RIDA®QUICK Clostridium difficile GDH

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Clostridium difficile GDH is a lateral flow immunochromatographic assay for the qualitative detection of Clostridium difficile-specific glutamate dehydrogenase (GDH) in stool specimens.

2. Summary and explanation of the test

Clostridium difficile, a strictly anaerobic spore-forming rod bacterium, is part of the normal bowel flora of humans. Under normal conditions, it is a relatively harmless bacterium. The rate of Clostridium difficile colonization is very high (up to 80%) in early childhood and decreases continuously with age. The average colonization rate in adults is normally only about 2 to 10 % but can increase to slightly over 30% under certain circumstances (e.g. in a hospital environment). The formation of high-molecular-weight protein toxins A (enterotoxin) and B (cytotoxin) is an essential factor in the pathogenesis of *Clostridium difficile* infection (CDI). Since not all strains of *Clostridium difficile* are toxin producers and because approximately 2 to 8 percent of healthy adults and up to 80 % of children under 2 years of age are colonized with Clostridium difficile, the only pathognomonic sign of suspected Clostridium difficile-associated diarrhea (CDAD) is the detection of *C. difficile* toxins A and B. The prerequisite for this is successful colonization of the large intestine with sufficient quantities of toxin-producing C. difficile strains, which is promoted by factors that result in a loss of normally protective bacterial populations in the intestine. In particular, the loss of protective flora due to antibiotics or other factors that lower intestinal immunity may result in the spread of C. difficile strains with a broader and stronger resistance to various antibiotics. Other virulence factors such as increased toxin production due to regulatory defects of some newly defined strains have allowed C. difficile to become a so-called "re-emerging pathogen", the pathogenicity of which is no longer restricted to persons who develop Clostridium difficile infection (CDI) in association with antibiotic use, but has also extended to an increasing number of untreated and non-hospitalized individuals. The growing importance of nosocomial pathogens has led to the development of new treatment approaches and, above all, to the development of new algorithms for the diagnosis of C. difficile infection. The primary goal is to detect the presence of the pathogen in order to prevent its spread to hospitalized patients, independent of whether toxigenic or non-toxigenic strains of Clostridium difficile are present. Glutamate dehydrogenase (GDH), an enzyme present in high copy numbers in many organisms, has proved to be a sensitive screening marker for Clostridium difficile. Since GDH is present in many intestinal bacteria, it is crucial that assay systems for glutamate dehydrogenase be accurate and highly sensitive for the detection of C. difficile-specific GDH. RIDA®QUICK Clostridium difficile GDH is a rapid test that meets both of these requirements to a high degree. Although it does not eliminate the need for the

detection of C. difficile toxins A and B, which is obligatory for the diagnosis of Clostridium difficile infection, RIDA®QUICK Clostridium difficile GDH improves the reliability of detection of this very consequential nosocomial pathogen when performed sequentially, i.e., before or parallel to the RIDA®QUICK Clostridium difficile Toxin A/B rapid test. Both the specific clinical symptoms and signs and the positive detection of C. difficile toxins A and B are required for diagnosis and adequate treatment decision-making in cases of suspected CDI.

3. Principle of the assay

This rapid test is a one-step lateral flow immunochromatographic assay employing both biotinylated and gold-labeled anti-GDH antibodies. When Clostridium difficile GDH is present in the specimen, immune complexes form with the gold-labeled anti-GDH antibodies and migrate through the reaction membrane. Streptavidin captures the migrating immune complexes at the test line (T line) via the biotin coupled to the anti-GDH antibodies, resulting in red-violet staining of the T line. Migrating gold-labeled antibodies not bound in the complex are bound later at the control line (C line). If Clostridium difficile GDH is not present in the specimen, the binding of gold-labeled immunocomplexes will not occur at the T line but only at the C line. The presence of a red C line confirms that the test was valid.

4. Reagents provided

Each kit contains sufficient reagents for 25 tests.

Cassette	25 tests	25 individually packaged test cassettes
Reagent A	13.5 ml	Specific anti-GDH antibodies; contains 0.05 % azide, ready for use, blue colored
Reagent B	13.5 ml	Specific anti-GDH antibodies; contains 0.05 % azide, ready for use, yellow colored
Pipette	25	Pouches of 1 disposable pipette each
Reagent vial	25	Pouches of 1 reaction vessel each
Pipette Tip	25	Pouches of 1 pipette tip each
Micro Pipette	1	Microliter pipette for 150 µl volumes

5. Reagents and their storage

Store the kit at 2 to 25 °C. Kit contents are stable until the expiration date printed on the

product label. The quality of the product cannot be guaranteed after the expiration date. Likewise, the usability of the cassettes can no longer be guaranteed if the cassette packaging is damaged.

