Bacterial Stool Panel	Positive	33	0	33	Pos. agreement: 77 %
	Negative	10	239	249	Neg. agreement: 100 %
	Total	43	239	282	

Tab. 14: Correlation of the *Campylobacter* spp. results with the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR and reference in-house real-time PCR.

		In-house real-time PCR			
		Positive	Negative	Total	Comments
RIDA®GENE Bacterial Stool Panel	Positive	41	1	42	Pos. agreement: 82 %
	Negative	9	231	240	Neg. agreement: 100 %
	Total	50	232	282	

13.2 Analytical sensitivity

The RIDA®GENE Bacterial Stool Panel multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica*.

The following figures 4, 5, 6 and 7 show dilution series of *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica* (each 10^5 - 10^1 DNA copies per µl) on the LightCycler® 480II.

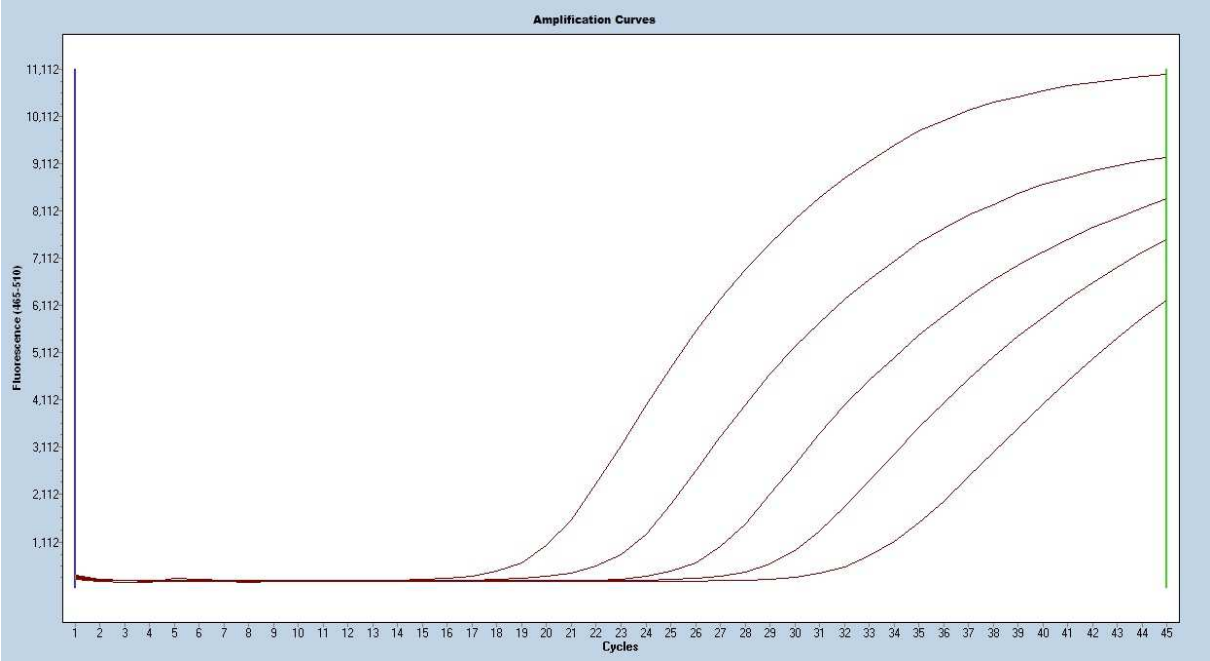

Fig. 4: Dilution series *Salmonella* spp. (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

