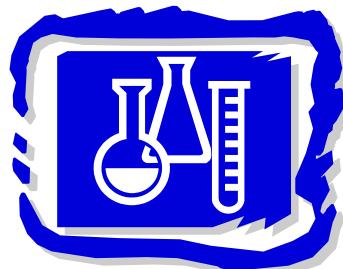


HP Ag

**Enzyme Immunoassay for the
qualitative/quantitative determination
of Helicobacter pylori Antigen
in human stools**

- for “in vitro” diagnostic use only -



DIA.PRO

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HP Ag

A. INTENDED USE

Enzyme Immunoassay (ELISA) for the one-step qualitative/quantitative determination of Helicobacter pylori Antigen (HP Ag) in human stools. The kit may be used for the follow-up of HP-infected patients and their pharmacological treatment. For "in vitro" diagnostic use only.

B. INTRODUCTION

Helicobacter pylori (Hp) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983. This bacterium is widely diffused in men, without limitations of sex and age; it has been found that infections can be transmitted directly by contact with contaminated biological fluids (saliva, stool, body secretions) and also from contaminated food and beverages.

H.pylori, and in particular some pathogenic strains (CagA +), is the etiological agent responsible of most of active infections and lesions of the gastric mucosa in man.

H.pylori infection moreover acts as cofactor in the development of tumoral pathologies of the gastric apparatus and it is suspected to be associated to some inflammatory pathologies of the genital female apparatus, evolving toward neoplastic transformation.

At the present time, the identification of Helicobacter pylori is mostly made with invasive histochemical techniques, with the determination of its urease activity on a isotopic substrate (breath test and mass analysis), with time-consuming bacteriological culture systems and with expensive molecular biology techniques (PCR).

ELISA for HP Ag have been only recently introduced as a specific, fast, non invasive (analysis of stools) and cheaper method of detection.

C. PRINCIPLE OF THE TEST

Stools from patients are used as a source of sample for the determination of HP antigen.

Microplates are coated with a cocktail of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter pylori antigens.

In the 1st incubation, the solid phase is treated with the sample, previously extracted from stools, and simultaneously with a mixture of monoclonal antibodies to Hp, conjugated with peroxidase (HRP).

After washing out all the other components of the sample, in the 2nd incubation the bound enzyme specifically present on the solid phase generates an optical signal that is proportional to the amount of H.pylori antigens present in the sample.

D. COMPONENTS

Code HPAG.CE contains reagents to perform 48 tests.

1. Microplate MICROPLATE

n° 1 - 6 strips x 8 breakable microwells, coated with anti HP Ag specific affinity purified mouse monoclonal antibodies and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Set: CAL ...

n° 4 vials - Lyophilized calibrators. To be dissolved with EIA grade water. When dissolved, Calibrators have the following concentrations: 0 - 0.1 - 0.5 - 1.0 ug/ml HP Ag

They contain fetal bovine serum, inactivated HP Ag, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.

Important Note: Calibrators when dissolved are not stable. Proceed as described in the proper section for storage.

3. Wash buffer concentrate WASHBUF 20X

1x60ml/bottle - 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 and 0.05% Tween 20.

4. Enzyme Conjugate CONJ

1x8ml/vial - Ready to use component. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HP Ag, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

The Enzyme Conjugate is color coded red.

5. Chromogen/Substrate SUBS TMB

1x16ml/vial - It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

6. Specimen Diluent: DILSPE

1x60ml/vial - Buffered solution for the extraction of HP Ag from the specimen and preparation of the sample. It contains 10 mM Tris-HCl buffer pH 7.4+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

The component is color coded blue.

7. Sulphuric Acid H₂SO₄ 0.3 M

1x10ml/vial - It contains 0.3 M H₂SO₄ solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Plate sealing foils: n° 2

9. Package insert: n° 1

Upon request:

HP Ag Extraction kit n° 1

The kit contains all what is necessary to prepare n° 48 samples extracted from stools collected by patients.

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated variable volume Micropipettes ranging 1000 ul and 200 ul; disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.
9. Disposable plastic micro-spoon stools collection container (available upon request from Dia.Pro s.r.l.)

