

## RIDA® GENE CD Toxin A/B

**REF** PG0825



## 1. Intended use

For *in vitro* diagnostic use. RIDA<sup>®</sup>GENE CD Toxin A/B is a real-time PCR for the direct, qualitative detection of *Clostridium difficile* toxin A (tcdA) and toxin B (tcdB) genes from human stool samples and culture. The RIDA<sup>®</sup>GENE CD Toxin A/B real-time PCR is intended for use as an aid in diagnosis of *Clostridium difficile* associated diarrhea (CDAD).

## 2. Summary and explanation of the test

*Clostridium difficile*, a gram-positive, spore-forming anaerobic bacterium was first described in 1935 by Hall and O'Toole as a component of the intestinal microflora in healthy neonates.<sup>1</sup> In the late 1970s, however, *Clostridium difficile* was identified as the cause of antibiotic-associated diarrhea and pseudomembranous colitis.<sup>2</sup> Today *Clostridium difficile* is one of the most common causes of nosocomial diarrhea. *Clostridium difficile* is responsible for 15 - 25% of antibiotic-associated diarrhea and nearly all cases of pseudomembranous colitis.<sup>3</sup> The predisposing risk factors for CDAD are for example antibiotic exposure, advanced age as well as number and duration of hospitalization. However, *Clostridium difficile* infection is also seen in an increasing number of non-antibiotic-treated and non-hospitalized individuals. The symptoms range from mild diarrhea to intestinal infections of variable severity, including pseudomembranous colitis, the most severe form of antibiotic-induced inflammatory bowel disease. Clinically symptomatic cases are caused by toxigenic *Clostridium difficile* strains that produce toxin A and toxin B. In recent years the incidence and severity of *Clostridium difficile* infections increased worldwide.<sup>4</sup> Real-time PCR permits a rapid, highly sensitive and specific detection of *Clostridium difficile* infection. An early and reliable diagnosis of *Clostridium difficile* infection makes it possible to administer specific treatment of CDAD patients and also to initiate hygiene measures to prevent nosocomial transmission.

### 3. Test principle

RIDA<sup>®</sup>GENE CD Toxin A/B multiplex real-time PCR is for the direct, qualitative detection of *Clostridium difficile* toxin genes A (tcdA) / toxin B (tcdB) in human stool and culture samples.

After DNA isolation, amplification of the gene fragments specific for toxin A and B of *Clostridium difficile* (if present) occurs. The amplified DNA targets 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<sup>®</sup>GENE CD Toxin A/B 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	<b>Reaction Mix</b>	2x	1050 µl	yellow
2	<b>Taq-Polymerase</b>	1x	80 µl	red
D	<b>Internal Control DNA</b>	2x	1700 µl	orange
N	<b>No Template Control</b>	1x	450 µl	white
P	<b>Positive Control</b>	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<sup>®</sup>GENE CD Toxin A/B multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

**Tab. 2:** Necessary equipment

Extraction platform	
R-Biopharm	RIDA <sup>®</sup> Xtract
Promega	Maxwell <sup>®</sup> RSC
bioMérieux	NucliSENS easy <sup>®</sup> MAG <sup>™</sup>
Real-time PCR instrument:	
Roche	LightCycler <sup>®</sup> 2.0, LightCycler <sup>®</sup> 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96 <sup>™</sup>
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](mailto:mdx@r-biopharm.de).

- RIDA<sup>®</sup>GENE Color Compensation II (PG0002) for use with the LightCycler<sup>®</sup> 2.0
- RIDA<sup>®</sup>GENE Color Compensation IV (PG0004) for use with the LightCycler<sup>®</sup> 480II
- 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](http://www.r-biopharm.com).

## 8. Collection and storage of samples

### 8.1 Sample preparation from stool samples

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

We 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. Use from the supernatant the appropriate volume according to the manufacturer's instruction.

The RIDA<sup>®</sup>GENE CD Toxin A/B 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 must **not** be added directly to the specimen. We also recommend to add 1 µl of the **Internal Control DNA** to the negative control and positive control PCR Mix.

