H. pylori IgA (EIA-3902)

INTRODUCTION

Helicobacter pylori (Hp) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983. Hp has been recognized to be the agent responsible of most of cases of gastric mucosal damage and to play a role in the evolution of gastric diseases to carcinoma. Hp causes an immunological response during infection and specific antibodies of the different classes of IgG, IgA and IgM are produced by the patient. ELISA are currently used to screen patients affected by gastritis or peptic ulcers for acute active infection due to some Helicobacter pylori virulent strains. In particular the presence of IgA and IgM antibodies seem to be correlated to the acute phase of illness while IgG antibodies are present at different titers shortly after primary infections and last in blood for many years after infection. Quantitative ELISA are also used in the follow-up of patients undergoing antibiotic therapy, useful in monitoring IgG titer variations during and after the pharmaceutical treatment

PRINCIPLE OF THE ASSAY

Microplates are coated with Helicobacter pylori specific immunodominant antigens. In the 1st incubation, the solid phase is treated with diluted samples and anti-Hp IgA are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-Hp IgA are detected by the addition of anti hIgA antibody, labelled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-Hp IgA antibodies present in the sample. IgA in the sample may be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (Uarb/ml) as no international standard is available. Interferences due to IgG are blocked by means of a Neutralizing Reagent directly added to the sample in the well. Interferences due to IgG may frequently be present if not blocked, and give origin to false negative results.

TEST CONDITIONS AND NOTICES

1. All the reagents contained in the kit are for "in vitro" diagnostic use only.
2. Do not use the kit or reagents after the expiry date stated on labels. Do not mix reagents of different lots.
3. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
4. Bring all the reagents to room temperature for at least 60 min, before the test is started.
5. Avoid any contamination of reagents when taking them out of vials. We recommend using automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
6. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "WASHING INSTRUCTIONS" section of this insert.
7. Ensure that the Substrate/Chromogen mixture does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
   When preparing the Substrate/Chromogen mixture for the analysis use only plastic, disposable, clean or sterile containers.
8. Samples and materials potentially infective have to be handled with care as they could transmit infection.
   All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121°C for 30 min or with sodium hypochlorite at a final concentration of 2.5% for 24 hr. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
9. Avoid any contact of liquids with skin and mucosas.
   Use always-protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.

CONTENT OF THE KIT

Strips Microplate (n° 1)

12 x 8 wells strips coated with Hp antigens.
The plate comes in a sealed bag with desiccant. Bring the plate to room temperature before use, to prevent any moisture formation inside the bag.

Enzymatic Tracer (n° 1 vial of 0.8 ml)

Proteic buffer solution containing a specific anti-hIgA antibody, labelled with HRP, 20x concentrated. It contains proteic stabilizers, 0.2 mg/ml gentamicine sulphate and 0.3% Kathon GC.

Tracer Diluent (n° 1 vial of 16 ml)
Proteic buffered solution for the dilution of the concentrated tracer. It contains proteic stabilizers, 0.2 mg/ml gentamicine sulphate and 0.3% Kathon GC as preservatives.

Washing Solution (n° 1 vial of 60 ml)
20 x concentrated solution to be diluted up to 1200 ml with EIA grade water. It contains a phosphate buffer, Tween 20 and Kathon GC as preservative. The diluted solution, when stored at room temperature, is stable for at least 1 week.

Chromogen (n° 1 vial of 8 ml)
The solution contains tetramethylbenzidine (TMB) with activators and stabilizers, diluted in a phosphate/citrate buffer. **Warning**: Store protected from light.

Substrate (n° 1 vial of 8 ml)
The solution contains stabilized hydrogen peroxide diluted in a phosphate/citrate buffer.

Stop Solution (n° 1 vial of 16 ml)
It contains a solution of 0.3 M sulphuric acid.
Warning : Irritant!

Sample Diluent (n° 2 vials of 60 ml)
Proteic solution for the preparation of samples. It contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.

Calibration Curve (n° 6 vials of 2 ml)
It contains the following ready-to-use standards calibrated in arbitrary units:
0-5-10-20-50-100 Uarb/ml

Neutralizing Reagent (n° 1 vials of 8 ml)
Proteic solution for the neutralization of IgG in samples. It contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.

Cardboard Sealers (n° 2)
Plastic transparent sealers to cover plates when incubating at +37°C. **Note:** All human serum derived materials have been tested as negative for HBsAg, and HIV antibodies with FDA approved kits. The Positive Control has been inactivated for HCV. Anyway, handle this component as potentially infective.

STORAGE AND STABILITY

1. The kit has to stored at 2-8°C and used before the expiration date declared on the external label.
2. Unused strips have to be returned into their bag containing the dessicant and firmly sealed with tape before store them back at 2-8°C. After the bag has been opened the first time, strips are stable till the dessicant turns pink.
3. The diluted washing solution is stable at room temperature for 1 week or for 3 weeks at 2-8°C.
4. The Chromogen/Substrate mixture is stable for 4 hr at r. t. when stored protected from light.
5. The diluted tracer is stable for 1 week at 2-8°C, when stored in a sterile disposable container.
6. All the other liquid reagents are stable at 2-8°C when handled carefully to avoid contaminations.

MATERIALS NOT PROVIDED

1. Micropipettes of 10, 100 and 1000 ul.
2. Vortex mixer and adsorbent papers.
3. ELISA grade water for dilutions.
4. Timer.
5. Photometric ELISA reader linear up to at least 2 OD and supplied with filters of 450nm and 620-630 nm.
6. Incubator set at +37°C
7. Automatic microplate washer or manual apparatus capable to deliver 300 ul and aspirate.

