RIDA®QUICK Clostridium difficile Toxin A/B

REF N0803

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Clostridium difficile Toxin A/B is an immunochromatographic rapid detection test for the qualitative detection of toxins A and B of *Clostridium difficile* in stool samples and in culture supernatants.

2. Summary and explanation of the test

Diarrhea illnesses are a relatively common side effect of antibiotic therapies. Particularly since the introduction of clindamycin in the early 1970s, there has been a higher incidence of more severe forms of the disease that manifested in pseudomembranous colitis (PMC) on a massive scale. This antibiotic-associated diarrhea (AAD) is mainly caused by *Clostridium difficile* and is therefore called *Clostridium difficile*-associated diarrhea (CDAD). It is one of the most common forms of nosocomial infections in the developed countries. The carrier rate in hospitalized patients has meanwhile increased to 16% to 35%. We have now identified strains with increasing virulence due to specific mechanisms of pathogenicity that have made a *Clostridium difficile* infection (CDI) a significant cost factor in health care. Production of the toxins A and B by toxigenic strains of *Clostridium difficile* plays a significant role in the clinical manifestation of the illness. These high-molecular-weight toxin proteins of about 300 kDa each are immunologically and functionally distinguishable. Toxin A is an enterotoxin, toxin B a cytotoxin. Both toxins act on their own, but also synergistically. Since some strains of *Clostridium difficile* do not produce toxins and about 2% to 8% of healthy adults and up to 80% of children under two years of age can be infected with *Clostridium difficile*, primarily the detection of toxins A and B in stool samples in conjunction with the occurrence of CDAD is very important for the diagnosis and treatment decision.

RIDA®QUICK Clostridium difficile Toxin A/B is a rapid detection test used for the specific detection of toxin A and toxin B simultaneously in the stool samples of patients using monoclonal antibodies. Reliable test results are available after just 15 minutes, making it possible to initiate effective therapeutic measures at an early stage.

3. Test principle

The present rapid detection test is a single-stage immunochromatographic lateral flow assay that uses both biotinylated as well as gold-labeled anti-toxin A and anti-toxin B antibodies. As soon as *Clostridium difficile* toxins A and/or B are present in a positive sample, immune complexes form with the labeled anti-toxin A and anti-toxin B antibodies, which then pass through the membrane. The streptavidin located on the T test line binds the circulating immune complexes via the biotin coupled to the anti-toxin A and anti-toxin B antibodies, causing a red-violet coloration of the T line. The continuous non-complexed gold-labeled antibodies that pass through are bound to the following control line C. In the event of negative samples, gold-labeled immune

complexes bind only to the C line and not to the T line. The red C line always shows whether the test process was valid.

4. Reagents provided

The reagents in the kit are sufficient for 25 determinations.

Table 1: Reagents provided

Cassette	25 assays	25 individually packed test cassettes
Reagent A	13.5 ml	Specific anti-toxin A and anti-toxin B antibodies (mouse); contains 0.05% sodium azide, ready for use, blue colored
Reagent B	13.5 ml	Specific anti-toxin A and anti-toxin B antibodies (mouse); contains 0.05% sodium azide, ready for use, yellow colored
Pipet	25 pcs.	Bag with 25 disposable pipettes
Reagent vial	25 pcs.	Bag with 25 reaction vials
Pipet Tip	25 pcs.	Bag with 25 pipette tips
Microlit Pipet	1 pcs.	Pipette for 150-µl volume

Hazardous materials are indicated according to labeling obligations. For further details, see the safety data sheets (SDSs) at www.biopharm.com.

5. Storage instructions

The package can be stored at 2 to 25°C and can be used until the printed date of expiration. After the expiration date, the quality guarantee is no longer valid. Similarly, the usability of cassettes can no longer be guaranteed if the cassette package is damaged.

6. Reagents required but not provided

6.1 Necessary reagents

No additional reagents are needed to perform this test.

6.2 Necessary laboratory equipment

The following equipment is needed to perform this test:

Equipment
Vortex mixer (optional)
Waste container with a 0.5% sodium-hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the instructions for use 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.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

Users are responsible for proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

The reagents contain 0.05% sodium azide as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

All reagents and materials that come into contact with potentially infectious samples must be treated in the same way as infectious samples, with suitable disinfectants (e.g., sodium hypochlorite) or autoclaved at 121°C for at least one hour.