## 8.2 Sample preparation from cultures

For DNA isolation from culture the following procedure is recommended: Add 1 ml PCR water into a preparation tube. Collect colonies with an inoculation loop and suspend them in the prepared PCR water. Cut or break the inoculation loop stem. Cap the preparation tube tightly and vortex strongly for 60 seconds. Heat and shake the preparation tube at 95 °C for 10 min in a heating block. Centrifuge for 1 min at 13.000 x g and apply the supernatant as sample.

**Note: Repeat the centrifugation step in case of strong turbidity (if necessary).**

The RIDA® GENE CD Toxin A/B 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-PCR water mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the **Internal Control DNA** to the negative control and positive control PCR Mix.

## 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
	<b>Total</b>	<b>20 µl</b>	<b>220 µl</b>

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
	<b>Total</b>	<b>21.0 µl</b>	<b>231.0 µl</b>

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 PCR-Mix of the negative control.

**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 PCR-Mix of the positive control.

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 (Tab. 5, Tab. 6, Tab. 7, Tab. 8).

### 9.3 PCR instrument set-up

#### 9.3.1 DNA real-time PCR profile

**Tab. 5:** DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

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

**Note: Annealing and Extension occur in the same step.**

**Tab. 6:** DNA real-time PCR profile for Mx3005P, ABI 7500 and CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	15 sec, 95 °C
Annealing/Extension	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 RNA real-time PCR assays in one run.**

**Tab. 7:** Universal real-time PCR profile for LightCycler® series

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	10 sec, 95 °C
Annealing/Extension	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™

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	15 sec, 95 °C
Annealing/Extension	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
Roche LightCycler® 2.0	<i>Clostridium difficile</i> Toxin A/B	530	RIDA® GENE Color Compensation Kit II (PG0002) is required
	ICD	560	
Roche LightCycler® 480II	<i>Clostridium difficile</i> Toxin A/B	465/510	RIDA® GENE Color Compensation Kit IV (PG0004) is required
	ICD	533/580	
ABI 7500	<i>Clostridium difficile</i> Toxin A/B	FAM	Check that passive reference option ROX is none
	ICD	VIC	
Agilent Techn. Mx3005P	<i>Clostridium difficile</i> Toxin A/B	FAM	Check that reference dye is none
	ICD	HEX	
Qiagen Rotor-Gene Q	<i>Clostridium difficile</i> Toxin A/B	Green	The gain settings have to be set to 5, according to the default settings
	ICD	Yellow	
Bio-Rad CFX96™	<i>Clostridium difficile</i> Toxin A/B	FAM	-
	ICD	VIC	

## 10. Quality control

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

The Positive Control CD Toxin A/B has a concentration of  $10^3$  copies/ $\mu$ l. In each PCR run it is used in a total amount of  $5 \times 10^3$  copies.

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

Sample	Assay result	ICD Ct	Target Ct
Positive control	Positive	NA <sup>*1</sup>	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

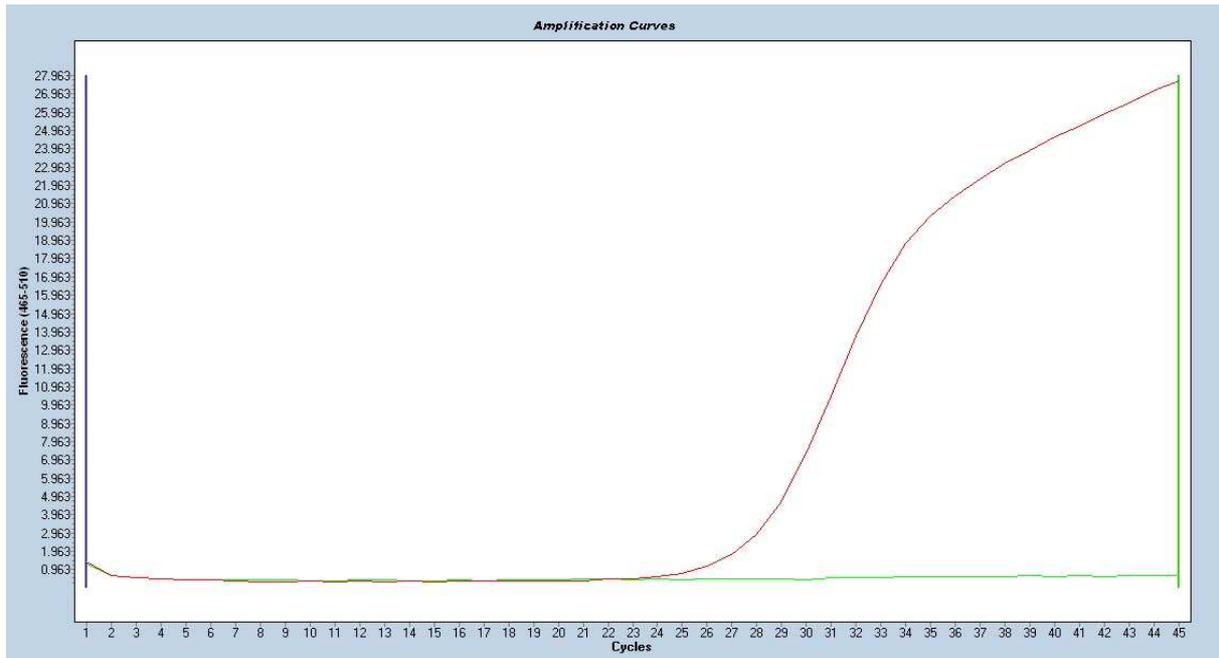
<sup>\*1</sup> No Ct value is required for the ICR to make a positive call for the positive control.

