VIROTECH Helicobacter pylori IgG LINE Immunoblot

(H. pylori IgG LINE-32)

Order No.: WE243G32

(H. pylori IgG LINE-96)

Order No.: WE243G96

VIROTECH Helicobacter pylori IgA LINE Immunoblot

(H. pylori IgA LINE-32)

Order No.: WE243A32

(H. pylori IgA LINE-96)

Order No.: WE243A96

FOR IN-VITRO DIAGNOSTIC ONLY

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Contents

1. Intended Use

LINE test kit for the qualitative detection of *Helicobacter pylori* specific IgG- respectively 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 sero-epidemiological 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. Principle of Test

Pathogen antigen proteins are transferred onto a nitrocellulose membrane by a special spraying process. The nitrocellulose membrane is then cut up into individual strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immune complexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alkaline phosphatase conjugated anti-human IgG-, respectively IgA-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualization of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-colored substrate, which forms blue-violet precipitates at each site („antigen bands“) where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with distilled or deionized water. Depending on the observed band pattern one can interpret the presence of specific IgG-, respectively IgA-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1. **IgG resp. IgA Nitrocellulose test strips** with sprayed antigen, (solid strips stabilized on a plastic foil), sorted in a booklet, ready to use **1x** 32 strips

2.	**IgG resp. IgA Cut off Control,** human serum, prediluted	**1x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**2x**	50 ml
4.	**IgG- resp. IgA- Conjugate** (100x conc.) **Anti-human**-(goat)-Alkaline Phosphatase, with preservative	**1x**	0.7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**1x**	1 pcs.

4.2 Kit for 96 determinations

1.	**IgG resp. IgA Nitrocellulose test strips** with applied antigen, (solid strips stabilized on a plastic foil), sorted in a booklet, ready to use	**3x**	32 strips
2.	**IgG resp. IgA Cut off Control,** human serum, prediluted	**2x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**4x**	50 ml
4.	**IgG- resp. IgA- Conjugate** (100x conc.) anti-human-(goat)- Alkaline Phosphatase, with preservative	**3x**	0.7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**3x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**3x**	1 pcs.

Also available on request:

IgG or IgA- Positive control, human serum, prediluted, 0.5 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE243P60 or IgA: WE243P40)

IgG/IgA- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG/IgA: WE243N20)

5. Storage and Stability of the test kits and the components

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. **Nitrocellulose test strips**: Use strips immediately after taken out of the bag. Close bag with the not required strips again safely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelf life
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	*or* room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic forceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additional required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic forceps
9. distilled or deionized water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

9. Test Procedure

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

9.1 Preparation of Samples

1. For each patient sample, 15 µl of serum/ plasma are required in IgG and 30 µl of serum/ plasma are required in IgA.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipemic, hemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000 x g), pipette clear supernatant and use for testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINE und EcoBlots products can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality distilled or deionized water and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
   The dilution-/ washbuffer is provided as a 10-fold concentrate. Dilute the dilution-washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well.

Both the concentrated and the diluted dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.

5. **IgG-, resp. IgA Conjugate**
   Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: The nitrocellulose test strips may only be tested in the approved Ig class (see label on blot booklet and indication on each individual test strip).

For the correct performance and evaluation of the Helicobacter pylori LINE, each test run should include the appropriate parameter and batch-specific cut off controls.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moisturized completely within one minute and can be incubated in supine, lateral position or face-down position.
5. Apply **15 µl portions of patient serum or plasma in IgG and 30 µl portions of patient serum or plasma in IgA;** or **100 µl of the cut-off / positive / negative control** by pipette, if possible at the upper, marked end of the strip. Incubate the patient serum and control for **30 minutes** on the rocking platform. Ensure that no cross-contamination occurs between individual patient samples during pipetting and subsequent pouring away.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulose paper.
7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for 30 minutes on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with distilled or deionized water**.**
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1.5 ml portions of ready-to-use **substrate solution** into the channels and develop for **10 ± 3 minutes** on the rocking platform.
14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml distilled or deionized water each**.**
15. Pour away the distilled or deionized water and let the strip dry on a clean cellulose paper. The background-coloring, that may be observed on the moisturized antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the enclosed evaluation record sheet for evaluation. The high specificity bands annotated on the record sheet facilitate evaluation of the patient samples.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot Processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE strip is fitted out with two controls:

1. **Serum control:**
   Only after the incubation with patient serum the serum incubation band appears below the mark line.
2. **Conjugate control:**
   The LINE strip is fitted out with a conjugate control band which appears after incubation with the respective conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed nitrocellulose test strip.

The position of the serum band and conjugate control band may be found on the record sheet.

10.1 Usage of the cut-off control

Bands with an intensity weaker than the cut-off band of the cut-off control are not considered for the interpretation.

