

ELISA

for the determination
of antibodies (IgG) against

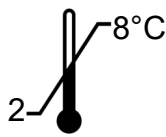
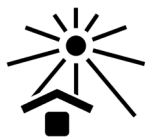
Helicobacter pylori

Directions for use

REF 0112FE00.FWD  12 x 8 determinations

IVD

CE



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The product described here complies with the requirements of the IVD Directive 98/79/EG.

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1. Introduction and background

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium which infects gastric mucosa (1). This infection is associated with various gastroduodenal diseases like active chronic gastritis and gastric and duodenal ulcers (2). In addition, evidence has been accumulated that *H. pylori* infection increases the risk of gastric carcinoma (3). Serological testing for *H. pylori* antibodies is a well-established method for the diagnosis of this infection (4). Several antimicrobial regimens (antibiotics combined with bismuth) have been successfully used to eradicate the bacterium.

The present enzyme-linked immuno sorbent assay (ELISA) is intended for the determination of IgG antibodies directed against *H. pylori* in human serum or plasma (cf. section 7). It may be used qualitatively for pre-endoscopy screening or quantitatively for therapy monitoring. The immobilised antigen is a purified extract from *H. pylori* ATCC-strain 43504, consisting of specific, native cell surface antigens, including the key antigen urease. Further, the preparation contains the Cag-A protein, coded by the cytotoxin-associated gene A, which is expressed only by the virulent strains of *H. pylori*.

The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). Six calibrators allow quantitative measurements; a negative and a positive control check the assay performance.

2. Warnings and precautions

The test kit is intended for in vitro diagnostic use only; not for internal or external use in humans or animals. It must be executed by trained personnel staff.

Do not use reagents beyond their expiration dates.

Adherence to the protocol is strongly recommended.

The sample buffer, calibrators and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂). The stop solution, 0,2 M sulfuric acid (H₂SO₄), is acidic and corrosive.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a manner complying with local/national regulations.

Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

The calibrators and controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.

However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Disease Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.

3. Principle of the test

The wells of the solid phase are coated with selected *H. pylori* antigens. On this surface, the following immunological reactions take place:

1st reaction: *H. pylori*-specific antibodies present in the sample bind to the immobilised antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.

2nd reaction: A second antibody, directed at human IgG antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed away from the solid phase.

3rd reaction: The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour development reflects the concentration of IgG antibodies against *H. pylori* in the sample.

4. Contents of the kit

- a. 1 microwell plate, coated with selected H. pylori antigens and hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.

MWP	12x8
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- b. Sample buffer, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.

BUF	SPL
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- c. Wash buffer, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.

BUF	WASH	10x
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- d. 6 calibrators, 2,0 mL each, 0 - 3,0 - 10 - 30 - 100 and 300 U H. pylori IgG / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.

CAL	1-6
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- e. Negative and positive control, 2,0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

CONTROL	-	CONTROL	+
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- f. Anti-human IgG HRP conjugate, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.

CONJ	IgG
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- g. Substrate solution, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H₂O₂. Contained in a vial impermeable to light.

SUBS	TMB
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- h. Stop solution (0,2 M H₂SO₄), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.

SOLN	STOP
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- i. Directions for use
- j. Lot-specific certificate of analysis

5. Materials required but not supplied

- a. Deionised or distilled water
- b. Graduated cylinder, 1000 mL
- c. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)
- d. Pipettes for 10, 100 and 1000 μL (1- and 8-channel pipettes recommended)
- e. Microwell plate washer (optional)
- f. Microwell plate photometer fitted with a 450 nm filter
- g. ELISA evaluation program (recommended)

6. Storage of the kit

Store kit at 2 - 8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

7. Reagent and sample preparation / specimen requirements

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is **crucially important** that no cross-contamination between the reagents occurs. Use only clean pipettes and do **not pour back** residues into the original flasks.

- a. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.

- b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).
- c. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA-, citrate- or heparin-treated plasma is suitable sample material as well.

Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.

Prepare samples using normal laboratory techniques and dilute them 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Mix thoroughly.

For rapid dispensing during the assay procedure, preparation of the calibrators, controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.

If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. For longer storage, -20°C or lower temperatures are recommended. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

8. Assay procedure

8.1. Manual operation

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, **careful washing** is essential (steps a, c and e). It is **crucially important to remove the wash solution completely**. For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

- a. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.
- b. Dispense the calibrators (2,0 mL each, ready-to-use, gradually blue), controls (2,0 mL each, ready-to-use, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended.

Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

- c. Wash the wells 4 times as in step a.
- d. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, red); 100 µL per well. Incubate the plate as in step b.
- e. Repeat wash step c.
- f. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colourless, black vial); 100 µL per well. Incubate the plate as in step b. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.
- g. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.
- h. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

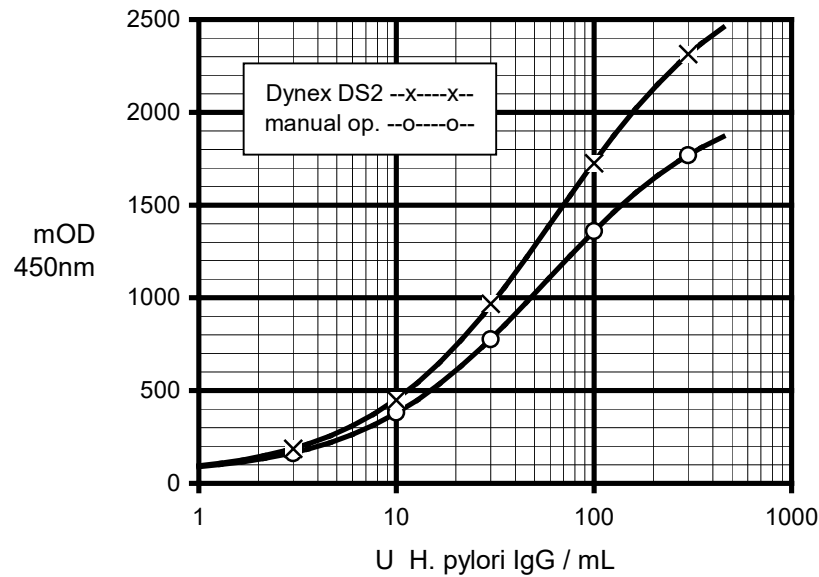
8.2. Dynex DS2 automated ELISA system

This product has been validated for use with the Dynex DS2 automated ELISA system. A description of the program flow for the assay execution and evaluation can be provided as a pdf file. The parameters of this program are merely a proposal and may need to be adapted by the operator to the requirements of the actual assay. In general terms, we have attempted to stick as close as possible to the protocol of manual operation, as above. However, due to the necessarily elevated temperature within the DS2, the substrate incubation period had to be shortened.

Section 11.8. gives a performance comparison between manual assay operation and the DS2 ELISA system.

9. Evaluation and quality control

Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.



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If no computer-supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into U H. pylori IgG per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance (= cut-off). It is determined according to the following formula:

$$\text{absorbance}_{\text{borderline}} = \text{absorbance}_{\text{positive control}} \times \text{factor}$$

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with each test kit. Example:

$$\begin{aligned} \text{absorbance}_{\text{positive control}} &= 1250 \text{ mOD} \\ \text{factor} &= 0,35 \\ \text{absorbance}_{\text{borderline}} &= 1250 \text{ mOD} \times 0,35 = 438 \text{ mOD} \end{aligned}$$

In order to gain an impression of how positive a particular sample is for H. pylori IgG, one may calculate the ratio, according to the formula:

$$\text{ratio} = \text{absorbance}_{\text{sample}} / \text{absorbance}_{\text{borderline}}$$

Example:

absorbanceborderline = 438 mOD
 absorbancesample = 1480 mOD
 ratio = 1480 mOD / 438 mOD = 3,4

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

10. Interpretation of results / limitations of the procedure

Based on the measurement of a blood donor and a positive collective of sera (see below), we suggest for the assessment of patient sera:

	quantitative evaluation U H. pylori IgG / mL sample	qualitative evaluation ratio
normal (negative) range	< 10,0	< 0,83
cut-off	13,0	1,00
equivocal range	10,0 - 16,9	0,83 - 1,19
positive range	> 16,9	> 1,19

These specifications are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the patient does not have an elevated level of IgG antibodies to H. pylori. It does not preclude the possibility of a recent infection. If clinical signs are observed, IgM or IgA antibodies directed at H. pylori may be determined.

A positive result may be considered as an indication for a H. pylori infection and a plausible explanation for a gastrointestinal disorder, as outlined in the beginning. It is not necessarily evidence for active disease.