6. Materials required but not provided

- Vortex mixer (optional)
- Waste receptacle containing 0.5 % sodium hypochlorite solution

7. Precautions

For in vitro diagnostic use only.
This assay should only be performed by trained laboratory personnel. The rules of good medical laboratory practice should be followed. Strict adherence to the test instructions is advised.

The reagents contain sodium azide as a preservative. Avoid contact with the skin and mucous membranes.

Do not pipette samples or reagents by mouth and avoid contact of reagents with injured skin or mucous membranes. Wear disposable gloves when handling samples and wash hands after completion of testing. Do not smoke, eat or drink in areas where samples or reagents are being handled.

All reagents and materials coming in contact with potentially infectious samples must be treated with appropriate disinfectants (e.g., sodium hypochlorite) or autoclaved at 121 °C for at least 1 hour.

8. Collection and storage of samples

Stool samples should be collected in clean containers without preservatives and stored at 2 to 8 °C until processed for testing. If the samples cannot be processed within 3 days, they should be stored at –20°C or colder. Frozen specimens must be thawed completely and equilibrated to room temperature before testing. Repeated freezing and thawing of samples should be avoided. If rectal swabs are to be used, ensure that sufficient fecal matter (ca. 50 mg) is available for performance of the assay.

9. Test procedure

9.1. General information

Bring all test specimens, reagents and test cassettes to room temperature (20 to 25 °C) before use. The test cassettes should not be removed from the packaging until immediately before use. Each test cassette is for single use only and cannot be reused. Do not perform the test in direct sunlight. Excess reagent must not be put back in the vial because this could cause contamination.

9.2. Sample preparation

Add **0.5 ml each** (about 12 to 14 drops) of Reagent A Reagent A and Reagent B Reagent B to a labeled reaction vial Reagent Vial. **Primarily** adhere to the 0.5 ml and 1.0 ml graduation marks on the reaction vessel, independent of the number of drops of reagent. Reagents A and B must be dispensed at a ratio of 1:1!

9.2.1 Preparation of stool samples

50 µl of **liquid stool specimen** is drawn into a disposable pipette Pipette (to the second bulge) and added to the reagents in the reaction vessel to yield a suspension.
If solid, approximately 50 mg of **solid stool specimen** is analogously processed to yield a suspension. Next, the reaction vessel is sealed carefully and the sample is homogenized by thorough mixing (and vortexing: optional). Subsequently, the homogenized stool suspension is allowed to stand for **5 minutes** to yield a largely particle-free supernatant. The reaction vessel can be placed in one of the openings of the reagent holder for sedimentation.

9.3. Sample testing

Remove the test cassette Cassette from the packaging and place on a flat surface. After placing an unused tip Pipette Tip on the microliter pipette Micro Pipette, remove 150 µl of the supernatant from the respective reaction vessel and dispense into the sample well of the test cassette. Ensure that the liquid flows freely through the membrane. If the test was performed correctly, the color band at control line C should appear within about 3 minutes. If no control line appears within 3 minutes, the test is invalid and must be repeated. In repeat testing, the test specimen should be better sedimented (optionally, by centrifuging it at 2000 g for 2 minutes), and the supernatant pipetted into the sample well of a new test cassette.
Always read the test result within **15 minutes** of applying the specimen to the sample well. Color development of the lines can intensify during the entire development time, and the color of the lines may change from red-violet to bluish/grayish violet as the strip dries.

10. Quality control – Signs of reagent deterioration

The test is invalid if the test cassette is damaged before application of the sample suspension into the sample well or if it any exhibits discoloration or color lines prior to use. The test is invalid if no red-violet control line appears within the 15-minute incubation period. If no control line appears, the following checks should be performed before repeating the assay:

- Check the expiration date of the test cassette and of the reagents used.
- Check to determine whether the test has been performed correctly.

- Check for contamination of the reagents.

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

11. Evaluation and interpretation

No more than two lines should appear, and they should appear in the following order relative to the sample well: One red-violet test line (T), or reaction band, and one red-violet control line (C), or control band. **If no control line appears, the test is invalid and must be repeated.**

Test results are interpreted as follows:

- **Clostridium difficile GDH-positive:** Both the test line and the control line appear.
- **Clostridium difficile GDH-negative:** Only the control line appears.
- **Invalid:** If no control line C appears, even if a test line is visible, the test is invalid. Likewise, if no significant color line develops until significantly later than 15 minutes after sample application, the test is also invalid and must be repeated.