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 (*C. difficile* toxin genes A/B) on the LightCycler® 480II

## 11. Result interpretation

The result interpretation is done according to Table 11.

**Tab. 11:** Sample interpretation

Target genes		
<i>C. difficile</i> toxin genes A/B	ICD	Result
positive	positive/negative	<i>C. difficile</i> toxin genes A/B detected
negative	positive	Target genes not detected
negative	negative	Invalid

*C. difficile* toxin genes A/B are detected, if the sample DNA and the **Internal Control DNA** show an amplification signal in the detection system.

*C. difficile* toxin genes A/B are also detected, if the sample DNA shows an amplification signal but none for the **Internal Control DNA** in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the **Internal Control DNA**.

*C. difficile* toxin genes A/B are not detected, if the sample DNA shows no amplification signal, but an amplification signal for the **Internal Control DNA** in the

detection system. An inhibition of the PCR reaction can be excluded by the detection of the **Internal Control DNA**.

A sample is invalid, if the sample DNA and **Internal Control DNA** show no amplification signal in the detection system. The sample contains 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**

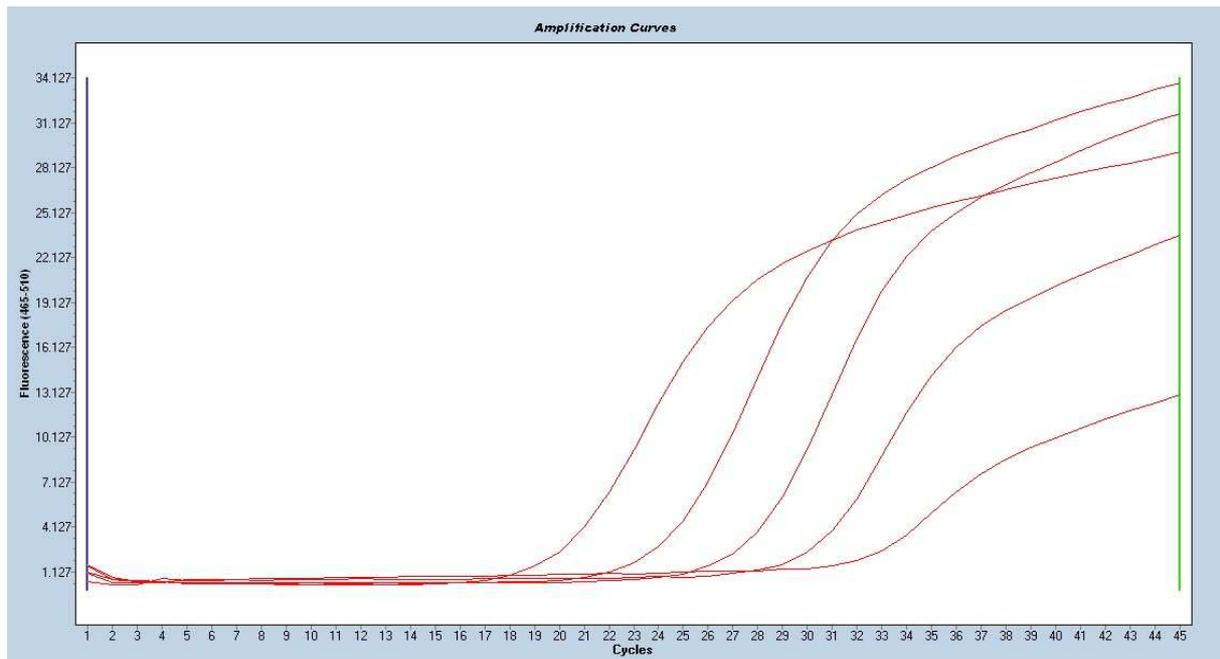
1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for stool and culture samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen 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<sup>®</sup>GENE CD Toxin A/B 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 *C. difficile* toxin A/B genes (tcdA/tcdB).