Specimens exhibiting results within the borderline range quoted above should be considered as equivocal and reported as such. It is recommended that a second sample be collected two weeks later and run in parallel with the first sample to document a possible change of antibody titer.

As with any serological test, the results should be interpreted in the light of the patient's symptoms and other diagnostic criteria.

11. Performance characteristics

11.1. Standardisation

The test is standardised with a purified serum preparation containing IgG antibodies specifically directed at *H. pylori*. This preparation is calibrated against a set of gradually positive sera, solely reserved for this purpose. The degree of sample reactivity is measured in arbitrary units (U/mL) since no international standard is available.

11.2. Analytical specificity

The test allows the specific determination of human IgG antibodies directed against *H. pylori*.

11.3. Detection limit (analytical sensitivity)

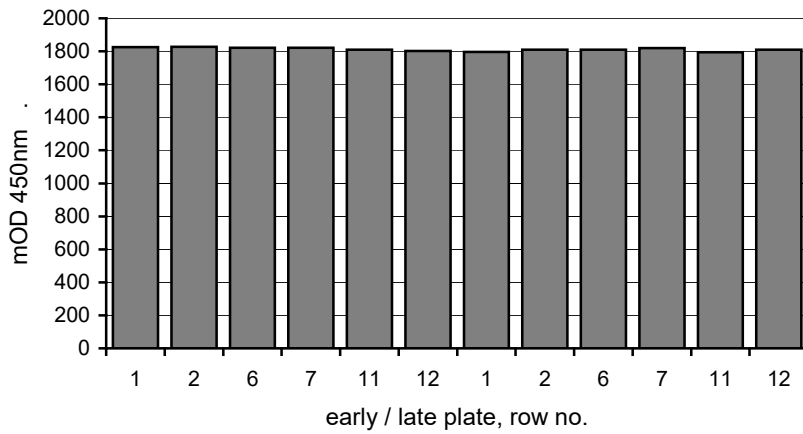
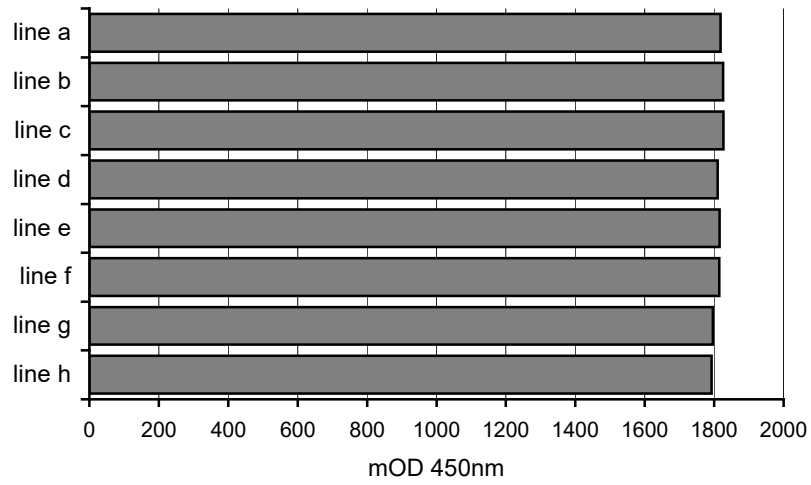
The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of sample buffer plus 3-fold standard deviation (s). It was determined as < 1 U *H. pylori* IgG per mL sample (n = 24).

Recommended measuring range: 2 - 200 U *H. pylori* IgG per mL sample

11.4. Homogeneity of the solid phase

Measurement of the solid phase homogeneity is a regular QC part of each production lot. This is determined by 288-fold measurement of an IgG-positive but non-saturating sample on 3 selected plates. Acceptance criterion: mOD-coefficient of variation (cv) over the plates < 8%. The figure below shows a representative excerpt (solid phase lot no. 12011) of such an analysis.

