RIDA®QUICK Campylobacter

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Campylobacter is an immunochromatographic rapid test for the qualitative detection of Campylobacter jejuni and Campylobacter coli antigens in human stool samples and cultures.

2. Summary and explanation of the test

Next to salmonellosis, campylobacteriosis has become one of the world's most common food-borne diarrheal diseases in humans. The high incidence of Campylobacter enteritis is facilitated by the widespread, cross-species distribution of the Campylobacter bacterium in wildlife, livestock and domestic animals (birds and mammals).
Campylobacter is a commensal of the intestinal tract of poultry. Therefore, the bacterium enters the food chain of humans mainly via these animals. However, other foodstuffs such as milk and ground meat as well as drinking water also function as vehicles for the transmission of the pathogen. Campylobacter is excreted into the environment in large quantities by numerous animal sources and is later transmitted to humans via contaminated food. Other possible routes of transmission of Campylobacter enteritis include direct contact with diseased animals infected with Campylobacter as well as the fecal-oral route, particularly in children. The infectious dose is relatively small—only 500 bacteria. Of the roughly 15 known Campylobacter species, C. jejuni and C. coli are the two most common causes of bacterial gastroenteritis in humans. After an incubation period of 2 to 10 days, the infectious agent is excreted in the stool of untreated infected individuals for up to 4 weeks. However, long-term excretion of Campylobacter can occur in individuals with immunodeficiency. Many Campylobacter infections are asymptomatic. In symptomatic infections, a prodrome of fever, headache, and myalgia and arthralgia is followed by typical symptoms of gastroenteritis, including diarrhea, cramps and abdominal pain. The diarrhea consists of mushy to watery and sometimes bloody stools. Late complications of the disease include arthritis and, infrequently, Guillain-Barré syndrome.
Most patients receive symptomatic treatment consisting of fluid and electrolyte replacement. Antibiotic therapy is indicated only in severe cases.
To successfully culture these sensitive bacteria, the stool samples should be as fresh as possible and should be transported to the laboratory quickly or under refrigerated conditions. This does not apply to modern antigen detection systems such as the RIDA®QUICK Campylobacter rapid test, which permits the specific detection of Campylobacter antigen in stool specimens, even in cases where the bacteria is no longer cultivable.

3. Principle of the assay

This rapid test is a one-step lateral flow immunochromatographic assay employing both biotinylated and gold-labeled anti-Campylobacter antibodies. When Campylobacter jejuni and

Campylobacter coli antigens are present in the specimen, immune complexes form with the gold-labeled anti-Campylobacter 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-Campylobacter 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 C. jejuni or C. coli antigens are 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-Campylobacter antibodies; contains 0.05 % azide, ready for use, blue in color
Reagent B	13.5 ml	Specific anti-Campylobacter antibodies; contains 0.05 % azide, ready for use, yellow in color
Pipette	50 pieces	Bag with 50 multifunction pipettes, graduated for pipetting liquid specimens and with a spatula for measuring out solid stool specimens
Reagent vial	25 pieces	Bag with 25 reaction vials

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 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. When 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. Preparing the samples

Prior to use, all stool specimens must always be thoroughly mixed to ensure homogeneous distribution of the antigens.

Please note:

For every specimen test, two graduated pipettes Pipette are available to be used as follows:

Pipette 1: for pipetting Reagent A Reagent │A and the specimen (50 µl or 50 mg with the spatula depending on the consistency of the specimen).

Pipette 2: for pipetting Reagent B Reagent │B and the mixture of Reagent A and B and the specimen.

9.3. Specimen testing

In a labelled reagent vial Reagent vial, pipette 0.5 ml Reagent A Reagent │A using **Pipette 1** and 0.5 ml Reagent B Reagent │B using **Pipette 2**. Add the previously homogenized stool specimen, either 50 mg of **Pipette 1** using the spatula (note the marking on the spatula) or 50 µl (first graduation on the pipette) depending on the consistency, to this

reagent mixture. Tightly seal the reaction vial and shake the contents well to mix (optional: vortex). Place the reaction vial in the frame from the test kit for at least 5 min. During this time, the specimen reacts with the reagent mixture while the solid stool components are deposited as sediment. In the meantime, take the room-temperature test cassette Cassette from its package and place in on a level surface.

As soon as the 5-minute reaction time has passed, carefully open the reaction vessel and use **Pipette 2** to remove 150 µl (second graduation on the pipette) of clarified supernatant and pipette it into the specimen funnel at the edge of the cassette. Make sure that the fluid runs through the membrane without obstruction. If performed correctly, the control band appears at control line C after approximately 3 minutes. If the control line is not visible after 3 minutes, the reaction vial must be closed again and centrifuged for 2 min at 2000 x g to sediment out any problematic solid particles. Afterwards, pipette 150 ml of the supernatant into the specimen funnel of a new cassette.

Read the test result after **15 minutes**. Over the entire development time and after drying of the strip, the colouring and intensity of the bands can change from red-violet to blue- to grey-violet.

9.3.1 Preparation of liquid and solid Campylobacter cultures

Add 50 µl of **nutrient broth** (e.g., Bolton broth) to 1.0 ml of the already prepared reagent mixture in the reaction vessel (0.5 ml of Reagent A and 0.5 ml of Reagent B) and mix. 150 µl of the mixture is used for sample testing (see 9.3.).