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
4. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
6. Check that the liquid components of the kit are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
7. Avoid cross-contamination between samples by using disposable tips and changing them after each sample.
8. Avoid cross-contamination between kit components by using disposable tips and changing them between the use of each one.
9. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
10. Treat all specimens as potentially infective, according to national regulations and laws concerning biological sample handling and wasting.
11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
12. Wastes produced during the use of the kit have to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, tools for the extraction of the sample from specimens, used microplates, etc.) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: COLLECTION, PREPARATION AND WARNINGS

- 1) It is recommended to collect fresh stools in the morning with the plastic collector provided on request together with the kit. Alternatively a conical bottomed disposable tube, provided by the laboratory to the patient, may be used.
- 2) The patient submitted to the test should not be under antibiotic or anti-bacterial treatments as this pharmaceutical therapy is known to affect H.pylori up to a certain extent, depending on the antibiotic used, giving origin to false interpretation.
- 3) The patient has to be asked to collect the specimen avoiding any possible contact with urine or water using the plastic spoon present in the stool collector and taking just the amount of specimen necessary to fill up the cavity of the spoon.
- 4) The patient is asked to deliver the specimen the same day to the laboratory. From the time of collection, the specimen

can be stored in the laboratory up to 24 hr at 2..8°C or kept frozen at -20°C for longer time.

- 5) Specimens, and then samples derived from them, have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is recommended when the number of samples on testing is pretty high.

Important Note: *Degradation of HP antigen heavily occurs in stools after 24 hrs generating false negative results, even if the specimen is stored at 2..8°C.*

The next following operations are described and represented in figures in the Instructions for Use of the Stool Extraction Kit provided together with the kit.
Operate according to the following instructions:

- 1) Open the stool collection device and introduce the extraction brush deeply into the specimen. Rotate the brush 2-4 times in order to collect the right amount of biological material (about 0.2 gr).
- 2) Transfer the brush carefully into the test tube supplied in the kit and then add 1 ml Specimen Diluent. Keeping the brush inside the tube, mix vigorously on vortex for 1 min +/-10% in order to dissolve H.pylori into solution.
- 3) Discard the brush and insert the filtering piston, supplied with the kit, into the tube. Push gently the piston down into the tube in order to collect not more than 150-200 ul of the liquid phase of the suspension, volume enough to carry out the test.

Important Notes:

- a)** *Be careful not to apply a too strong manual pressure on the piston. The piston could break the tube and spills could be generated. If this should happen, use a paper towel soaked with an hospital disinfectant to clean up the contaminated surfaces.*
- b)** *Avoid any addition of preservatives to samples, especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.*

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.

In this case, call Dia.Pro's customer service.

Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2..8°C.

Important Note: *After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Set:

Add the volume of ELISA grade water reported in the label to the lyophilized powder of each Calibrator. Let fully dissolve the content and then gently mix on vortex.

Important Note: *When dissolved, Calibrators are not stable. Store Calibrators frozen in aliquots at -20°C, carefully labeled with the content of HP Ag present in each of them.*

Wash buffer concentrate:

The concentrated solution has to be diluted 20x with ELISA grade water and mixed gently end-over-end before use. During

preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Important Note: Once diluted, the wash solution is stable for 1 week at +2..8°C.

Enzyme Conjugate:

Ready to use. Mix well on vortex before use.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Specimen Diluent:

Ready to use. Mix well on vortex before use.

Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning H statements:

H315 – Causes skin irritation.

H319 – Causes serious eye irritation.

Precautionary P statements:

P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.

P332 + P313 – If skin irritation occurs: Get medical advice/attention.

P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337 + P313 – If eye irritation persists: Get medical advice/attention.

P362 + P363 – Take off contaminated clothing and wash it before reuse.

positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions provided by the manufacturer.

Important Note: Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the washer do not get blocked by the presence of stool bodies.

4. Incubation times have a tolerance of $\pm 5\%$.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 2.0 ; repeatability $\geq 1\%$. Blanking is carried out on the well identified in the section "Internal Quality Control". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.

Important Note: Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the workstation do not get blocked by the presence of stool bodies. We strongly suggest to use disposable sample tips in order to avoid any block or damage of fix probes.