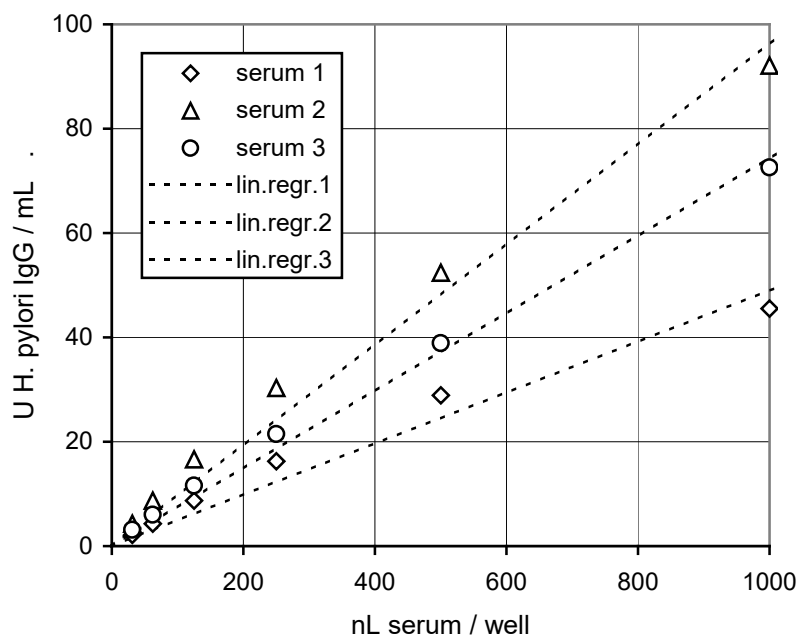
plate	early (n/10)						late (9n/10)						mean	cv%
row	1	2	6	7	11	12	1	2	6	7	11	12		
line a	1843	1836	1802	1815	1835	1813	1793	1817	1812	1810	1827	1819	1819	0,8
line b	1825	1828	1828	1844	1826	1817	1810	1821	1805	1838	1817	1845	1825	0,7
line c	1833	1836	1843	1843	1837	1820	1806	1830	1831	1834	1797	1814	1827	0,8
line d	1830	1818	1835	1829	1797	1802	1800	1798	1820	1814	1781	1802	1811	0,9
line e	1802	1830	1833	1844	1811	1799	1809	1811	1803	1846	1794	1804	1816	1,0
line f	1830	1837	1827	1824	1791	1814	1799	1819	1823	1817	1780	1813	1815	0,9
line g	1819	1828	1799	1786	1786	1791	1787	1795	1785	1804	1777	1803	1797	0,8
line h	1823	1808	1809	1795	1799	1757	1773	1792	1807	1790	1781	1772	1792	1,1
mean	1826	1828	1822	1823	1810	1802	1797	1810	1811	1819	1794	1809	1813	
cv%	0,7	0,6	0,9	1,2	1,1	1,1	0,7	0,8	0,8	1,0	1,0	1,1		1,1



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11.5. Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor > 0,98. A typical result is depicted below.



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11.6. Precision

For the assessment of the test precision, the variability of results under the following conditions was determined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	mean U/mL	variability (cv, %)	
		intra-assay	inter-assay
1	23	3,3	3,5
2	41	2,4	3,2
3	83	4,0	5,2

b. Operator to operator variability (n = 12)

sample	mean U/mL	variability (cv, %)
1	26	1,8
2	44	3,6
3	92	5,1

c. Variability between 2 kit lots (n = 6)

sample	mean U/mL	variability (cv, %)
1	21	1,6
2	41	4,8
3	90	4,6

11.7. Frequency distribution of H. pylori IgG

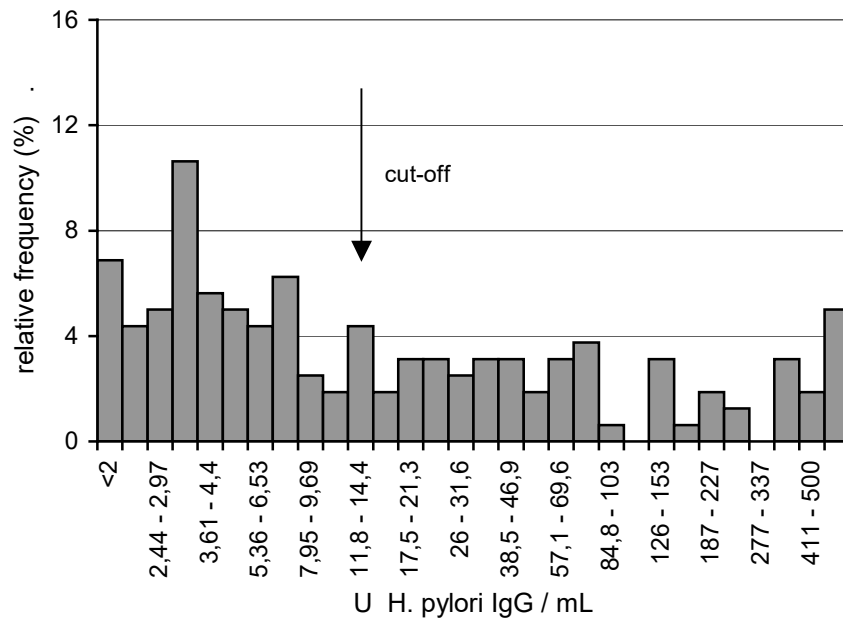
This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of sera found positive according to a CE-compliant reference ELISA. The following distribution of the analyte was observed:

