RIDASCREEN® Helicobacter

REF C2302

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1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® Helicobacter is an enzyme immunoassay 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* are 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.

RIDASCREEN® Helicobacter is an enzyme immunoassay in microtiter plate format 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

The RIDASCREEN® Helicobacter test employs monoclonal antibodies in a sandwich-type method. The well surface of the microtiter plate is coated with monoclonal antibodies against *H. pylori*-specific antigens.
A pipette is used to place a suspension of the stool specimen to be examined as well as the controls into the well of the microtiter plate together with biotinylated anti-*Helicobacter* antibodies (Conjugate 1) for incubation at room temperature (20 - 25 °C). After a washing stage, streptavidin poly-peroxidase conjugate (Conjugate 2) is added and incubated again at room temperature (20 - 25 °C). In the presence of *H. pylori*-specific antigens in the specimen, a sandwich complex of immobilized antibodies, antigens, and conjugated antibodies forms. A further washing stage removes the unattached streptavidin poly-peroxidase conjugate. In positive samples, the addition of a substrate changes the bound enzyme from a colorless solution to a blue solution. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of *H. pylori*-specific antigens in the specimen.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96 determinations	Microtiter plate, 12 microwell strips (breakable) in the strip holder; coated with monoclonal antibodies (mouse) against *H. pylori*-specific antigens.
Diluent | 1	100 ml	Sample dilution buffer 1, protein-buffered NaCl solution, ready to use, blue colored
Wash buffer	100 ml	Washing buffer, phosphate-buffered NaCl solution (concentrated 10-fold); contains 0.1 % thimerosal
Control | +	2 ml	Positive control; inactivated *H. pylori* antigen; ready to use; red-pink colored
Control | -	2 ml	Negative control; negative control (sample dilution buffer 1); ready to use
Conjugate | 1	13 ml	Biotin-conjugated antibodies (mouse) against *H. pylori*-specific antigens; ready to use; green colored
Conjugate | 2	13 ml	Streptavidin poly-peroxidase conjugate in stabilized protein solution; ready for use; orange colored
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop reagent; 1 N sulfuric acid; ready for use

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

5. Storage instructions

All reagents must be stored at 2 - 8 °C and can be used until the date printed on the label. Providing the diluted washing buffer is stored at 2 - 8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.
The aluminum bag must be opened with scissors in such a way that the clip seal is not separated. Any microtiter strips which are not required must immediately be returned to the aluminum bag and stored at 2 - 8 °C.

The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Reagents required but not provided

6.1 Necessary reagents

The following reagents are required to perform the RIDASCREEN® Helicobacter Test:

Reagents
Distilled or deionized water

6.2 Necessary laboratory equipment

The following equipment is required to perform the RIDASCREEN® Helicobacter test:

Equipment
Sample vials
Disposable pipettes (Article no.: Z0001)
Vortex mixer (optional, see 9.3.)
Micropipette for 50 - 100 µl and 1 ml volume
Graduated cylinder
Stopwatch
Washing device for microtiter plates (art. no. Z50TS8V or Z800TS) or multichannel pipette (300 µl).
Photometer for microtiter plates (450 nm, reference filter 620-650 nm)
Filter paper (laboratory towels)
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 safety equipment (suitable gloves, gown, safety glasses) when handling the specimens, and wash hands after finishing the test. Do not smoke, eat, or drink in areas where samples are being processed.

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

The kit includes a positive control that contains a recombinant *H. pylori*-specific antigen. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the patient specimens.

The washing buffer contains 0.1 % thimerosal 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

Until it is used, store the test material at 2 - 8 °C. If the material cannot be used for a test within three days, we recommend storage at 20 °C or colder. Avoid freezing and thawing the specimen repeatedly. After diluting a stool specimen in the sample dilution buffer 1:11, it can be stored at 4 °C for use within three days (Table 1). Stool samples and rectal smears should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® Helicobacter test. If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.

Table 1: Specimen storage

Undiluted stool specimen		Diluted stool specimen
2 - 8 °C	≤ -20 °C	4 °C
≤ 3 days	> 3 days	≤ 3 days

9. Test procedure

9.1. General information

All reagents and the microtiter Plate must be brought to room temperature (20 - 25 °C) before use. The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (in sealed bags) and the reagents must be stored again at 2 - 8 °C.
Once used, the microwell strips must not be used again. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

In order to prevent cross-contamination, the samples must be prevented from coming into direct contact with the kit components.
The test must not be carried out in direct sunlight.
We recommend covering the microtiter plate or sealing with plastic wrap to prevent evaporation losses.