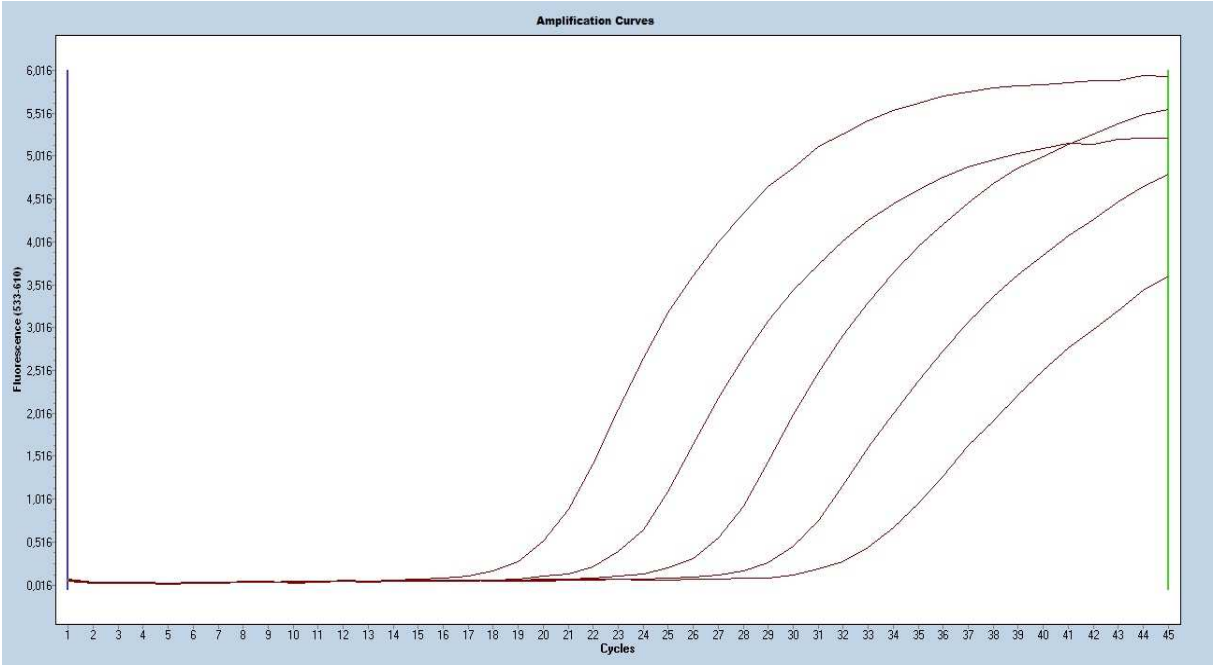

Fig. 5: Dilution series *Yersinia enterocolitica (ystA)* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

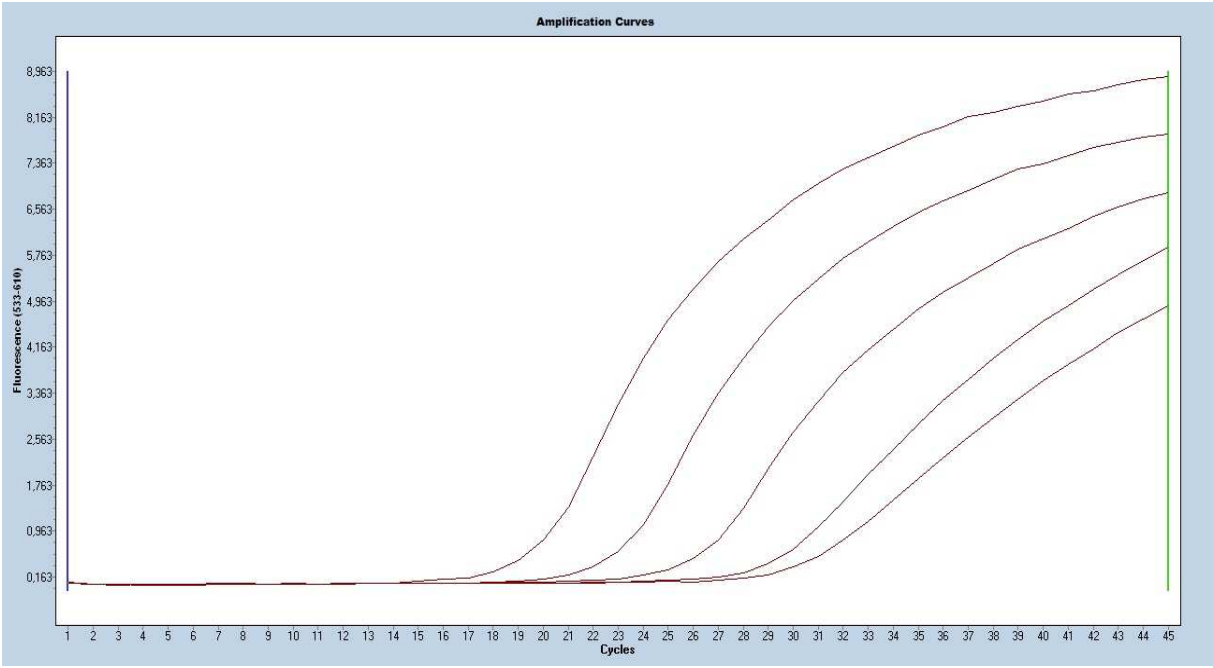

Fig.6: Dilution series *Yersinia enterocolitica (ystB)* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

