RIDA®QUICK Helicobacter

REF N2303

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Helicobacter is an immunochromatographic rapid test for the qualitative detection of *Helicobacter-pylori*-specific antigen in human stool specimens.

2. Summary and explanation of the test

In 1984 Marshall and Warren were able to detect the presence of a *Campylobacter*-like organism in the mucosa of the gastric antrum and corpus in patients with histologically confirmed gastritis and peptic ulcers of the duodenum. We now recognize that *Helicobacter pylori* is causally involved in the development of gastrointestinal diseases. Infections by *H. pylori* result in inflammations that have a causal relationship with chronic gastritis, gastric ulcers, ulcers of the small intestine, and gastric cancers. This premise is confirmed by the healing of gastritis and ulcers which is usually successful following eradication therapy. *H. pylori* has developed various defense mechanisms to survive in the acidic, bactericidal environment of the stomach. The enzyme urease converts urea into ammonia and carbon dioxide, and thereby neutralizes gastric acid. The production of catalase and superoxide dismutase protects *H. pylori* from being attacked by neutrophils. Many *H. pylori*-positive patients develop gastritis, and about 10 % of patients develop ulcers. Of patients with ulcers of the small intestine or stomach, 90 % are *H. pylori* positive, regardless of age. There are two basic approaches for diagnosing *H. pylori* infections: direct detection of the organism and indirect determination through the detection of the antibodies patients produce in response to *H. pylori*. Direct, albeit invasive detection methods of an infection include the rapid urease test, histology, PCR, and the cultivation of the organism from biopsy material. Cultivating *H. pylori* from biopsy material is a difficult and tedious process. Technical difficulties can lead to false-negative results, which means low sensitivity. In addition, *H. pylori* tends to colonize the gastric mucosa in an island pattern, which is why the sensitivity of histology increases with an increasing number of obtained biopsies. Another direct detection method of *H. pylori* is the urea breath test. This test detects the carbon dioxide produced by bacterial urease. The breath test has high sensitivity and specificity but requires special testing devices and the ingestion of isotope-labelled urea by patients. When these methods are used, however, the test accuracy of the urease-dependent tests (rapid urease test and urea breath test) is heavily influenced by the presence of interfering factors. A commonly used detection tool is the serological determination of *H. pylori*-specific antibodies. This is an indirect detection method that detects the antibodies produced by the patient in response to *H. pylori*. The test for monitoring the success of eradication therapy using serological methods is simply insufficient since the antibody titer decreases only slowly over several months.

RIDA®QUICK Helicobacter is an immunochromatographic rapid test for the direct, non-invasive detection of *H. pylori* antigens in human stool. The test is based on

monoclonal antibodies, preventing fluctuations between the individual lots. The direct detection of antigens can be used to support the formulation of an initial diagnosis as well as to check the success of therapy four to six weeks after the end of eradication therapy or detect the re-occurrence of an infection.

3. Test principle

This rapid test is a single-stage immunochromatographic lateral flow test in which biotinylated as well as gold-labelled anti-*H. pylori* antibodies are used. Once *H. pylori*-specific antigens are present in a positive specimen, immune complexes are formed containing the labelled anti-*H. pylori* antibodies, which pass through the membrane. The streptavidin located at the T test line binds the immune complexes passing through the test line via the biotin coupled to anti-*H. pylori* antibodies, resulting in a red-violet coloring of the T line. The non-complex gold-labelled antibodies passing through are bound to the subsequent control line C. If specimens are negative, the gold-labelled immune complexes will not bind to the T line; they will bind only to the C line. The red C line always indicates whether the test process was valid.

4. Reagents provided

The reagents in the kit are sufficient for 25 determinations.

Cassette	25 determinations	25 individually packed test cassettes
Reagent | A	13.5 ml	Specific anti-*Helicobacter pylori* antibody (mouse); contains 0.05% sodium azide, ready to use, blue color
Reagent | B	13.5 ml	Specific anti-*Helicobacter pylori* antibody (mouse); contains 0.05% sodium azide, ready to use, yellow color
Pipet	50 pcs.	Bag containing 50 multifunctional pipettes, graduated for pipetting liquid specimens and having a spatula for measuring out stool specimens
Reagent vial	25 pcs.	Bag with 25 reaction vials

Dangerous substances are indicated according to labelling obligations. For more details, refer to the Safety Data Sheets (SDS www.r-biopharm.com).

5. Storage instructions

The kit can be stored at 2 °C to 30 °C and can be used until the printed date of expiration. After the expiry date, the quality guarantee is no longer valid. Similarly, the usability of the 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 containing 0.5 % 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 user instructions for carrying out this test. Do not pipette samples or reagents by mouth. Avoid contact with broken skin and mucous membranes. Wear personal protective equipment (suitable gloves, apron, protective glasses) when handling reagents and samples and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are being processed.
For more details, refer to the Safety Data Sheets (SDS www.r-biopharm.com).

