RIDASCREEN® Helicobacter IgA, IgG

Article no: K2311 (IgA)
K2321 (IgG)

CE

1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Helicobacter tests are enzyme immunoassays for the quantitative determination of IgA or IgG antibodies against *Helicobacter pylori* in human serum.

The tests should be used for confirmation purposes when there is a suspected case of infection with *Helicobacter* pylori or for clarifying the immune status.

2. Summary and explanation of the test

After infection with helicobacter, specific antibodies are formed against the pathogen as a result of the response from the immune system. By using immunological methods, it is possible to determine the antibodies in the serum. Both the test method used and the choice of the pathogen-specific antigen have a significant influence on the test result.

3. Test principle

Purified antigens are coated to a microwell plate. Antibodies in the patient samples bind to the antigens and are determined during the second incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The enzyme converts the colourless substrate (H_2O_2/TMB) to a blue end product. The enzyme reaction is stopped by adding sulphuric acid and the colour of the mixture switches from blue to yellow at the same time. The final measurement is carried out at 450 nm on a photometer using a reference wavelength $\geq$ 620 nm.

4. Reagents provided

Table 1: Pack contents (there are enough reagents in a pack for 96 determinations)

			K2311 IgA	K2321 IgG
Plate	96 det.	Microwell plate; 12 microwell strips (can be divided) in the strip holder; coated with the *H. pylori* antigen	X	X
SeroPP	110 ml	Sample buffer, ready for use; phosphate-buffered NaCl solution; coloured yellow	X	X
SeroWP	100 ml	Wash buffer, 10-fold concentrate; tris-buffered NaCl solution	X	X
Control IgA + *blue lid*	2.5 ml	Standard control IgA, ready for use; diluted human serum; coloured blue	X	
Control IgG + *green lid*	2.5 ml	Standard control IgG, ready for use; diluted human serum; coloured green		X
Control IgA − *colourless lid*	1.2 ml	Negative control IgA, ready for use; diluted human serum	X	
Control IgG − *colourless lid*	1.2 ml	Negative control IgG, ready for use; diluted human serum		X
Control IgA A *blue lid*	1.2 ml	Quality control control A IgA, ready for use; diluted human serum	X	
Control IgA B *blue lid*	1.2 ml	Quality control B IgA, ready for use; diluted human serum	X	
Control IgG A *green lid*	1.2 ml	Quality control A IgG, ready for use; diluted human serum		X
Control IgG B *green lid*	1.2 ml	Quality control B IgG, ready for use; diluted human serum		X
SeroA LD *blue lid*	12 ml	Anti-human IgA conjugate LD (goat), ready for use; peroxidase conjugated antibodies in stabilised protein solution	X	
SeroG HD *green lid*	12 ml	Anti-human IgG conjugate HD (goat); ready for use; peroxidase conjugated antibodies in stabilised protein solution		X
SeroSC	12 ml	Substrate; H_2O_2/tetramethylbenzidine; ready for use	X	X
Stop	12 ml	Stop reagent 0.5 M sulphuric acid; ready for use	X	X

Details of hazardous substances according to labeling obligations. For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com.

5. Storage instructions

The test kit must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for one week when stored at room temperature (20 – 25 °C). After the expiry date, the quality guarantee is no longer valid.

The aluminium bag containing the microwell plate must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must be immediately returned to the aluminium bag and stored at 2 – 8 °C.

The reagents also must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

6. Additional necessary reagents – and necessary equipment

6.1. Reagents

− distilled or deionised water

6.2. Accessories

− Incubator at 37 °C
− Test tubes
− Vortex mixer
− Micropipettes for 10-100 µl and 100-1000 µl capacities
− Measuring cylinder (1000 ml)
− Timer
− Microplate washer or multichannel pipette
− Microplate reader (450 nm, reference wavelength $\geq$ 620 nm)
− Filter paper (laboratory towels)
− Waste container containing 0.5 % sodium hypochlorite solution

7. Precautions for users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com

The control sera (standard control, negative control, quality control A and quality control B) in the kit have been tested for HIV- and HCV-Ab as well as HbsAg with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

8. Specimen collection and storage

The test has been developed for testing human serum samples. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested. Repeated freezing and thawing of the samples and microbial contamination must be prevented at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid samples can lead to false results.

Table 2: Sample storage

Undiluted serum		Diluted serum
2 – 8 °C	-20 °C	2 – 8 °C
1 week	>1 week	7 hours

9. Test procedure

9.1. General information

All reagents and the microwell plate must be brought to room temperature (20 – 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage at 2 – 8 °C.

Take only the volume of reagents that is needed for test procedure. Do not pour reagents back into vials as reagent contamination may occur.