7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.
8. Upon request, Dia.Pro srl offers a sample preparation device able to produce a particle free sample showing excellent performances in the assay. Please inquire.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm 2\%$.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm 0.5^\circ\text{C}$) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Prepare the sample from stools as described in section G and represented in the Instructions for Use of the HP Ag Extraction Kit.
2. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
3. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

4. Dilute all the content of the 20x concentrated Wash Solution as described above.
5. Dissolve the Calibrator Set as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a qualitative method.

A. Quantitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1+B1 wells empty for blanking purposes.
2. Pipette 100 µl Calibrators in duplicate into the calibration wells (see the example of dispensation reported below).
3. With the Pasteur pipette supplied aspirate the liquid filtered up into the inner chamber of the piston and dispense 2 drops (about 100 µl) of sample into the sample well. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
4. Dispense then 100 µl Enzymatic Conjugate in all wells, except for A1+B1, used for blanking operations.

Important note: Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

5. Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and incubate the microplate for **120 min at +37°C**.

Important notes: Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.

6. When the first incubation is over, wash the microwells as previously described (section I.3)
7. Pipette 200 µl Chromogen/Substrate into all the wells, A1+B1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min**.

Important note: Do not expose to strong direct light as a high background might be generated.

8. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

9. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

An example of dispensation scheme is reported below:

Microplate						
	1	2	3	4	5	6
A	BLK	CAL4				
B	BLK	CAL4				
C	CAL1	S1				
D	CAL1	S2				
E	CAL2	S3				
F	CAL2	S4				
G	CAL3	S5				
H	CAL3	S6				

Legenda: BLK = Blank CAL = Calibrator S = Sample

A. Qualitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1well empty for blanking purposes.
2. Pipette 100 µl Calibrator 1 in duplicate, 100 µl Calibrator 2 in duplicate, 100 µl Calibrator 4 in single and then 100 µl samples. Check for the presence of samples in wells as reported before.
3. Dispense 100 µl Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

4. Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and then incubate the microplate for **120 min at +37°C**.

Important notes: Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.

5. When the first incubation is over, wash the microwells as previously described (section I.3)
6. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.
8. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

An example of dispensation scheme is reported below:

Microplate						
	1	2	3	4	5	6
A	BLK	S3				
B	CAL1	S4				
C	CAL1	S5				
D	CAL2	S6				
E	CAL2	S7				
F	CAL4	S8				
G	S1	S9				
H	S2	S10				

Legenda: BLK = Blank CAL = Calibrator S = Sample

Important notes:

- If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading
- Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.

N. ASSAY SCHEME

Operations	Procedure
Calibrators&samples	100 ul
Enzyme Conjugate	100 ul
1st incubation	120 min
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200ul
2nd incubation	20 min
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Ensure that:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
CAL 0 ug/ml	< 0.200 mean OD450nm value after blanking
CAL 0.1 ug/ml	OD450nm > OD450nm CAL 0 ug/ml + 0.100
CAL 1 ug/ml	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
CAL 0 ug/ml > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of the negative one); 4. that no contamination of the calibrator or of the wells where the calibrator was dispensed

	has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 0.1 ug/ml OD450nm < Cal 0 ug/ml + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative calibrator instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 1 ug/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator (dispensation of negative calibrator instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF RESULTS

Quantitative Assay:

Calculate the mean OD450nm value of the calibrators. Then draw a calibration curve possibly using a 4 parameters fitting curve system. Then calculate on the curve the concentration of HP antigen in the sample.

Qualitative Assay:

The test results are calculated by means of a cut-off value determined from the O450nm value of the CAL 0 ug/ml (CAL 0) and the OD450nm of the CAL 0.1 ug/ml (CAL 0.1) with the following formula:

$$\text{Cut-Off} = (\text{CAL 0} + \text{CAL 0.1}) / 2$$

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

In the quantitative assay, samples showing a concentration of H.pylori antigen higher than 0.05 ug/ml are considered positive. For the qualitative assay, test results are interpreted as a ratio of the sample OD450nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by H.pylori.

Any patient showing an equivocal result should be retested on a second sample.
A positive result is indicative of HP infection and therefore the patient should be treated accordingly.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
2. Any positive result should be confirmed first by repeating the test and then, if still positive, by an alternative method before a diagnosis of HP infection is confirmed.
3. When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.
4. Diagnosis of HP infection has to be taken and released to the patient by a suitably qualified medical doctor. This should be done taking also into account other diagnostic evidences of infection.