8. Collection and storage of samples

Stool samples must be collected in clean containers without additives and stored at 2 to 8°C before the beginning of the test. If stored for more than three days, the sample must be frozen at -20°C (Table 2). In this case, the sample is fully thawed and brought to room temperature before testing. Avoid repeated freezing and thawing of the sample.

If rectal smears are used, make sure that the amount of stool material is sufficient (approx. 50 mg) for the test.

Table 2: Specimen storage

Undiluted stool samples	
2 to 8°C	≤ -20°C
≤ 3 days	> 3 days

9. Test procedure

9.1 General information

The samples, reagents, and test cassettes must be brought to room temperature (20 to 25°C) before use. The test cassettes should not be taken out of the outer packaging until shortly before use. Once used, the cassettes must not be re-used. Do not carry out the test procedure in direct sunlight. Do not return excess reagent to the vials because contamination can result.

9.2 Preparing for sample testing

Transfer **0.5 ml** (about 12 to 14 drops) each of reagent A [Reagent A] and reagent B [Reagent B] into a labeled reaction vial [Reagent vial]. During this step, the graduation of 0.5 ml and 1.0 ml on the reaction vial takes **precedence** regardless of the respective number of drops of reagents A and B. Reagents A and B must be in a **1:1** ratio.

9.2.1 Use of stool samples

For **liquid** stool samples, use the disposable pipette [Pipet] to suspend 50 µl (up to the second bulge) in the pre-pipetted reagent mix.
For **solid** stool samples, suspend about 50 mg in the same way. Then, seal tight the reaction vial, and homogenize the sample by mixing thoroughly (or by vortexing). Afterwards, the homogeneous suspension needs to settle for **5 minutes** to allow an essentially particle-free supernatant to form. For sedimentation, the reaction vial can be placed in one of the middle openings of the reagent insert.

9.2.1 Use of liquid and solid cultures of *Clostridium difficile*

Pipette and mix 50 µl of a **nutrient broth** (e.g., thioglycollate broth) in 1.0 ml of the reagent mix prepared previously in the reaction vial, consisting of reagent A (0.5 ml) and reagent B (0.5 ml). Of the mixture, 150 µl will be used for sample testing (Section 9.3).

When **solid culture media** are used, remove as many colonies as possible from the culture medium plate and then completely suspend in 1 ml distilled water or saline solution (0.9% NaCl). Next, pipette and mix 50 µl of this suspension in 1.0 ml of the reagent mix prepared previously in the reaction vial, consisting of reagent A (0.5 ml) and reagent B (0.5 ml). Of the mixture, 150 µl will be used for sample testing (Section 9.3).

9.3 Sample testing

Remove the test cassette Cassette from the outer packaging and place it on a level surface. Then, place a new pipette tip Pipet Tip on the Microlit pipette Microlit Pipet, and take 150 µl of supernatant from the respective reaction vial and pipette it into the application area of the test cassette. Make sure that the liquid can flow through the membrane without difficulty. If the test is performed correctly, the control band appears at control line C after approximately 3 minutes. If the control line is not visible after 3 minutes, a newly prepared sample will need to be better sedimented (optionally, by 2-minute centrifugation for 2,000 g) and pipetted into the application area of a new test cassette.
Always wait **15 minutes** to read the test result. The coloration of the bands and their intensity can change from red-violet to blue-violet to gray-violet during the entire development phase and after the drying of the strip.