## 13. Performance characteristics

### 13.1 Analytical sensitivity

The RIDA<sup>®</sup> GENE CD Toxin A/B multiplex real-time PCR assay has a detection limit of  $\geq 10$  DNA copies per reaction.

The following figure 2 shows a dilution series of *C. difficile* toxin genes A/B (each  $10^5 - 10^1$  DNA copies per  $\mu\text{l}$ ) on the LightCycler<sup>®</sup> 480II.



**Fig. 2:** Dilution series *C. difficile* toxin A/B genes ( $10^5 - 10^1$  DNA copies /  $\mu\text{l}$ ) on the LightCycler<sup>®</sup> 480II

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

## 13.2 Analytical specificity

The RIDA<sup>®</sup> GENE CD Toxin A/B multiplex real-time PCR is specific for *Clostridium difficile* in human stool samples. No cross-reaction could be detected for the following species (see Tab.12):

**Tab. 12:** Cross-reactivity testing

Adenovirus 1, human, strain Adenoid 71	-	<i>Clostridium bifermentans</i>	-	<i>Giardia lamblia</i>	-
Adenovirus 7, human, strain Gomen	-	<i>Clostridium novyi</i>	-	<i>Klebsiella oxytoca</i>	-
Adenovirus 40, human, strain Dugan	-	<i>Clostridium perfringens</i>	-	Norovirus GI	-
Adenovirus 41, human, strain Tak	-	<i>Clostridium septicum</i>	-	Norovirus GII	-
<i>Arcobacter butzleri</i>	-	<i>Clostridium sordelli</i>	-	<i>Proteus vulgaris</i>	-
<i>Aeromonas hydrophila</i>	-	<i>Clostridium sporogenes</i>	-	<i>Pseudomonas aeruginosa</i>	-
Astrovirus	-	<i>Cryptosporidium muris</i>	-	Rotavirus	-
<i>Bacillus cereus</i>	-	<i>Cryptosporidium parvum</i>	-	<i>Salmonella enteritidis</i>	-
<i>Bacteroides fragilis</i>	-	<i>E.coli</i> (O157:H7)	-	<i>Salmonella typhimurium</i>	-
<i>Campylobacter coli</i>	-	<i>E.coli</i> (O26:H-)	-	<i>Serratia liquefaciens</i>	-
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	-	<i>E.coli</i> (O6)	-	<i>Shigella flexneri</i>	-
<i>Campylobacter jejuni</i>	-	<i>Entamoeba histolytica</i>	-	<i>Staphylococcus aureus</i>	-
<i>Campylobacter lari</i> subsp. <i>lari</i>	-	<i>Enterobacter cloacae</i>	-	<i>Staphylococcus epidermidis</i>	-
<i>Campylobacter upsaliensis</i>	-	<i>Enterococcus faecalis</i>	-	<i>Vibrio parahaemolyticus</i>	-
<i>Candida albicans</i>	-	<i>Giardia intestinalis</i> Portland1	-	<i>Yersinia enterocolitica</i>	-
<i>Citrobacter freundii</i>	-	<i>Giardia intestinalis</i> WB Clone 6	-		

### 13.3 Analytical reactivity

The reactivity of the RIDA<sup>®</sup> GENE CD Toxin A/B multiplex real-time PCR assay was tested with *Clostridium difficile* (see Tab. 13). All *Clostridium difficile* strains tested were detected by the RIDA<sup>®</sup> GENE CD Toxin A/B multiplex real-time PCR assay or by sequence alignment.