12. Limitations of the method

RIDA®QUICK Clostridium difficile GDH specifically detects Clostridium difficile-specific glutamate dehydrogenase in stool specimens. No correlation between the intensity of the specific color lines and the occurrence or clinical severity of clinical symptoms can be derived from the test result. **The test results must always be interpreted in conjunction with the clinical signs and symptoms.**

A **positive** result does not rule out the possibility of other infectious agents or causes.

A **negative** result does not rule out the possibility of Clostridium difficile infection. The test may be negative due to intermittent pathogen excretion or because specific GDH concentrations in the sample are below the limit of detection. If there is justified suspicion of infection with the target pathogen based on the case history, another stool sample should be tested.

Excessive amounts of stool sample can result in the test strip developing brown stains, which may cover the red-violet color of the specific test and control lines. In the event of such staining, the test should be repeated with a smaller quantity of stool sample; alternatively, stronger centrifugation of the stool suspension can be performed to determine whether C. difficile-specific glutamate dehydrogenase is indeed present in the sample but masked by an excess of stool matrix.

13. Performance characteristics

13.1 Clinical sensitivity and specificity

In a validation study, a total of 80 frozen stool specimens were tested by the RIDA®QUICK Clostridium difficile GDH lateral flow immunochromatographic assay versus a real-time polymerase chain reaction (RT-PCR) assay for the 16 S rDNA gene of C. difficile and a C. difficile GDH ELISA. The results are summarized in the table below.

Table 1:
Sensitivity and specificity of RIDA®QUICK Clostridium difficile GDH versus RT- PCR and ELISA

		RIDA®QUICK Clostridium difficile GDH	
		+	-
RT-PCR / ELISA	+	**30**	**0**
	-	**1**	**49**

Relative sensitivity: 100.0 %
Relative specificity: 98.0 %

13.2 Precision

The precision of the RIDA®QUICK Clostridium difficile GDH test was measured on the basis of intra-assay reproducibility (10 replicates / 1 day / 1 operator / 1 lot), inter-day reproducibility (3 replicates / 10 days / 1 operator / 1 lot), inter-operator reproducibility (3 replicates / 1 day / 3 operators / 1 lot), and inter-lot reproducibility (3 replicates / 1 day / 1 operator / 3 lots). Five references were measured for each test: one negative, two weakly positive, and two moderately positive. The RIDA®QUICK Clostridium difficile GDH test yielded the expected result in all measurements.

13.3 Cross-reactivity

Various pathogenic bacteria of the intestinal tract were tested with the RIDA®QUICK Clostridium difficile GDH test and exhibited no cross-reactivity. The tests were performed using bacteria suspensions (10^5 to 10^9 CFU/ml), parasite cultures (10^7 to 10^9 organisms/ml), cell-culture supernatants from virus-infected cells, and a stool specimen.

The results are summarized in the following table.

Test organism	Origin / Source	Result
Adenovirus	Cell culture supernatant	Negative
Aeromonas hydrophila	Culture	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 bifermentas	Culture	Negative
Clostridium difficile	Culture	**Positive**
Clostridium novyi	Culture	Negative
Clostridium perfringens	Culture	Negative
Clostridium septicum	Culture	Negative
Clostridium sordellii	Culture	Negative
Clostridium sporogenes	Culture	Negative
E. coli (O26:H-)	Culture	Negative
E. coli (O6)	Culture	Negative
E. coli (O157:H7)	Culture	Negative
Enterobacter cloacae	Culture	Negative
Enterococcus faecalis	Culture	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	Negative
Staphylococcus epidermidis	Culture	Negative
Vibrio parahaemolyticus	Culture	Negative
Yersinia enterocolitica	Culture	Negative

13.4 Interfering substances

The following substances exhibited no significant effect on the test results when mixed into

Clostridium difficile GDH-positive and -negative stool samples at the specified concentrations:

Barium sulfate (18.5 % w/w), loperamide (antidiarrheal, 0.02 % w/w), Pepto-Bismol (antidiarrheal, 6.3 % v/w), mucin (5 % w/w), cyclamate (artificial sweetener, 1.3 % v/w), human blood (5 % v/w), stearic acid / palmitic acid (1:1 ratio, 40 % w/w), metronidazole (antibiotic, 3 % w/w), diclofenac (0.1 % w/w), and vancomycin (antibiotic, 3 % w/w).

13.5 Analytical sensitivity

Analytical sensitivity of RIDA®QUICK Clostridium difficile GDH was determined to be approximately 4.6 ng Clostridium difficile GDH per ml sample by two operators by dilution series testing in two lots of product. The limit of detection was confirmed to be 4.6 ng per ml sample, as determined in 60 measurements over 5 days in two lots of product tested by two operators, yielding 100% positive results.

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