9.2. Preparing the washing buffer

Mix 1 part washing buffer concentrate Wash buffer with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

9.3 Preparing the specimens

Fill a labelled test tube with 1 ml RIDASCREEN® sample dilution buffer Diluent | 1. Using a spatula or disposable inoculation loop, remove approx. 50 to 100 mg of stool specimen and suspend it in the diluent.
If the stool specimen is liquid, use a disposable pipette (article no. Z0001) to aspirate approx. 100 µl of the sample to just above the second marking and suspend it in the buffer.
Homogenize the stool suspension by suction and ejection from the disposable pipette, or alternatively, by mixing in a vortex mixer. Let the suspension stand a short period of time (10 minutes) for the coarse stool particles to settle, and use this clarified supernatant of the stool suspension directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant **must** be particle-free. In this case, it is advisable to centrifuge the sample at 2500 x g for 5 minutes.

Note:
Stool samples diluted in Diluent | 1 can be used in any other RIDASCREEN® ELISA, provided that it also uses Diluent | 1.

9.4. First incubation

After inserting a sufficient number of wells in the strip holder, add 100 µl of the positive control Control | +, the negative control Control | -, and the stool specimen suspension to the wells. Then add 100 µl of the biotin-conjugated antibody Conjugate | 1, mix (by tapping lightly on the edge of the plate), and incubate for 60 minutes at room temperature (20 - 25 °C).

9.5. Washing

Careful washing is important in order to achieve the correct results and should therefore be carried out strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container for disposal in accordance with official regulations. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. Then wash the plate five times using 300 µl washing buffer each time. Make sure that the wells are emptied completely by

knocking them out after each wash onto a part of the absorbent paper which is still dry and unused.

If a microplate washer or fully automated ELISA is used, ensure that the machine is correctly adjusted or request the settings from the manufacturer if necessary. Appliances delivered by R-Biopharm are already programmed with validated settings and work protocols. To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see Item 9.3., Preparing the samples). Also make sure that all of the liquid is aspirated during each washing phase.

9.6. Second incubation

Use a pipette to fill 100 µl streptavidin poly-peroxidase conjugate Conjugate | 2 into the wells, then incubate for 30 minutes at room temperature (20–25 °C).

9.7. Washing

Wash as described in Item 9.5.

9.8. Third incubation

Fill all wells with 100 µl substrate Substrate. Then incubate the plate for 15 minutes in darkness at room temperature (20 - 25 °C). Next, fill all wells with 50 µl stop solution Stop in order to stop the reaction. After blending carefully by tapping lightly on the side of the plate, measure the extinction at 450 nm (optional: 450/620 nm). Blank value adjustment should be done in air, i.e. without the microtiter plate.

Note:
High-positive patient samples may cause black-colored precipitates of the substrate.

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

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the reagents are stable and that the test is conducted correctly. The test has been carried out correctly if the extinction rate (O.D.) for the negative control is less than 0.200 at 450 nm (less than 0.160

at 450/620 nm) and the measured value for the positive control is greater than 0.800 at 450 nm or at 450/620 nm. A value of the negative control greater than 0.200 (0.160) may indicate insufficient washing. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, may indicate that the reagents have expired.
If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g., calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1. Calculating the cut-off

In order to establish the cut-off, 0.150 extinction units are added to the measured extinction for the negative control.

Cut-off = extinction for the negative control + 0.150

11.2. Test results

Assessment of the sample is **positive** if the extinction rate is more than 10 % higher than the calculated cut-off value.

Assessment of the sample is **marginal** and the test needs to be repeated if the extinction rate ranges from 10 % less to 10 % greater than the cut-off value. If the repeat examination with a fresh stool sample again falls within the grey zone, the sample should be considered negative.

Samples which are more than 10 % below the calculated cut-off must be considered **negative**.

Negative OD values can occur if bichromatic measurement methods (450/620 nm) are used.

12. Limitations of the method

The RIDASCREEN® Helicobacter test detects *H. pylori*-specific antigens in human stool specimens. It is not possible to associate the determined level of extinction 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.

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

A **marginal** result may be due to non-homogeneous distribution of the antigens in the stool sample. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested from the patient.

13. Performance characteristics

13.1. Test quality

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

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

		Competitor EIA (CLIA)	
		pos.	neg.
RIDASCREEN® Helicobacter	pos.	43	3
	neg.	6	209

Positive agreement: 90.5 %

Negative agreement: 97.9 %

13.2. Cross-reactivity

A variety of pathogenic micro-organisms from the intestinal tract were examined using the RIDASCREEN® Helicobacter ELISA and demonstrated no cross-reactivity. These studies were conducted with bacterial suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. Virus culture supernatants and stool samples are listed accordingly. The results of that study are listed in Table 3.