10. Quality control – Indication of instability or expiration of reagents

The test should be evaluated only if the test cassette is intact prior to pipetting the sample suspension and no color changes or bands can be seen. Furthermore, after an incubation period of 15 minutes, at least the red-violet control band must be visible. If it does not appear, check the following things prior to repeating the test:

- Expiration date of the test cassettes and the reagents used
- Correct test procedure
- Contamination of the reagents

If the control band is still not visible after repeating the test using a new test cassette, please contact the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

A maximum of two bands must appear, viewed from the sample application field, in the following sequence: A red-violet reaction band at test line T and a red-violet control

band at control line C. **If the control band is absent, the test cannot be evaluated and is invalid!**

The following interpretations are possible:

- ***Clostridium-difficile* toxin positive:** both bands are visible.
- ***Clostridium difficile* toxin negative:** only the control band is visible.
- **Invalid:** no bands are visible or there is a constellation other than the one mentioned above. Similarly, band discolorations that appear much later than 15 minutes have no diagnostic value and must not be assessed.

12. Limitations of the method

RIDA®QUICK Clostridium difficile Toxin A/B detects toxin A and/or B of *Clostridium difficile* in stool samples or in *C. difficile* cultures after enrichment. It is not possible to associate the intensity of the visible specific bands to the occurrence or severity of clinical symptoms. **The results obtained must always be interpreted in combination with the complete clinical symptoms.**

A **positive** result does not rule out the presence of other infectious pathogens or causes.

A **negative** result does not rule out the possibility of *Clostridium difficile* infection. Such a result may be due to intermittent excretion of the pathogen or due to an insufficient amount of toxin in the sample. If the patient history supports a suspicion of infection with the target pathogen, the examination should be repeated with another stool sample from the patient.

An excess of stool sample can cause the test strip to turn a brownish color on top of the red-violet coloring of the specific test band. In these cases, the test needs to be repeated with a smaller amount of stool or a suspension that is better sedimented through centrifugation to determine whether the suspected *Clostridium difficile* toxins are actually present in the sample, but were superimposed by excessive stool matrix used.

13. Performance characteristics

13.1 Clinical sensitivity and specificity

RIDA®QUICK Clostridium difficile Toxin A/B was compared with two commercially available lateral flow assays. For the comparison, 61 stool samples were analyzed with the three assays. The results are summarized in Table 3.

Table 3: Clinical performance of RIDA®QUICK Clostridium difficile Toxin A/B

		Assay 1		Assay 2	
		Positive	Negative	Positive	Negative
RIDA®QUICK Clostridium difficile Toxin A/B	Positive	35	0	35	0
	Negative	4	22	0	26

Positive agreement:	94.6%	100%
Negative agreement:	91.7%	100%

13.2 Analytical sensitivity

Analytical sensitivity of the RIDA®QUICK Clostridium difficile Toxin A/B rapid detection test was determined by diluting defined concentrations of pure toxins A and B in series. This dilution series was used to determine a provisional limit of detection (LoD) that was verified by 30 measurements using the provisional LoD. The results are summarized in Table 4.

Table 4: Analytical sensitivity of RIDA®QUICK Clostridium difficile Toxin A/B

Toxin concentration Toxin A [ng/ml]	RIDA®QUICK Clostridium difficile Toxin A/B		Toxin concentration Toxin B [ng/ml]	RIDA®QUICK Clostridium difficile Toxin A/B	
	Lot 1	Lot 2		Lot 1	Lot 2
8.00	Positive	Positive	4.00	Positive	Positive
7.00	Positive	Positive	3.00	Positive	Positive
6.00	Positive	Positive	**2.00**	**Positive**	**Positive**
5.00	**Positive**	**Positive**	1.00	Negative	Positive
4.00	Positive	Negative	0.50	Positive	Negative
3.00	Negative	Negative	0.25	Positive	Negative
2.00	Negative	Negative	0.13	Negative	Negative
1.00	Negative	Negative	0.06	Negative	Negative
0.50	Negative	Negative	0.03	Negative	Negative
0.25	Negative	Negative	0.02	Negative	Negative

According to the measurements for verification, the LoD of RIDA®QUICK Clostridium difficile Toxin A/B was determined to be 5 ng/ml for toxin A and 2 ng/ml for toxin B in the sample. Therefore, the LoD of the prepared reaction mix, which is a 1:21 dilution of the sample in reagent A and reagent B, is 0.24 ng/ml for toxin A and 0.10 ng/ml for toxin B.