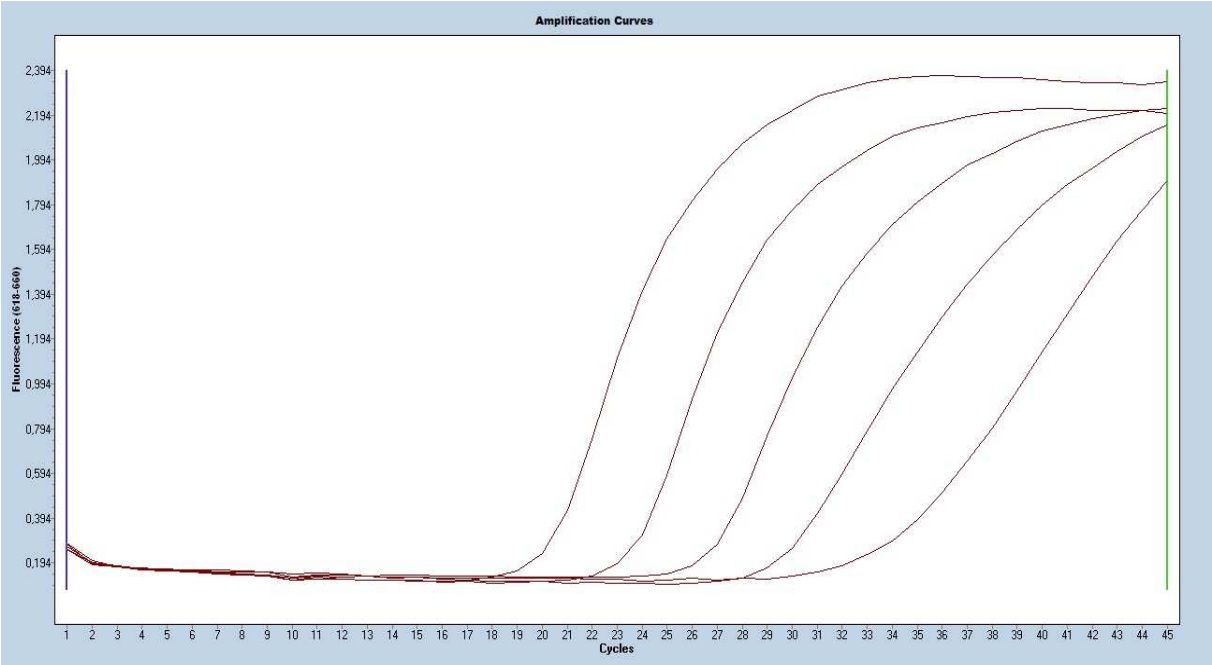

Fig.7: Dilution series *Campylobacter* spp. (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.3 Analytical specificity

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

Tab. 15: Cross-reactivity testing

Adenovirus 40	-	*Clostridium bifermentans*	-	*E. coli* (O6)	-	*Serratia liquefaciens*	-
Adenovirus 41, human, strain Tak	-	*Clostridium difficile*	-	*Entamoeba histolytica*	-	*Shigella flexneri*	-
Aeromonas hydrophila	-	*Clostridium novyi*	-	*Enterobacter cloacae*	-	*Staphylococcus aureus*	-
Arcobacter butzleri	-	*Clostridium perfringens*	-	*Enterococcus faecalis*	-	*Staphylococcus epidermidis*	-
Astrovirus	-	*Clostridium septicum*	-	*Giardia lamblia*	-	*Vibrio parahaemolyticus*	-
Bacillus cereus	-	*Clostridium sordellii*	-	*Klebsiella oxytoca*	-	*Yersina frederiksenii*	-
Bacteroides fragilis	-	*Clostridium sporogenes*	-	Norovirus GG I	-	*Yersina kristensenii*	-
Candida albicans	-	*Cryptosporidium muris*	-	Norovirus GG II	-	*Yersinia pseudotuberculosis*	-
Campylobacter fetus subsp. *fetus*	-	*Cryptosporidium parvum*	-	*Proteus vulgaris*	-	*Yersinia rohdei*	-
Campylobacter upsaliensis	-	*E. coli* (O157:H7)	-	*Pseudomonas aeruginosa*	-	*Yersinia ruckeri*	
Citrobacter freundii	-	*E. coli* (O26:H-)	-	Rotavirus, strain Wa	-		

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR was evaluated against multiple *Salmonella* serotypes, *Campylobacter* species and *Yersinia enterocolitica* (see Tab. 16). All *Salmonella* serotypes, *Campylobacter* species and *Yersinia enterocolitica* of the panel were detected by the RIDA®GENE Bacterial Stool Panel multiplex real-time PCR.

Tab. 16: Analytical reactivity testing

***Salmonella* serotypes**					
S. abony	+	*S. hadar*	+	*S. oranienburg*	+
S. agona	+	*S. heidelberg*	+	*S. paratyphi A*	+
S. anatum	+	*S. infantis*	+	*S. paratyphi B*	+
S. arizonae	+	*S. javiana*	+	*S. paratyphi C*	+
S. Bareilly	+	*S. kedougou*	+	*S. saintpaul*	+
S. bongori	+	*S. mississippi*	+	*S. schwarzengrund*	+
S. choleraesuis	+	*S. montevideo*	+	*S. typhi*	+
S. derby	+	*S. muenchen*	+	*S. typhimurium*	+
S. diarizonae	+	*S. newport*	+	*S. worthington*	+
S. Dublin	+	*S. nottingham*	+		
S. enteritidis	+	*S. ohio*	+		
***Yersinia* species**					
Y. enterocolitica	+	*Y enterocolitica* subsp. *palearctica*	+		
***Campylobacter* subspecies**					
C. coli	+	*C. lari*	+	*C. jejuni*	+

14. Version history

Version number	Chapter and designation
2018-06-20	Previous version
2020-01-06	General revision 1. Intended use 3. Test principle 6. Additional necessary reagents and necessary equipment 9.3 PCR instrument set-up 9.4 Detection channel set-up 10. Quality control 12. Limitations of the method 13. Performance characteristics

15. Explanation of symbols

General symbols

	For in vitro diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Not applicable

16. Literature

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