blood donor sera		positive sera	
n:	160	n:	77
mean:	80 U/mL	mean:	230 U/mL
mean + s:	250 U/mL	mean - s:	4,6 U/mL
mean + 2s:	410 U/mL	mean - 2s:	< 0 U/mL
median:	9,4 U/mL	median:	140 U/mL
95 th percentile:	490 U/mL	5 th percentile:	26 U/mL

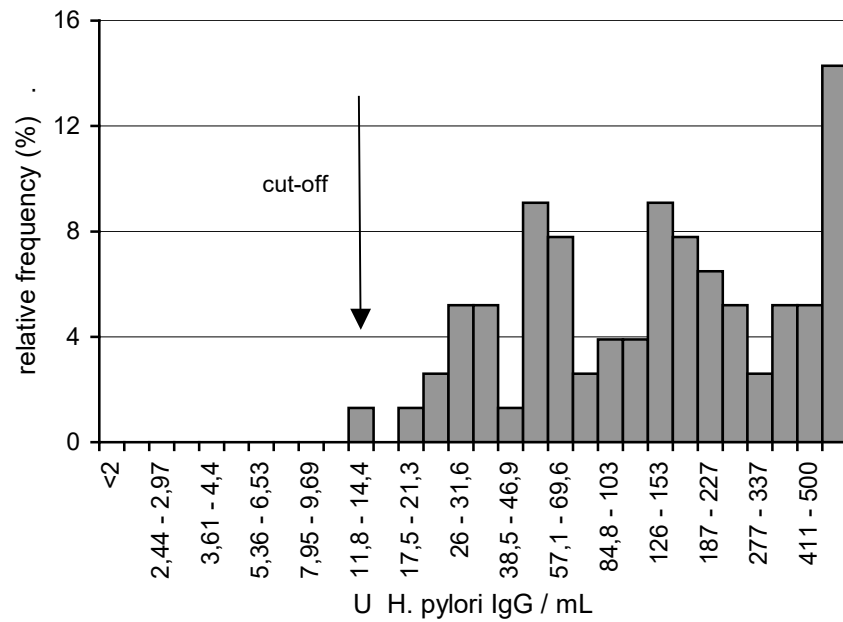
ROC-analysis of these data was used to determine the cut-off as 13,0 U/mL (5). Based on this definition, the reference collective was classified positive as a whole, the weakest serum scoring just above the cut-off. The data presented here suggest a diagnostic specificity and sensitivity of the ELISA of about 55 and nearly 100 %, respectively.

The low specificity value rather reflects the well-known prevalence of H. pylori infection (which is highly dependent on the population examined and increases with age) than a lack of specificity of the ELISA. Published data range from about 25 up to 60 % in developed countries. In accordance with these findings, 45 % of the above blood donor collective are found positive when the suggested cut-off is applied, as shown below.