IgG and IgA cut-off band: Cag A

10.2 Meaning of the Antigens

Antigen/ Description	**Molecular-weight**	**Meaning of the antigen**	**Specificity of the antibodies with the LINE**	**Do occur in *H. pylori***
Cag A **(C**ytotoxin-**a**ssociated-**g**ene **A)**	140 kD	CagA infiltrates the host cells, thus destroys amongst others the acid-producing gastric cells and the incidence of gastric-ulcera and gastric carcinoma is favored. Characteristic for the particular virulent strains of type I; missing in the less virulent strains of type II. High-immunogenic protein	High-specific	type I
Vac A **(Va**cuolating **C**ytotoxin **A)**	87 kD	Is transferred to the circumfluent medium and causes cell damages of the gastric mucosa; may take a local immunosuppressive effect (10). Characteristic for the particular virulent strains of type I; missing in the less virulent strains of type II. Ab-response less constant compared to CagA.	High-specific	type I
p30	30 kD	Not yet characterised protein	High-specific	type I and type II
UreA **Urease A subunit**	26 kD	Urease A does not show affinity with ureases of other organisms and thus is a high-specific marker for a Helicobacter-Infection.	High-specific	type I and type II
p25	25 kD	Membrane-protein, that interferes the connection of Helicobacter pylori to the epithelial cells of the stomach (11).	High-specific	type I and type II
p19	19 kD	Not yet closer characterized membrane-protein.	High-specific	type I and type II

10.3 Interpretation Criteria

The interpretation of serological results should always include the clinical picture, epidemiological data and all further available laboratory findings.

Recommended IgG-, IgA interpretation

bands more largely or directly cut off	interpretation
No band or only one band of p30, p19	negative
only one band of Vac A, UreA, p25	borderline
Cag A or **Arise from ≥2 bands of the following:** Vac A, p30, UreA, p25, p19	positive

10.4 Limits of the Test

1. In rare cases patient sera may show “inverse” bands (dark background, white bands), these are not to be interpreted, means the immunoblot is in those cases not interpretable. The serum should be checked using other serological methods.
2. IgA-antibodies may persist 6 months to 3 years after successful treatment. Normally IgG-antibodies persist for many years.

11. Performance Data

11.1 Sensitivity and Specificity

For the detection of the sensitivity and specificity, routine sera and blood donor sera have been tested with the Helico LINE and with the Westernblot:

IgG: Routine Sera (n=42) + Blood Donor Sera (n=77)

Helicobacter pylori LINE IgG

Helicobacter pylori EcoBlot (n=119)	negative	borderline	positive
negative	60	0	1
borderline	2	0	2
positive	0	0	54

5 discrepant sera (3 blood donor sera, 2 routine sera) are not considered for the assessment. They have been tested on a second reference assay. The two reference systems did not show matching results.

IgA: Routine Sera (n=41) + Blood Donor Sera (n=79)

Helicobacter pylori LINE IgA

Helicobacter pylori EcoBlot (n=120)	negative	borderline	positive
negative	72	1	0
borderline	7	0	1
positive	3	2	34

3 discrepant sera (1 blood donor serum, 2 routine sera) are not considered for the assessment. They have been tested on a second reference assay. The two reference systems did not show matching results.

11.2 Cross-Reactivity

Cross-reactions with *Campylobacter* may not generally be excluded due to the existing antigen relatedness (14).

11.3 Prevalence (Expected Values)

The following table shows the determination of 77 blood donor sera in IgG and 79 sera in IgA:

IgG	Amount (n=77)	%
negative	50	64,9
borderline	0	0
positive	27	35,1

IgA	Amount (n=79)	%
negative	60	75,9
borderline	1	1,3
positive	18	22,8

11.4 Intra-Assay-Precision (Repeatability)

For the determination of the repeatability, 30 blot strips of a nitrocellulose-membrane have been incubated in an IgG- and IgA-examination run with a positive serum. The serum showed equally strong intensities on the entire nitrocellulose-sheet each for IgG and IgA.

11.5 Inter-Assay-Precision (Reproducibility)

For the determination of the reproducibility two positive sera and one negative serum have been tested for IgG and IgA. The determination has been affected in 10 different test runs proceeded by 3 different test persons.
In all examinations, the serological specifications have been exactly met.

11.6 Accuracy

21 interlaboratory-test-sera have been tested for IgG and 19 interlaboratory-test-sera have been tested for IgA:

IgG	Helicobacter pylori LINE IgG	
Instant-target specification	positive	negative
positive	13	0
negative	0	8

IgA	Helicobacter pylori LINE IgA		
Instant-target specification	positive	borderline	negative
positive	6	0	0
borderline/positive	0	1	0
negative	0	0	10
negative/borderline	0	2	0

All interlaboratory target specifications have been reached.

12. Literature

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13. Symbols

 => refer to user manual!

14. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl patient serum/ plasma in IgG; 30 µl patient serum/ plasma in IgA / 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	**3 x 5 minutes**	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	**30 minutes**	with 1,5 ml working dilution (1 + 100)
Washing	**3 x 5 minutes** **1 x 1 minutes**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml substrate solution each
Stopping	**3 x without incubation in between**	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515ml	3,03ml	4,545ml	6,06ml	7,575ml	9,09ml	11,11ml	12,12ml	14,14ml	15,15ml

Number of strips	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17ml	18,18ml	20,2ml	21,21ml	23,23ml	24,24ml	26,26ml	27,27ml	29,29ml	30,3ml

Number of strips	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	32,32ml	33,33ml	35,35ml	36,36ml	38,38ml	39,39ml	41,41ml	42,42ml	44,44ml	45,45ml

Number of strips	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47ml	48,48ml	50,5ml	51,51ml	53,53ml	54,54ml	56,56ml	57,57ml	59,59ml	60,6ml