Table 3: Cross-reactivity with pathogenic microorganisms

Organism	Origin	MV [OD 450/620]
Adenovirus	Cell culture supernatant	-0.005
Aeromonas hydrophila	Culture	0.005
Arcobacter butzlerii	Culture	0.002
Astrovirus	Cell culture supernatant	0.004
Bacillus cereus	Culture	0.000
Bacteroides fragilis	Culture	0.000
Campylobacter coli	Culture	0.004
Campylobacter fetus	Culture	0.028
Campylobacter jejuni	Culture	-0.003
Campylobacter lari	Culture	-0.001
Campylobacter upsaliensis	Culture	-0.005
Candida albicans	Culture	-0.002
Citrobacter freundii	Culture	-0.007
Clostridium difficile	Culture	-0.003
Clostridium sordellii	Culture	-0.005
Cryptosporidium parvum	Culture	-0.004
Escherichia coli (O157:H7)	Culture	-0.010
Escherichia coli (O26:H-)	Culture	-0.007
Escherichia coli (O6)	Culture	-0.007
Escherichia coli	Culture	-0.002
Entamoeba histolytica	Stool	-0.009
Enterobacter cloacae	Culture	-0.001
Enterococcus faecalis	Culture	-0.004
Giardia lamblia	Stool	0.003
Helicobacter cinaedi	Culture	-0.008
Helicobacter heilmannii	Culture	-0.008

Klebsiella oxytoca	Culture	-0.004
Norovirus	Posko	-0.006
Proteus vulgaris	Culture	0.001
Pseudomonas aeruginosa	Culture	-0.003
Rotavirus	Cell culture supernatant	-0.011
Salmonella enteritidis	Culture	-0.006
Salmonella typhimurium	Culture	-0.011
Shigella flexneri	Culture	-0.004
Shigella sonnei	Culture	-0.009
Staphylococcus aureus	Culture	-0.004
Staphylococcus epidermidis	Culture	-0.006
Vibrio parahaemolyticus	Culture	-0.005
Yersinia enterocolitica	Culture	-0.010

13.3. Precision

The reproducibility of the RIDASCREEN® Helicobacter ELISA was tested with six references representing the complete measurement range from weak to high positive. To determine the intra-assay reproducibility, 40 replicates of these references were assayed. The mean values and the variation coefficients (VC) were determined for three lots. For the inter-assay reproducibility, references from 10 different working days were assayed in duplicates, with 2 runs per day. The measurements were determined in 3 lots by 4 technicians. The inter-lot reproducibility was determined for all 3 lots. The results are shown in Table 4.

Table 4: Results for the reproducibility/precision of RIDASCREEN® Helicobacter ELISA

Reference Mean value / VC		Intra-assay			Inter-assay			Inter-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1–3
1	MV	3.193	3.225	3.034	3.327	3.206	3.109	3.214
	VC (%)	3.57 %	1.85 %	3.76 %	2.14 %	4.71 %	5.40 %	5.37 %
2	MV	2.530	2.443	2.123	2.884	2.646	2.590	2.707
	VC (%)	5.36 %	3.04 %	4.37 %	5.66 %	8.18 %	10.19 %	9.78 %
3	MV	1.452	1.430	1.337	1.776	1.570	1.567	1.638
	VC (%)	9.68 %	5.19 %	8.76 %	7.48 %	11.93 %	12.96 %	12.86 %
4	MV	0.826	0.712	0.722	0.971	0.853	0.833	0.886
	VC (%)	12.30 %	4.30 %	8.55 %	8.67 %	11.44 %	13.15 %	13.70 %
5	MV	0.423	0.443	0.398	0.616	0.516	0.527	0.553
	VC (%)	12.00 %	4.47 %	11.22 %	9.73 %	11.02 %	13.82 %	15.02 %
6	MV	-0.005	-0.008	-0.007	0.014	-0.003	-0.004	0.002
	VC (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

13.4 Analytical sensitivity

To determine the analytical sensitivity of the RIDASCREEN® Helicobacter ELISA, the limit of blank (LoB) was determined with 90 assays of negative specimens. The limit of detection (LoD) was then determined with 30 assays of a recombinant *Helicobacter* antigen preparation. The results of these measurements are shown in Table 5.

Table 5: Results for the analytical sensitivity of the RIDASCREEN® Helicobacter ELISA

	MV [OD 450/620]	ng/ml
LoB	0.077	-
LoD	-	0.4

13.5 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:

Mucin	5.0 % w/w	Diclofenac	0.1 % v/w
Human blood	5.0 % v/w	Cyclamate/saccharin mix	1.3 % v/w
Barium sulphate	18.5 % w/w	Iberogast	0.09 % v/w
Loperamide	0.02% w/w	Quadruple therapy clarithromycin + metronidazole + amoxicillin + lansoprazole	 1.50 % w/w + 1.20 % w/w + 3.00 % w/w + 0.09 % w/w
Pepto-Bismol	6.3 % v/w		
Stearic acid/ palmitic acid	40 % w/w (1:1)		

Only extremely fatty stool specimens can produce slightly elevated OD values.

14. Version history

Version number	Chapter and designation
2018-06-07	Release version

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

Plate	Microtiter plate
Diluent | 1	Sample dilution buffer 1
Wash buffer	Washing buffer
Control | +	Positive control
Control | -	Negative control
Conjugate | 1	Biotin-conjugated antibodies
Conjugate | 2	Streptavidin poly-peroxidase conjugate
Substrate	Substrate
Stop	Stop solution

16. References

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