The microwell strips cannot be used more than once. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

Some of the reagents in the kit are not test specific. The reagents labelled Sero (such as SeroPP) can also be used with other RIDASCREEN® Sero ELISA with the corresponding reagents.

The control sera relate to the lot. Control sera from kits with different lot numbers must not be exchanged.

RIDASCREEN® Sero ELISA quality controls A and B are provided as additional components to the respective RIDASCREEN® Sero ELISA test kits. These are controls for additional quality control purposes which can be used optional. They contain human control serum with different antibody concentrations.

9.2. Preparing the wash buffer

1 part wash buffer concentrate SeroWP is mixed with 9 parts distilled water. In order to do this, place 100 ml of the concentrate in a 1000 ml measuring cylinder and make up the solution to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved before-hand by warming in a water bath at 37 °C. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for one week when stored at room temperature (20 – 25 °C).

9.3. Preparing the samples

Dilute the serum samples to be tested with sample buffer SeroPP before starting the test 1:50.

e.g. 10 µl serum + 490 µl SeroPP

Note:
The negative control and standard control, quality control A and quality control B are ready for use and must NOT be diluted or absorbed.

9.4. First incubation

After insertion of a sufficient number of wells into the frame, pipette 100 µl diluted sera and 100 µl ready-to-use control into each of the corresponding wells leaving Position A1 (reagent blank value) empty. Add the negative control Control IgA - or Control IgG - once and the standard control Control IgA + or Control IgG + in duplicate. Add the quality control Control IgA A and Control IgA B or the quality control Control IgG A and Control IgG B once. Cover the plate and incubate at 37 °C for 30 minutes in an incubator. During this process, the bottoms of the wells must not be in contact with heat-conductive materials. The microwell plate must be covered during incubation.
The controls which correspond to the determination (IgA or IgG) are to be used.

A1	Reagent blank value
B1	Negative control
C1	Standard control
D1	Standard control
E1	Quality control A
F1	Quality control B
G1, H1	Patient serum 1 and 2 etc.

Note:
The microwell plate must not be placed in a cold incubation container reaching 37 °C during incubation. The temperature of the container must be adjusted to 37 °C beforehand.

9.5. Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. Then tap the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 4 times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by tapping them on an unused part of the absorbent paper after each wash.

When using a microplate washer, make sure that the machine is correctly adjusted to the type of plate being used. After washing, tap the plate onto clean absorbent paper in order to remove any residual moisture.

9.6. Second incubation

Add 100 µl conjugate Anti-human IgA conjugate LD SeroA LD or Anti-human IgG conjugate HD SeroG HD to the corresponding wells (including A1). Next, cover the plate and incubate at 37 °C for 30 minutes in an incubator (see Section 9.4).

9.7. Washing

Wash 4 times in accordance with Section 9.5.

9.8. Third incubation

Add 100 µl substrate SeroSC to each well. Then, cover the plate and incubate at 37 °C for 30 minutes in a moist chamber / incubator. After this, stop the reaction by adding 100 µl stop reagent Stop to each well. After mixing carefully (by lightly tapping the side of the plate), measure the absorbance at 450 nm (reference wavelength ≥ 620 nm) in a plate photometer. Calibrate the reagent blank value (Position A1) to zero.

Note:
The underside of the microwell plate must be wiped off to remove condensation water before the measurement is carried out.

10. Quality control and indications of instability or deterioration

For quality control purposes, the standard control (in duplicate) and the negative control must be used every time the test is carried out. The test has been carried out correctly if the average absorbance for the standard control at 450/620 nm is within the range stated on the enclosed data sheet. If the two individual measurements deviate from the average by more than 20 %, the test must be repeated. The absorbance for the negative control at 450/620 nm must be < 0.3.

RIDASCREEN® Sero ELISA quality controls A and B are additional controls for quality control purposes which can be used optional. The target values are stated on the enclosed, lot-specific quality assurance certificate. The obtained values (U/ml, IU/ml or mIU/ml) are recommended as reference values for the quality assurance in accredited laboratories.

If the values differ from those required, if the reagent is turbid or the substrate has turned blue before adding to the wells, it 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 contact your local R-Biopharm distributor.

11. Evaluation and interpretation

The test can be evaluated using three different methods:

1. By using the standard curve provided in the kit
2. By using the table of values (see data sheet provided in the kit)
3. Mathematically using the 4-parameter method or the α method

The reagent blank value must be subtracted from each measured value before the evaluation.

