VIROTECH Helicobacter pylori IgA ELISA
(H. pylori IgA ELISA)

Order No.: EC143A00

VIROTECH Helicobacter pylori IgG ELISA
(H. pylori IgG ELISA)

Order No.: EC143G00

Color Coding: **IgA: blue metallic/black**
IgG: blue metallic

FOR IN VITRO DIAGNOSIS ONLY

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Contents

1. Intended Use

The ELISA testkit is intended for the qualitative and semiquantitative detection of *Helicobacter pylori*-specific IgG- and IgA-antibodies in human serum.

2. Diagnostic Relevance

Helicobacter (H.) pylori is a Gram-negative bacterium specialising in the gastric mucosa, which causes about 500,000 deaths worldwide due to gastric carcinoma (1, 6).

The infection is usually acquired in childhood. Transmission is from person to person, and close contact within the family and socioeconomic status play an important part. Accordingly, prevalence is much higher in developing countries than in industrial nations (7). In Germany the infection rate in adults is about 30% (5).

H. pylori infection persists throughout life and causes chronic gastritis, which often remains without clinical symptoms. However, in 20% of those affected, complications occur in the form of a gastric ulcer, duodenal ulcer, gastric carcinoma or MALT lymphoma (mucosa-associated lymphatic tissue) (5).

Healing of the ulcer disease and MALT lymphoma is possible with antibiotic therapy in the early stage (5). Early treatment can counteract the development of gastric carcinoma, i.e. diagnosis of *H. pylori* infection as soon as possible should be attempted. In patients who have not had previous treatment, the bacteria can be fully eliminated in 80%-96% of cases (2). Recurrent infection after successful *H. pylori* eradication is very low (approx. 1% per year) (7). If antibiotic therapy has already been used once, the success rates are lower on further treatment because of the increasing antibiotic resistance. For this reason, it is recommended to detect *H. pylori* from culture of gastric biopsies in patients who have previously been treated once and to carry out sensitivity testing (7,8).

Invasive and non-invasive methods can be used to detect *H. pylori* infection. The invasive methods include the rapid urease test, histology, culture and PCR. In these methods, the pathogen is found in biopsies. The non-invasive tests include the urea breath test, the stool antigen test and detection of antibodies in the serum. All tests have advantages and disadvantages and are not absolutely accurate on their own. The method should therefore be selected according to the query (7).

Serology is employed

- as an initial test in patients who have not been previously treated (2)
- in monitoring therapy long-term (1)
- in seroepidemiological investigations (3).

Serology is indicated in cases with a reduced number of bacteria e.g. in:

- marked atrophy of the gastric mucosa
- gastric haemorrhage
- use of proton pump inhibitors.

While all other methods can give false-negative results in these cases, detection of specific H. pylori antibodies with maintained sensitivity is possible (2).

3. Test Principle

ELISA for detection of human serum IgG and IgA antibodies

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

Immunoblot procedure for the detection human serum IgG- and IgA-antibodies

The *Helicobacter pylori* LINE from VIROTECH has the advantage that antibody production against specific virulence factors – e.g. CagA and VacA – of *H. pylori* can be detected.

4. Package Contents

4.1 IgG Testkit

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised

2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 IgA Testkit

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml,** pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgA negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgA cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgA positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgA-Conjugate 2 (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgA-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.

10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgA-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE(\text{positive control}) = \frac{OD(\text{positive control})}{OD(\text{cut - off control})} \times 10$$

$$VE(\text{patient serum}) = \frac{OD(\text{patient serum})}{OD(\text{cut - off control})} \times 10$$

9.3 Interpretation Scheme IgG and IgA

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody

concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.

3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Both IgA and IgG *Helicobacter pylori* LINE results should be taken into consideration for the diagnosis of patient's sera suspected to have a *Helicobacter pylori* infection.
3. IgA-antibodies may persist 6 months to 3 years after successful treatment. Normally IgG-antibodies persist for many years.
4. Cross-reactivities with other Gram-negative pathogens, e.g. due to flagellin homologies, cannot be excluded.

10. Performance Data

10.1 Sensitivity and specificity

To determine the result clinically defined sera (n=19), interlaboratory test sera (n=19), routine sera (n=40) and blood donor sera (n=80) were tested for IgG with a commercially available ELISA and the VIROTECH Helicobacter pylori LINE.
Samples with discrepant results were additionally characterised using a commercially available immunoblot.

Sera collective (n=158)		**Helicobacter pylori ELISA IgG**		
		negative	borderline	positive
result	negative	82	1	0
	borderline	0	1	0
	positive	0	0	74

This results in a sensitivity and specificity of >99.9% for IgG.
The borderline results were not included in the analysis.

To determine the result clinically defined sera (n=20), interlaboratory test sera (n=19), routine sera (n=32) and blood donor sera (n=80) were tested for IgA with a commercially available ELISA and the VIROTECH Helicobacter pylori LINE.

Samples with discrepant results were additionally characterised using a commercially available immunoblot.

Sera collective (n=151)		**Helicobacter pylori ELISA IgA**		
		negative	borderline	positive
result	negative	83	4	9
	borderline	5	0	7
	positive	3	3	37

This results in a sensitivity of 92.5% and specificity of 90.2% for IgA.

10.2 Infection (expected values)

To obtain the expected values (infection), 80 sera were tested for IgG and IgA. In a blood donor population, an infection rate of about 30% can be expected. About 90% show an IgA response .

	IgG		IgA	
n=80	**No.**	**%**	**No.**	**%**
Negative	58	72.5	60	75.0
Borderline	0	0	2	2,5
Positive	22	27.5	18	22.5

10.3 Intra-assay variation coefficient (repeatability)

In one assay strips from different plates of one lot were tested with one serum. The variation coefficient obtained thus is < 9% for IgA and IgG.

10.4 Inter-assay variation coefficient (reproducibility)

3 sera were tested in 10 independent test batches in different laboratories and by different test persons. The variation coefficient obtained thus is < 15% for IgA and IgG.

11. Literature

1. Helicobacter pylori – Von der Grundlage zur Thearpie (1996) Herausgeber P. Malfertheiner, Thieme Verlag
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3. Zöller et al (1993) Nachweis der Helicobacter pylori-Infektion: Rolle der Immundiagnostik. *Klin. Lab.* 39: 45-54
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5. Kist M., Glocker E., *Helicobacter-pylori*-Infektionen: Studie ResiNet zur Resistenzentwicklung - aktuelle Ergebnisse, Epidemiologisches Bulletin, 2005, Nr. 24
6. Kist M., Glocker E., Suerbaum S., Pathogenese, Diagnostik und Therapie der *Helicobacter-pylori*-Infektion, Bundesgesundheitsblatt, 2005
7. *Helicobacter-pylori* und gastroduodenale Ulkuskrankheit, AWMF-Leitlinien-Register, Nr. 021/001, 2008

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

IgG/IgA-Samples – Dilution 1:101

e.g.:

10 µl serum/plasma + 1000 µl Dilution Buffer

(Serum Dilution Buffer is ready to use)

Test procedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgA
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**