When using **solid culture media**, first remove and completely suspend as many colonies from the culture plate in 1 ml of distilled water or saline solution (0.9% NaCl). Then add 50 µl of this suspension to 1.0 ml of the already prepared reagent mixture in the reaction vessel (0.5 ml of Reagent A and 0.5 ml of Reagent B) and mix. 150 µl of the mixture is used for sample testing (see 9.3.).

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:

- **Campylobacter-positive:** Both the test line and the control line appear.
- **Campylobacter 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

The RIDA®QUICK Campylobacter rapid test detects Campylobacter antigens in human stool samples and Campylobacter cultures. 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 result must always be interpreted in conjunction with the clinical signs and symptoms.**

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

A **negative** test does not rule out the possibility of Campylobacter infection. The test may be negative due to intermittent pathogen excretion or to antigen concentrations below the limit of detection. If there is justified suspicion of C. jejuni or C. coli infection based on the case history, another stool sample should 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 the target Campylobacter antigens are indeed present in the sample but masked by an excess of stool matrix.

13. Performance characteristics

13.1 Test quality

A study was conducted to compare the performance of the RIDA®QUICK Campylobacter rapid test with that of the gold standard, culture of the bacteria on charcoal cefoperazone

deoxycholate (CCD) agar under microaerophilic conditions. A total of 574 stool specimens from the daily diagnostic workload of a contract laboratory were tested with RIDA®QUICK Campylobacter for this purpose. The results are summarized below in Table 1.

Table 1: Comparison of RIDA®QUICK Campylobacter with culture on CCD agar plates

		Culture (CCD agar)	
		Positive	Negative
RIDA®QUICK Campylobacter	Positive	62	7
	Negative	1	504

Sensitivity:	98.4 %
Specificity:	98.6 %
Positive predictive value:	89.8 %
Negative predictive value:	99.8 %

13.2 Analytical sensitivity

The analytical detection limit of the assay was determined separately for C. jejuni and C. coli. The limit of detection (LOD) is the lowest pathogen concentration distinguishable as positive (visible band) by RIDA®QUICK Campylobacter. The LOD, as determined by 3 different readers with cassettes from 2 different lots and 60 replicates (95% confidence interval), was 2.1 x 10^4 colony-forming units (CFU) per ml for Campylobacter jejuni and 8.5 x 10^5 CFU/ml for Campylobacter coli.

13.3 Precision

Precision of the RIDA®QUICK Campylobacter rapid test was evaluated in terms of intra-assay reproducibility, inter-day reproducibility (10 days), inter-operator reproducibility (3 operators), and inter-lot reproducibility (3 lots). Replicates of the following 5 reference samples were tested in each case: one negative, two moderately positive and two weakly positive samples. The RIDA®QUICK Campylobacter rapid test yielded the expected result in all measurements.

13.4 Cross-reactivity

Various pathogenic bacteria of the intestinal tract were tested with the RIDA®QUICK Campylobacter rapid test and exhibited no cross-reactivity with C. jejuni or C. coli. The tests

were performed using bacteria suspensions (10^6 to 10^9 CFU/ml), parasite cultures (10^7 to 10^9 organisms/ml), and infectious culture supernatants from virus-infected cells. The results are summarized below in Table 2. None of the tested organisms except Campylobacter jejuni and Campylobacter coli exhibited reactivity in the RIDA®QUICK Campylobacter rapid test.

Table 2: Cross-reactivity with pathogenic bacteria of the intestinal tract

Test Organism	Origin / Source	Result
Adenovirus	Infectious culture supernatant	Negative
Aeromonas hydrophila	Culture	Negative
Astrovirus	Infectious culture supernatant	Negative
Bacillus cereus	Culture	Negative
Bacteroides fragilis	Culture	Negative
Campylobacter coli	Culture	Positive
Campylobacter fetus	Culture	Negative
Campylobacter jejuni	Culture	Positive
Campylobacter lari	Culture	Negative
Campylobacter upsaliensis	Culture	Negative
Candida albicans	Culture	Negative
Citrobacter freundii	Culture	Negative
Clostridium difficile	Culture	Negative
Clostridium perfringens	Culture	Negative
Clostridium sordellii	Culture	Negative
Cryptosporidium muris	Culture	Negative
Cryptosporidium parvum	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
Giardia lamblia	Stool specimen	Negative
Klebsiella oxytoca	Culture	Negative
Proteus vulgaris	Culture	Negative
Pseudomonas aeruginosa	Culture	Negative
Rotavirus	Infectious 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

14. Interfering substances

The following substances were tested with the RIDA®QUICK Campylobacter rapid test and exhibited no significant effect on the test results when mixed with Campylobacter-positive and Campylobacter-negative stool samples at the specified concentrations:
Barium sulfate (5 % w/w), loperamide (antidiarrheal, 5 % w/w), Pepto-Bismol (antidiarrheal, 5 % v/w), mucin (5 % w/w), cyclamate (artificial sweetener, 5 % v/w), human blood (5 % v/w), stearic acid / palmitic acid (1:1 ratio, 40 % w/w), metronidazole (0.5) (antibiotic, 5 % v/w), and diclofenac (0.00263 % v/w).

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