blood donor sera



positive sera



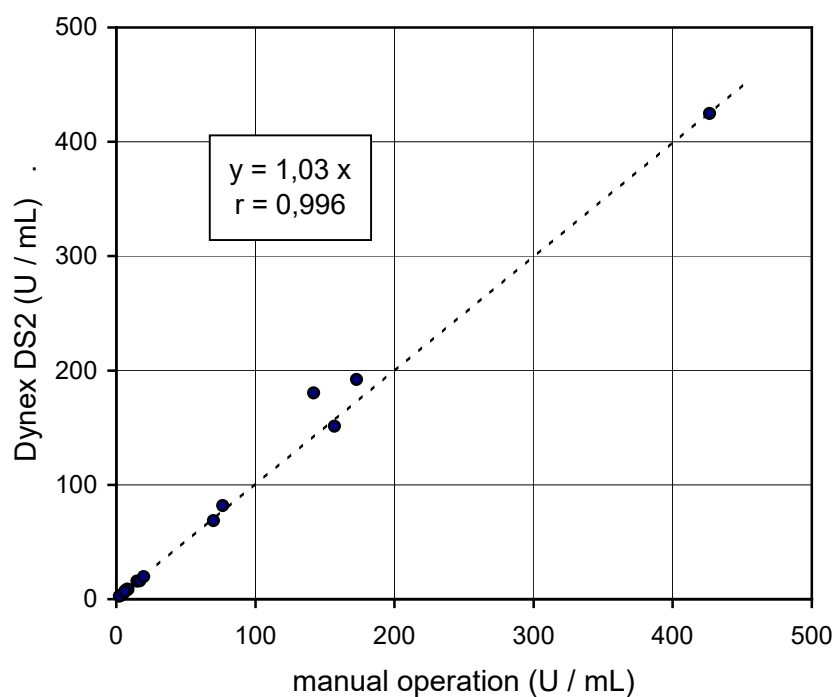
11.8. Manual operation vs. Dynex DS2 automated ELISA system

Variability: Using specimen of one and the same kit lot, the variability of assay results were compared between manual operation and the Dynex DS2 automated ELISA system:

	manual operation	Dynex DS2
intra-assay variability (n = 16)	mean cv = 3,1 %	mean cv = 3,4 %
inter-assay variability (n = 48)	mean cv = 4,5 %	mean cv = 3,8 %

Standard curve: depicted in section 9

Correlation:



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12. Warranty

Steffens biotechnische Analysen GmbH (SBA) guarantees that the product delivered has been thoroughly tested to ensure that its properties specified herein are fulfilled. No further warranties are given.

The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case SBA disclaims all warranties whether expressed, implied or statutory. Moreover, SBA accepts no liability for any damage, whether direct, indirect or consequential, which results from inappropriate use or storage of the product.

13. Symbols



Article code



Batch code



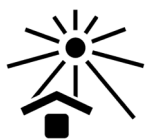
Contains x determinations



For *in vitro* diagnostic use



Conformité Européenne



Store shielded from sunlight



Store at 2 - 8°C



Expiration date



Read "Directions for Use"



Warning



Biological risk



Manufactured by

14. References

1. Dunn, B. E., et al.: Helicobacter pylori. Clin Microbiol Rev 10 (1997), 720 - 741
2. Mitchell, H. M.: The epidemiology of Helicobacter pylori. Curr Topics Microbiol Immunol 241 (1999), 12 - 30
3. Asaka, M., et al.: Long-term Helicobacter infection - from gastritis to gastric cancer. Aliment Pharmacol Therapeutics 12 (1998), 9 - 15
4. Azuma, T., et al.: Review: Diagnosis of Helicobacter infection. J Gastroenterol Hepatol 11 (1996), 662 - 669
5. Sommer, R., and Eitelberger, F.: Wertigkeit der Gliadin-Antikörper im Serum zur Diagnose der Zöliakie. Wien Klin Wochenschr 104/4 (1992), 86 - 92

15. Summary flow chart

- a. Dilute the samples 1/100 in sample buffer (100 mL, ready-to-use, orange) and mix.
- b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with water and mix.
- c. Wash the wells once with 350 μ L wash buffer each. Dispense 100 μ L of the calibrators (2,0 mL each, ready-to-use, gradually blue) and controls (2,0 mL each, ready-to-use, green and red) and of the diluted samples into the wells of the solid phase. Duplicate measurements are recommended. Incubate for 30 minutes at room temperature ($23 \pm 3^{\circ}\text{C}$).
- d. Wash the wells 4 times with 350 μ L wash buffer each.
- e. Dispense 100 μ L of the conjugate (14 mL, ready-to-use, red) into the wells. Incubate as in step c.
- f. Repeat washing step d.
- g. Dispense 100 μ L of the substrate solution (14 mL, ready-to-use, black vial) per well. Incubate as in step c. Then, add 100 μ L stop solution (14 mL, ready-to-use, colourless) per well and agitate the plate briefly.
- h. Immediately measure the absorbance at 450 nm.
- i. Quantitative evaluation: determine the standard curve and, using this curve, transform the absorbance of the samples into their respective antibody concentration (U/mL).
- j. Qualitative evaluation: determine the borderline absorbance by multiplying the absorbance of the positive control with the factor shown in the certificate of analysis. Then, calculate the ratio of the samples by dividing their absorbance by the borderline absorbance.