SAMPLES AND TREATMENT

Sera and plasma

Either fresh sera or plasma can be used for the assay. Samples have to be diluted 1:101 (es.: 10ul + 1000 ul) with the Sample Diluent. If not used immediately plasma and sera can be stored at 2-8°C for 1 week. In case of longer storage freeze samples at –30°C. Samples should be clear and not contaminated by microrganisms. If necessary, remove contaminating particles by centrifugation at 2000 g x 20 min at r.t. or by filtration on 0.22u filters. Highly lipemic, icteric or hemolized samples should not be used as they can give false results in the assay. Do not freeze and thaw samples more than one time, as IgA may get damaged.

Stool

When stool is used as sample, 0.2 gr of specimen has to be transferred into a plastic disposable tube and 1 ml of Sample Diluent to be added. Mix on vortex for 1 min and let the sample stand for 5-10 min. so that the heavy particals of the sediment will settle. Afterwards aspirate the volume necessary for the test from the top of the suspension and use it for the assay.

H. PREPARATION OF REAGENTS

Washing Solution

The concentrated solution has to be diluted 20x in ELISA grade water before use.

Tracer

Dilute the concentrated tracer 1:20 with the Tracer Diluent. Mix gently on vortex before use. Prepare only the volume necessary to the test.

Chromogen/Substrate

About 5 minutes before use, prepare this reagent in a disposable plastic container, according to needs, by mixing 1 volume of Chromogen with 1 volume of Substrate. Prepare only the volume necessary to the test.

WASHING INSTRUCTIONS

A good washing procedure is essential to obtain correct and precise analytical data. We therefore recommend using a good quality ELISA microplate washer, maintained at the best level of washing performances. Generally, 4-5 automatic washing cycles of 300 µl/well are sufficient to avoid false positive reactions and high background. Anyhow, we recommend calibrating the washing system on the kit itself so to match the declared analytical performances. In case of manual washing, we suggest to carry out 5 cycles, dispensing 300 µl/well and aspirating the liquid for 5 times. The liquid aspirated from the strips must be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hrs, before liquids are wasted in an appropriate way.

ASSAY PROCEDURE

Important notes: At least 1 hr before use, bring all the components of the kit to r.t. and mix the liquid reagents carefully on vortex. Do not mix reagents of different lots. We recommend distributing the calibrators in duplicate. Distribution and incubation times should be the same for all the wells. Avoid long interruptions among the different steps of the assay. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad. The color developed in the last incubation is stable for maximum 1 hr in the dark. We recommend to read the microplate at 450 nm (reading filter) and subtract the

blank at 620-630 nm (blanking filter). Use well A1 to carry out blanking operations. Leave the A1 well empty for blanking operations. Dilute and treat samples as reported. Do not dilute standards, as they are prediluted and ready to use. Dispense 50 ul Neutralizing Reagent in the entire sample wells; do not dispense in standard wells and in A1. We suggest then dispensing standards and samples into wells according to the following table:

A1+B1	**Wells for blanking**
C1+D1	**100 ul Standard 1**
E1+F1	**100 ul Standard 2**
G1+H1	**100 ul Standard 3**
A2+B2	**100 ul Standard 4**
C2+D2	**100 ul Standard 5**
E2+F2	**100 ul Standard 6**
G2…H12	**100 ul Samples**

Cover the microplate with the plate sealer and incubate strips for 60 min at +37°C. Peel out the plate sealer and wash the microplate according to instructions. In the meantime prepare the Tracer by diluting it 1:20 as described. Add 100 ul diluted Tracer to all the wells, but A1. Incubate the microplate sealed for 60 min at +37°C. Peel out the plate sealer and wash the microplate according to instructions. In the meantime prepare the Chromogen/Substrate mixture as described. Add 100 ul of the Chromogen/Substrate mixture to all the wells, A1 included. Incubate the microplate for 20 min at r.t., protected from light. Stop the enzymatic reaction by adding 100 ul Stop Solution to all the wells, A1 included. Read the microplate at 450nm and 620-630nm blanking the instrument on A1 well.

M. VALIDITY OF THE ASSAY

The assay is considered valid if:

1. The OD450nm of the A1 blank well is < 0.100.
2. After blanking on A1, the OD450nm mean value of the Standard 0 Uarb/ml is < 0.200.
3. The OD450nm mean value of the Standard 100 Uarb/ml is > 0.500.
4. The OD450nm mean value of the Standard 5 Uarb/ml is higher than NC.
   In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of controls and samples.

CALCULATION OF RESULTS

If the test turns out to be valid, elaborate the standard curve with a qualified curve fitting system and then calculate the concentration of samples on the curve. A gray zone has been set between 5 and 10 arbU/ml. Values higher than 10 arbU/ml are considered significantly positive for the presence of IgA anti H.pylori in the sample tested. Anti H.pylori IgA concentrations over 50 arbU/ml may be considered a serological marker of a recent exposure to this agent or of an ongoing infection.

EXAMPLE OF STANDARD CURVE

0	Uarb/ml	OD450nm	0.050
5	Uarb/ml	OD450nm	0.200
10	Uarb/ml	OD450nm	0.350
20	Uarb/ml	OD450nm	0.600
50	Uarb/ml	OD450nm	1.500
100	Uarb/ml	OD450nm	2.300

ASSAY PERFORMANCES

Sensitivity: The sensitivity of the assay has been calculated on a panel of positive samples by comparing with a FDA approved kit on the market. The test shows a sensitivity > 98%.

Specificity: It has been calculated on panels of negative and positive samples, preclassified with an FDA approved kit present on the market. The assay shows a specificity > 98% on plasma and sera.

Reproducibility: It has been calculated on the Negative and Positive Controls tested in replicates in different days. CV's between 4-12% have been obtained depending on their OD450nm value.

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