13.3. Precision

The precision of the RIDA®QUICK Clostridium difficile Toxin A/B assay was determined by examining intra-assay precision, inter-day precision, inter-operator precision, and inter-lot precision. For each test, five references were measured: one negative, two weakly positive (toxin A and B), and two moderately positive (toxin A and B).

13.3.1 Intra-assay precision

Intra-assay precision was determined by having one operator measure the five references in ten replicates each. RIDA®QUICK Clostridium difficile Toxin A/B showed 100% intra-assay precision.

13.3.2 Inter-day precision

Inter-day precision was determined by having two operators measure the five references in triplicate on ten different days. Inter-day precision was 100% for all positive references and 93% for all negative references, which is within an acceptable limit.

13.3.3 Inter-operator precision

Inter-operator precision was carried out by two operators within the scope of inter-day precision. The assay provided reproducible results between different operators (see 13.3.2).

13.3.4 Inter-lot precision

The five references were each analyzed by the same operator in triplicate in three kit lots. Inter-lot precision was 100%.

13.4. Cross-reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined using the RIDA®QUICK Clostridium difficile Toxin A/B assay and, aside from *Staphylococcus aureus*, they demonstrated no cross-reactivity. These tests were conducted using bacterial suspensions (10^7 to 10^9 CFU/ml), parasite cultures (10^7 to 10^9 organisms/ml), cell culture supernatants of virus-infected cells, and one stool sample. The results are shown in Table 5.

Table 5: Cross-reactivity of RIDA®QUICK Clostridium difficile Toxin A/B

Organism	Origin	Result
Adenovirus	Cell culture supernatant	Negative
Aeromonas hydrophila	Culture	Negative
Astrovirus	Cell culture supernatant	Negative
Bacillus cereus	Culture	Negative
Bacteroides fragilis	Culture	Negative
Campylobacter coli	Culture	Negative
Campylobacter jejuni	Culture	Negative
Candida albicans	Culture	Negative
Citrobacter freundii	Culture	Negative
Clostridium difficile	Culture	Negative
Clostridium perfringens	Culture	Negative
Clostridium sordellii	Culture	Negative
Cryptosporidium parvum	Culture	Negative
E. coli (O26:H-)	Culture	Negative
E. coli (O6)	Culture	Negative
E. coli (O157:H7)	Culture	Negative
Entamoeba	Positive control material	Negative
Enterobacter cloacae	Culture	Negative
Enterococcus faecalis	Culture	Negative
Giardia lamblia	Stool sample	Negative
Klebsiella oxytoca	Culture	Negative
Proteus vulgaris	Culture	Negative
Pseudomonas aeruginosa	Culture	Negative
Rotavirus	Cell culture supernatant	Negative
Salmonella enteritidis	Culture	Negative
Salmonella typhimurium	Culture	Negative
Serratia liquefaciens	Culture	Negative
Shigella flexneri	Culture	Negative
Staphylococcus aureus	Culture	Positive
Staphylococcus epidermidis	Culture	Negative
Vibrio parahaemolyticus	Culture	Negative
Yersinia enterocolitica	Culture	Negative

13.5 Interfering substances

The substances listed below had no effect on the test results when mixed into Clostridium difficile-toxin A/B-positive and -negative stool samples in the specified concentrations:

Loperamide	0.02% (w/w)	Barium sulphate	18.5% (w/w)
Pepto-Bismol	6.3% (v/w)	Cyclamate	1.3% (v/w)
Human blood	5% (v/w)		
Stearic acid/palmitic acid (1:1)	40% (w/w)	Metronidazole 0.5% solution	3% (v/w)
Mucin	5% (w/w)	Diclofenac	0.01% (v/w)
Vancomycin	3% (v/w)		

14. Version history

Version number	Section and designation
2010-04-20	Previous version
2020-08-10	General revision 2. Summary and explanation of the test 4. Reagents provided 7. Warnings and precautions for the users 8. Collection and storage of samples 13. Performance characteristics

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Batch number
	Use before
	Store at
REF	Item number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Cassette	Test cassette
Reagent A	Reagent A
Reagent B	Reagent B
Pipet	Disposable pipette
Reagent vial	Reaction vial
Pipet Tip	Pipette tip
Microlit Pipet	Micropipette

16. References

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