**Tab. 13: Analytical reactivity testing**

<i>Clostridium difficile</i>					
<i>C. difficile</i> ribotype 001	+	<i>C. difficile</i> ribotype 023	+	<i>C. difficile</i> ribotype 075	+
<i>C. difficile</i> ribotype 002	+	<i>C. difficile</i> ribotype 027	+	<i>C. difficile</i> ribotype 078	+
<i>C. difficile</i> ribotype 012	+	<i>C. difficile</i> ribotype 046	+	<i>C. difficile</i> ribotype 0126	+
<i>C. difficile</i> ribotype 017	+	<i>C. difficile</i> ribotype 056	+	<i>C. difficile</i> ribotype 0131	+
<i>C. difficile</i> ribotype 020	+				
<i>C. difficile</i> toxinotype 0	+	<i>C. difficile</i> toxinotype X	+	<i>C. difficile</i> toxinotype XXI	+
<i>C. difficile</i> toxinotype I	+	<i>C. difficile</i> toxinotype XIa	+	<i>C. difficile</i> toxinotype XXII	+
<i>C. difficile</i> toxinotype II	+	<i>C. difficile</i> toxinotype XIb	+	<i>C. difficile</i> toxinotype XXIV	+
<i>C. difficile</i> toxinotype IIIa	+	<i>C. difficile</i> toxinotype XIc	+	<i>C. difficile</i> toxinotype XXV	+
<i>C. difficile</i> toxinotype IIIb	+	<i>C. difficile</i> toxinotype XIId	+	<i>C. difficile</i> toxinotype XXVI	+
<i>C. difficile</i> toxinotype IIIc	+	<i>C. difficile</i> toxinotype XII	+	<i>C. difficile</i> toxinotype XXVII	+
<i>C. difficile</i> toxinotype IV	+	<i>C. difficile</i> toxinotype XIII	+	<i>C. difficile</i> toxinotype XXVIII	+
<i>C. difficile</i> toxinotype V	+	<i>C. difficile</i> toxinotype XIVa	+	<i>C. difficile</i> toxinotype XXIX	+
<i>C. difficile</i> toxinotype VI	+	<i>C. difficile</i> toxinotype XIVb	+	<i>C. difficile</i> toxinotype XXX	+
<i>C. difficile</i> toxinotype VII	+	<i>C. difficile</i> toxinotype XVI	+	<i>C. difficile</i> toxinotype XXXI	+
<i>C. difficile</i> toxinotype VIII	+	<i>C. difficile</i> toxinotype XVII	+	<i>C. difficile</i> toxinotype XXXII	+
<i>C. difficile</i> toxinotype IXa	+	<i>C. difficile</i> toxinotype XVIII	+	<i>C. difficile</i> toxinotype XXXIII	+
<i>C. difficile</i> toxinotype IXb	+	<i>C. difficile</i> toxinotype XIX	+	<i>C. difficile</i> toxinotype XXXIV	+
<i>C. difficile</i> toxinotype IXc	+	<i>C. difficile</i> toxinotype XX	+		

## 14. Version history

Version number	Chapter and designation
2013-01-29	Release version
2018-05-22	General revision
2018-05-22	4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 8. Collection and storage of samples 9. Test procedure 10. Quality control 13. Performance characteristics 14. Version history 15. Explanation of symbols

## 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

1. Hall IC and O'Toole E. Intestinal flora in new-born infants: with a description of a new pathogenic anaerobe, *Bacillus difficilis*. *Am J Dis Child* 1935, 49: 390–402.
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3. Bartlett JG and Gerding DN. Clinical Recognition and Diagnosis of *Clostridium difficile* Infection. *CID* 2008, 46: S12-18.
4. Bartlett JG. Narrative Review: The new Epidemic of *Clostridium difficile*-Associated Enteric Disease. *Ann Intern Med* 2006; 145:758-764.