11.1. Evaluation by using the standard curve provided in the kit

In order to carry out the evaluation using the standard curve, a correction must be made to the average value for the standard control first in order to take into account any fluctuations which may occur from one day to the next. The correction factor F is calculated from the current measured average value for the standard control and its target value. The target value, which depends on the lot, is recorded on the enclosed data sheet.

$$F = \frac{\text{standard control target value}}{\text{standard control measured average value}}$$

All OD values for the samples must be multiplied by the factor F. The corresponding U/ml values are then read off the standard curve using the corrected values.

11.2. Evaluation by using the table of values

	U/ml		Range of values for the standard control		
			0.89 - 0.94		
-	< 10.0		< 0.17		
?	10.0 - 16.0		0.17 - 0.26		
	16.1 - 30.0		0.27 - 0.47		
	30.1 - 60.0		0.48 - 0.80		
+	60.1 - 110.0		0.81 - 1.27		
	110.1 - 210.0		1.28 - 1.84		
	210.1 - 400.0		1.85 - 2.39		
	> 400.0		> 2.39		

Figure 1: Example of an IgG determination
      (Extract from a lot-specific data sheet)

The absorbance for the standard control is used to identify the column in the table with the range of values which applies to the current measurement. The measured absorbance for the sample is assigned to the appropriate range of values and then the titer in U/ml is read from the second column to the left in the table.

For example, the absorbance for the standard control for a certain measurement is 0.91. In this case, the column in the table with the range 0.89 - 0.94 is the one to use to determine the results. A patient sample with an absorbance of 0.61 therefore corresponds to the titer range 30.1 - 60.0 U/ml. (The values cited are merely to be regarded as examples and may differ from the current values on the data sheet.)

The evaluation for the determined results (positive (+), negative (-) or equivocal (?)) must be taken from the first column of the table of values.

11.3. Mathematical evaluation

The required values for mathematical evaluation according to the 4-parameter method or the α-method are recorded on the enclosed data sheet.

11.4. Test result

Table 3: Evaluation of the determined units

	IgA	IgG
negative	< 10 U/ml	< 10 U/ml
equivocal	10 - 13 U/ml	10 - 16 U/ml
positive	> 13 U/ml	> 16 U/ml

12. Limitations of the method

The RIDASCREEN® Helicobacter ELISA detects IgA or IgG antibodies against *H. pylori*. The test cannot be used to derive a relationship between the extinction determined and occurrence of serious clinical symptoms. The results obtained must always be interpreted in combination with the clinical picture.

A negative result in the ELISA cannot rule out infection with *Helicobacter pylori*. When infection with *Helicobacter pylori* is suspected for clinical reasons and the serological findings are negative, further sampling and testing should be carried out after four weeks.

An equivocal result could indicate the start of an immune response. Antibodies against *Helicobacter pylori* are present. Progressive seroconversion, which indicates an acute development of the disease, must be clarified by repeated testing after two to four weeks. An uncertain result may also be observed after therapy and recovery after infection.

Two consecutive samples of sera should always be collected from a patient and subjected to serological testing in order to improve the quality of the diagnostics. The progress of the titre is important for interpreting the findings.

A positive result in the ELISA does not always mean that the disease is actively developing. Antibodies can also be determined during *Helicobacter pylori* colonisation without clinical manifestations.

We recommend carrying out the RIDA®LINE *Helicobacter* test in order to confirm the ELISA result and identify highly virulent Helicobacter pylori strains by determining the antibodies against the pathogenicity factors CagA and VacA.

Only a few tests currently exist for the significance of IgA antibodies for an infection with Helicobacter pylori. We recommend carrying out this test when the IgG findings are unclear.

A positive result does not rule out the presence of another infectious pathogen as the cause of the disease.

13. Performance characteristics

Table 4: Inter-assay variation (n = 30)

Inter-assay variation	IgA		IgG	
	OD	CV	OD	CV
Serum 1	0.189	8.7 %	0.192	7.1 %
Serum 2	0.388	8.5 %	0.683	9.7 %
Serum 3	0.872	8.3 %	1.166	8.2 %

Table 5: Intra-assay variation (n = 24)

Intra-assay variation	IgA		IgG	
	OD	CV	OD	CV
Serum 1	0.169	9.4 %	0.170	4.5 %
Serum 2	0.350	9.1 %	0.615	4.8 %
Serum 3	0.832	7.7 %	1.152	4.6 %

Table 6: Sensitivity and specificity in comparison with two other commercial ELISAs

	IgA	IgG
Sensitivity	100.0 %	100.0 %
Specificity	94.7 %	100.0 %

Table 7: Results from testing 200 blood-donor sera taken from a blood donor center in Germany

200 blood donor sera	IgA	IgG
negative	66.5 %	77.5 %
equivocal	10.5 %	4.5 %
positive	23.0 %	18.0 %

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