An example of qualitative method is reported below.

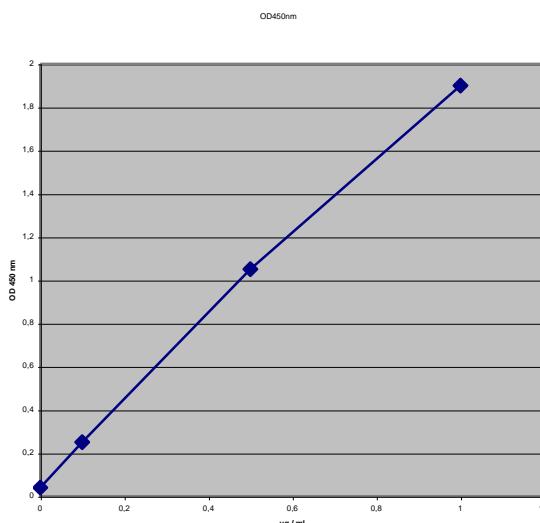
Note: The following data must not be used instead of real figures obtained by the user.

Cal 0 ug/ml: 0.040 – 0.060 OD450nm
Mean Value: 0.050 OD450nm
Lower than 0.200 – Accepted

Cal 0.1 ug/ml: 0.210-0.230 OD450nm
Mean Value: 0.220 OD450nm
Higher than Cal 0 ug/ml + 0.100 – Accepted
Cut-Off = (CAL 0 + CAL 0.1) / 2 = 0.135
Calibrator 1 ug/ml: 2.000 OD450nm
OD450nm higher than 1.000 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.1 = positive

An Example of Calibration curve is reported below :



R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances was conducted by testing negative and positive samples in an external clinical site and in the own laboratories as well.

1. Limit of detection

The limit of detection of the assay was calculated by examining serial dilution of HP antigen in Sample Diluent.

Results show that the analytical sensitivity of the assay is better than **0.05 ug/ml** when the limit of dilution is considered mean OD450nm CAL 0 ug/ml + 5 SD.

2. Diagnostic performances:

The diagnostic performances of the kit were studied in some external trials against (a) the "breath test", considered by the medical literature the Gold Standard for HP Ag determination and (b) against a commercial CE-marked ELISA, as well.

In the studies were the breath test was used as reference stools were collected from patients the same day of the breath test. In the study carried out with a commercial ELISA as reference the same samples were extracted with the specific devise supplied in combination with kits.

In the studies conducted in reference to the **breath test** the following mean values were obtained from two centers, one in Italy and one in Spain:

- Sensitivity: 98%
- Specificity: 96%

No confirmation by ELISA was possible on discrepant samples.

In the studies conducted in reference to a CE-marked commercial **ELISA**, similarly based on monoclonal antibodies to H.pylori, the following mean values were obtained:

- Sensitivity: 100%
- Specificity: 93%

The discrepant samples (7% "false positive") turned to be positive in the breath test, revealing a better overall performance of our kit respect to the commercial reference one, when compared on the gold standard method.

Due to the highest specificity shown by the monoclonal antibodies used in the kit no cross-reaction was observed with Campylobacter species.

3. Precision:

The variability shown in the tables did not result in sample misclassification. CV values ranging 4-8%, depending on OD450nm values were observed.

S. LIMITATIONS

False negative results were obtained from samples extracted from specimens stored for more than 1 day at 2-8°C. This finding is explain by the fact that the devise detects "live" H.pylori as confirmed by the results of excellent comparison to the breath test.

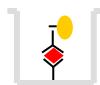
False positive results were mostly obtained from samples of liquid stools, difficult to handle and extract.

ASSAY GRAPHICAL SCHEME

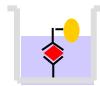
Add 100 μ l Calibrators, samples and conjugate to the plate and then incubate for 120 min at +37°C



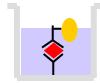
Wash as described in the proper section



Add 200 μ l Chromogen/Substrate and incubate 20 min at r.t.



Add 100 μ l Sulphuric Acid



Read the plate at 450nm (reading) and at 620-630nm (blanking)



All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:

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