



REF 6010

July 26, 2007

Helicobacter pylori Antigen

- 48 determinations -



IVD In vitro diagnostic device

Enzyme immunoassay for the determination of Helicobacter pylori antigen in fecal specimens

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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INTENDED USE

Helicobacter pylori Antigen is used for the qualitative detection of Helicobacter pylori antigen in fecal specimens.

The human pathogen *Helicobacter pylori* (*H. pylori*) is a gram-negative spiral shaped bacterium that has been found in the stomachs of humans in all parts of the world. In developing countries, 70 to 90% of the population carries *H. pylori* mostly already acquired before the age of 10 years. In developed countries the prevalence of infection ranges from 25 to 50%. *H. pylori* infections are transmitted via the oral-oral, the fecal-oral route or iatrogenic. A substantial reservoir for *H. pylori* aside from the human stomach has not been confirmed so far (1).

H. pylori is characterized by a strong urease activity and some strains additionally produce a cytotoxin (Vac A). These extracellular products contribute to the pathogenesis by direct damage of the gastric epithelium accompanied by a chronic inflammation with enhanced levels of inflammation mediators. Patients infected with *H. pylori* often develop autoantibodies to parietal cells. The key-role of these autoantibodies in the development of atrophic gastritis as precursor of gastric cancer has been reviewed (1, 2).

About 10% of the infected persons develop *H. pylori* associated gastritis and secondary diseases like chronic active gastritis, Ulcus ventriculi, Ulcus duodeni, gastric cancer and MALT lymphoma. Patients suffering from gastric malignomas and Ulcus duodeni are infected with *H. pylori* in nearly 100% of the cases. About 80% of patients with chronic gastritis, 70% of patients with Ulcus ventriculi and 60% of patients with gastric cancer are infected with *H. pylori* (2).

Diagnosis is usually based on gastroendoscopy combined with the detection of the pathogen in biopsy material by culture, histology and rapid urease test. Culture from biopsy material is difficult and not always successful. The ¹⁴C breath test which is often performed as follow up test, detects CO₂ released by the bacterial urease from radioactive labeled urea in patients breath. This method is not invasive but the need for special equipment and the uptake of radioactive urea by the patients are disadvantageous.

Immunological tests on the basis of specific anti-*H. pylori* antibodies are now available. They enable the direct detection of *H. pylori* antigens from stool specimens and may be used for therapeutic surveillance. Specific antibodies to *H. pylori* may show a lifelong persistence and even after eradication therapy they are usually still detectable. Therefore therapeutic monitoring by detection of specific antibodies is of limited meaning and does only make sense when paired serum samples are investigated (1, 3).

1. Dunn BE, Cohen H, Blaser MJ: Helicobacter pylori. Clin Microbiol Rev 1997, 720-741
2. Hahn H, Falke D, Ullmann U: Medizinische Mikrobiologie und Infektiologie. Springer Verlag, Berlin-Heidelberg-New York, 1999
3. Antos D, Crone J, Konstantopoulos N, Koletzko S: Evaluation of a novel monoclonal rapid one-step immunochromatographic assay for detection of Helicobacter pylori antigen in stool samples from children. J Clin Microbiol 2005, 43, 2598-601

PRINCIPLE OF THE TEST

Helicobacter pylori Antigen is an indirect two-site-immunoassay for the qualitative determination of Helicobacter pylori antigen based on polyclonal antibodies.

Biotinylated anti-*H. pylori* antibodies are dispensed simultaneously with specimens and the positive and negative control into the wells of the microplate coated with anti-*H. pylori* antibodies. After 60 minutes at 22-25°C non-bound material is removed by a washing step.

Subsequently solid phase captured antigen-antibody-biotin complexes specifically react with streptavidin-horseradish peroxidase (HRP) during a second incubation period of 30 min at 22-25°C. Non-bound material is separated by a subsequent washing step.

HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 15 min incubation at 22-25°C turning the solution from blue to yellow.