The reagents contain 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 specimens must be treated with suitable disinfectants (e.g., sodium hypochlorite) or autoclaved at 121 °C for at least one hour.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

8. Collection and storage of specimens

Stool specimens must be collected in clean standard containers and may be stored for up to three days at 2 °C to 8 °C until they are used in the test. If longer storage is necessary before use, the stool specimens must be stored at -20 °C (Table 1). Avoid freezing and thawing the specimen repeatedly. Do not collect the stool specimens in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since such substances may interfere with the RIDA®QUICK Helicobacter test.

Table 1: Specimen storage

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

9. Test procedure

9.1. General information

The specimens, reagents, and test cassettes must be brought to room temperature (20 °C to 25 °C) before use. To do so, remove the test cassettes needed and other necessary components from the test kit packaging at least one hour prior to performing the test. The test cassettes themselves should not be taken out of the package until shortly before use. Once used, the test cassettes must not be re-used. The test procedure must not be carried out in direct sunlight. Do not return excess reagent to the containers, because contamination can result.

9.2 Preparing the specimens

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

Please note:
For every specimen test, two graduated multifunctional pipettes [Pipet] are available to be used as follows:

Pipette 1: for pipetting reagent A [Reagent | A] and the specimen (50 µl for liquid specimens) or 50 mg using the spatula at the other end of the pipette for solid specimens.

Pipette 2: for pipetting reagent [Reagent | B] and the mixture from reagents A and B and the specimen onto the test cassette.

9.3 Specimen testing

In a labelled reaction vial [Reagent vial], pipette **0.5 ml** (third graduation) [Reagent A] using pipette 1 and **0.5 ml** (third graduation) [Reagent B] using pipette 2. Add to this reagent mixture 50 mg using the spatula of pipette 1 or 50 µl (first graduation) of the previously homogenized stool specimen. Tightly seal the reaction vial and shake the contents well to mix (optional: vortex). Then place the reaction vial in the frame included in the test kit for 5 minutes. During this time, the specimen reacts with the reagent mixture while the solid stool components are deposited as sediment. In the meantime, remove the room-temperature test cassette [Cassette] from its packaging and place it on a level surface.
As soon as the 5-minute reaction time has passed, carefully open the reaction vial and use pipette 2 to remove 150 µl (second graduation) of the 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 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, the reaction vial must be closed again and centrifuged for 2 minutes at 2,000 x g to sediment out any problematic solid particles in the specimen. After sedimentation, a new test cassette must be used to repeat the test.
Always wait **15 minutes** to read the test result. Over the entire development time and after drying of the strip, the coloring and intensity of the bands can change from red-violet to blue- to grey-violet.

10. Quality control – indication of instability or deterioration of reagents

The test should be evaluated only if the test cassette is intact prior to pipetting the sample suspension and no changes in color or bands can be seen. Furthermore, after an incubation period of 15 minutes, at least the red-violet control bands must be visible. If they do not appear, check the following items prior to repeating the test:
- Shelf life of the test cassettes and the reagents used
- Correct test procedure
- Contamination of the reagents

If the conditions are still not fulfilled after the test is repeated, please consult the manufacturer or your local distributor.

11. Evaluation and interpretation

A maximum of two bands must appear, viewed from the specimen 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 control band C is absent, the test cannot be evaluated and is invalid!**

The following interpretations are possible:
- ***H. pylori* positive:** Control and test bands are visible.
- ***H. pylori* negative:** Only the control band is visible.
- **Invalid:** No band is visible, or there is a different constellation than the one described above. Likewise, changes in band color which do not appear until some time after 15 minutes have no diagnostic value and should not be assessed.

12. Limitations of the method

RIDA®QUICK Helicobacter detects the specific antigen of *H. pylori* in human stool specimens. The intensity of the specific band visible bears no association to the occurrence or severity of clinical symptoms. **The results obtained must always be interpreted in combination with the clinical signs and symptoms.**

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

A **negative** result does not rule out a possible infection with *H. pylori*. Such a result may be due to intermittent excretion of the specific antigen or due to an insufficient amount of antigen in the specimen. If the patient history supports a suspicion of infection by the target pathogen, the examination should be repeated with another stool specimen from the patient.

13. Performance characteristics

13.1. Test quality

The diagnostic performance of RIDA®QUICK Helicobacter was tested with 266 stool specimens in a routine laboratory. The specimens came from patients with suspected infection by *H. pylori*. The routine diagnostic assay used in the laboratory (CLIA) was performed as a reference. Five specimens were borderline in the routine diagnostic testing and were excluded. The results of that study are shown in Table 2.

Table 2: Comparison of RIDA®QUICK Helicobacter with the EIA (CLIA) of routine diagnostic testing at the study center

		Competitor EIA (CLIA)	
		pos.	neg.
RIDA®QUICK Helicobacter	pos.	37	6
	neg.	12	206

Positive agreement: 80.4 %

Negative agreement: 95.8 %

13.2. Precision

For determination of the precision of the RIDA®QUICK Helicobacter test, the 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) were examined. Five references were measured for each examination: one negative, two weakly positive, and two moderately positive. The RIDA®QUICK Helicobacter test produced the expected result in 100 % of the measurements for intra-assay, inter-day, and inter-operator testing. A divergent result was found for the weakly positive reference in one lot in the inter-lot precision. Therefore, the test delivers precise results under all conditions tested.

13.3. Cross-reactivity

A variety of pathogenic micro-organisms from the intestinal tract were examined using the RIDA®QUICK Helicobacter test and demonstrated no cross-reactivity. The examinations were performed using bacterial suspensions (10^6 to 10^9 CFU/ml), cell culture supernatants from virus-infected cells, and viral capsid preparations.

The results of that study are listed in Table 3.

Table 3: Potentially cross-reactive micro-organisms in RIDA®QUICK Helicobacter

Organism	Origin	Results
Arcobacter butzleri	Culture	Negative
Adenovirus	Cell culture supernatant	Negative
Astrovirus	Cell culture supernatant	Negative
Bacillus cereus	Culture	Negative
Bacteroides fragilis	Culture	Negative
Campylobacter coli	Culture	Negative
Campylobacter fetus	Culture	Negative
Campylobacter jejuni	Culture	Negative
Campylobacter lari	Culture	Negative
Campylobacter upsaliensis	Culture	Negative
Candida albicans	Culture	Negative
Citrobacter freundii	Culture	Negative
Clostridium difficile	Culture	Negative
Clostridium sordellii	Culture	Negative
Cryptosporidium parvum	Culture	Negative
Entamoeba histolytica	Stool 1:10	Negative
Enterobacter cloacae	Culture	Negative
Enterococcus faecalis	Culture	Negative
Escherichia coli EHEC	Culture	Negative
Escherichia coli EPEC	Culture	Negative
Escherichia coli ETEC	Culture	Negative
Escherichia coli STEC	Culture	Negative
Giardia lamblia	Stool 1:10	Negative
Klebsiella pneumoniae	Culture	Negative
Norovirus	Viral capsid	Negative
Proteus vulgaris	Culture	Negative

Pseudomonas aeruginosa	Culture	Negative
Salmonella enteritidis	Culture	Negative
Salmonella typhimurium	Culture	Negative
Shigella flexneri	Culture	Negative
Aeromonas hydrophila	Culture	Negative
Helicobacter cinaedi	Culture	Negative
Helicobacter heilmannii	Culture	Negative
Rotavirus	Cell culture supernatant	Negative
Shigella sonnei	Culture	Negative
Staphylococcus aureus	Culture	Negative
Staphylococcus epidermidis	Culture	Negative
Vibrio parahaemolyticus	Culture	Negative
Yersinia enterocolitica	Culture	Negative

13.4 Interfering substances

The substances listed below showed no effects on the test results when they were mixed into *Helicobacter*-positive and -negative stool specimens in the described concentrations:

Loperamide	0.02 % w/w	Barium sulphate	18.50 % w/w
Pepto-Bismol	6.30 % w/w	Iberogast	0.09 % w/w
Human blood	5.00 % v/w	Sweetener	1.30 % w/w
Stearic acid/palmitic acid	40.00 % w/w	Quadruple therapy clarithromycin + metronidazole + amoxicillin + lansoprazole	 1.50 % w/w + 1.20 % w/w + 3.00 % w/w + 0.09 % w/w
Mucin	5.00 % v/w		
Diclofenac	0.10 % v/w		

13.5 Analytical sensitivity

The analytical sensitivity of RIDA®QUICK Helicobacter was determined as a limit of detection by two operators in two lots of the product by testing a dilution series of approximately 4.8 ng *Helicobacter* antigen/ml. The limit of detection was confirmed by 60 measurements over five days in two lots by two operators using 4.8 ng/ml with 100 % positive results.

14. Version history

Version number	Chapter and designation
2018-06-07	Release version
2018-07-30	13.5 Analytical sensitivity

15. Explanation of symbols

General symbols

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

Test-specific symbols

Cassette	Test cassette
Reagent | A	Reagent A
Reagent | B	Reagent B
Pipet	Pipette
Reagent vial	Reaction vial

16. Literature

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