The optical density (OD) of the solution read at 450 nm is directly proportional to the specifically bound amount of *Helicobacter pylori* antigen. For optimal results a reference filter (620 nm wavelength) should be used. Results are interpreted as positive or negative considering a cut-off value .

SAMPLE PREPARATION

Specimen collection and storage

The stool samples should be stored at 2-8°C immediately after collection and processed within 48 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Formalin-preserved stool samples should not be used in this assay.

Sample preparation

Quickly thaw frozen samples; warm samples to room temperature and mix well.

Pipette 500 µl of sample diluent into a clean tube.

Using a disposable stirring rod transfer about 100 mg (diameter about 2-3 mm) of faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly.

If necessary, sediment floating particles by a centrifugation step.

TEST COMPONENTS FOR 48 WELLS

A Ag 48	Microtiter plate , 6 breakable strips per 8 wells coated with polyclonal antibodies to <i>Helicobacter pylori</i> (rabbit)	1 vacuum sealed with desiccant
B BUF WASH 10x	Concentrated wash buffer sufficient for 500 ml solution	50 ml concentrate capped white
C DIL	Sample diluent	50 ml ready for use capped black
D A-HP	Anti-HP biotinylated polyclonal anti- <i>Helicobacter pylori</i> antibodies (rabbit)	7 ml ready for use capped white
E CONJ	Conjugate containing streptavidin labelled with poly-HRP	7 ml ready for use capped brown
F SOLN TMB	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	7 ml ready for use capped blue
G H2SO4 0.25 M	Stop solution 0.25 sulfuric acid	7 ml ready for use capped yellow
P CONTROL	Positive control <i>H. pylori</i> antigen positive sample, inactivated	1 ml ready for use capped red +
N CONTROL	Negative control <i>H. pylori</i> antigen negative sample	1 ml ready for use capped green -

Materials required but not provided

- micropipettes
- multi-channel pipette or multi-pipette
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water
- glassware
- centrifuge (optional)

Size and storage

Helicobacter pylori Antigen has been designed for 48 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box labels.

Upon receipt, all components of the *Helicobacter pylori* Antigen have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with distilled or de-ionized water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- Dilute samples with sample diluent (C) 1 + 5 (w/v), e.g. 100 mg stool + 0.5 ml sample diluent (C)
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (20-25°C) before use. Mix gently without causing foam.
2. Dispense **3 drops** (or 120 µl) of anti-HP (antibody-biotin conjugate) (D) into all respective wells
3. Dispense **3 drops (or 120 µl)** of negative control (N) **3 drops (or 120 µl)** of positive control (P) **100 µl** of diluted samples into the respective wells, mix
4. Cover plate, incubate **60 min** at 22-25°C
5. Decant, then wash each well **five times** using **300 µl** wash solution (made of B).
6. Dispense **3 drops (or 120 µl)** of streptavidin-HRP conjugate (E) into the respective wells
7. Seal plate, incubate **30 min** at 22-25°C
8. Decant, then wash each well **five times** using **300 µl** wash solution (made of B).
9. Add **3 drops (or 120 µl)** of substrate (F) to each well.
10. Incubate **15 min protected from light** at 22-25°C.
11. Add **3 drops (or 120 µl)** of stop solution (G) to each well and mix gently.
12. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

Cut-off determination

OD of negative control + 0.10 OD units

Samples with absorbances lower than or equal to the cut-off value are considered negative, samples with absorbances higher than the cut-off value are considered positive for *Helicobacter pylori* antigen.

REFERENCE VALUES

Helicobacter pylori	
Negative	≤ Cut-off
Positive	> Cut-off

Example of typical assay results

Wells	OD (a)	OD (b)	OD (mean)
Negative control	0.087	0.094	0.090
Positive control	1.916	1.934	1.925
Cut-off	> (0.090 + 0.10) = 0.190		
Sample 1	2.012	2.076	2.044 – positive
Sample 2	0.118	0.126	0.122 – negative

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.20
- the mean OD of the positive control is ≥ 1.20

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control.

Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. Fermented samples with pH values below 5 after resuspension may produce false negative results.

A negative test result in the *Helicobacter pylori* ELISA does not exclude an infection:

The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

CHARACTERISTIC ASSAY DATA

Precision

Intra-assay coefficient of variation (cv) in the *Helicobacter pylori* Antigen ELISA calculated from 8-fold determination of samples:

sample	mean OD	standard deviation	cv (%)
I	1.090	0.056	5.1
II	2.491	0.122	4.9
III	0.552	0.019	3.4
IV	0.203	0.006	2.8

Inter-assay coefficient of variation (cv) in the *Helicobacter pylori* Antigen ELISA from 5 different test runs from 8-fold determination of samples:

sample	mean OD	standard deviation	cv (%)
I	1.069	0.061	5.7
II	2.511	0.104	4.1
III	0.562	0.030	5.4
IV	0.188	0.009	4.8

Lower detection limit

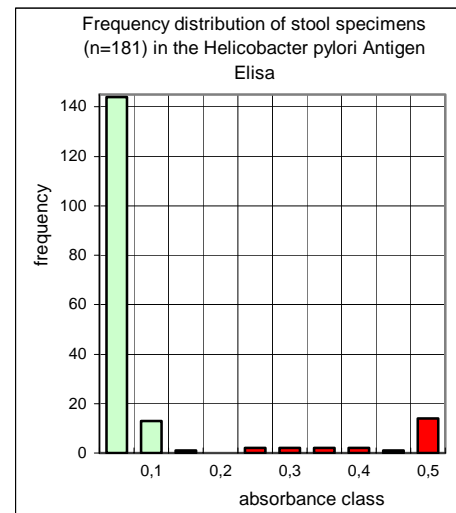
The lower detection limit of the *Helicobacter pylori* Antigen ELISA has been determined by titration of stool specimens spiked with purified *H. pylori* antigen.

The lower detection limit is < 15 ng/ml.

Determination of the cut-off value

The frequency distribution of the absorbances of 145 stool specimens from a microbiological routine laboratory has been investigated in order to fix the cut-off value. The cut-off has been determined with

OD negative control + 0.1



Specificity and sensitivity

Specificity and sensitivity of the *Helicobacter pylori* Antigen Elisa were determined in a retrospective study in comparison to another commercially available Elisa.

	Comparative-ELISA positive	Comparative-ELISA negative
Helicobacter pylori ELISA positive	21	0
Helicobacter pylori ELISA negative	2	46

Specificity: 100%
Sensitivity: 91%

INCUBATION SCHEME

Helicobacter pylori Antigen (6010)

Dilute patients sample

100 mg sample + 0.5 ml sample diluent (C)

1.	Bring all reagents to room temperature (RT, 20-25°C)		
		Controls (P, N)	Specimens
2.	Dispense Anti-HP Biotin conjugate (D)	3 drops (or 120 µl)	3 drops (or 120 µl)
3.	Dispense Controls (P, N) 1+5 (w/v) prediluted fecal specimens	3 drops (or 120 µl)	100 µl
4.	Cover plate and incubate 60 minutes, RT (22-25 °C)		
5.	Wash Decant, 5 x 300 µl wash solution (made of B)		
6.	Dispense conjugate (E)	3 drops (or 120 µl)	3 drops (or 120 µl)
7.	Cover plate and incubate 30 minutes, RT (22-25 °C)		
8.	Wash Decant, 5 x 300 µl wash solution (made of B)		
9.	Dispense substrate (F)	3 drops (or 120 µl)	3 drops (or 120 µl)
10.	Incubate protected from light 15 minutes, RT (22-25 °C)		
11.	Dispense stop solution (G)	3 drops (or 120 µl)	3 drops (or 120 µl)
12.	Read at 450 nm against 620 (690) nm within 30 min		

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots (except: diluent, washing buffer, substrate and stop solution).
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.