RESULTS
Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgA.
Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE
- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL
Subtract the value of the blank from all the other readings. The OD values of cut off must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity
116 human sera were analyzed by this Helicobacter pylori IgA Elisa and Westernblot reference method. Out of 116 samples, 16 were positive for the presence of IgA antibodies to H.pylori by DIAsource Elisa and Westernblot showed 15 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
DIA	16	100	0	1
WB	15	101		

2. Precision

2. Inter-assay Study			
No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean (OD's)	1.29	0.91	0.23
SD	0.121	0.089	0.025
CV%	**9.37**	**9.78**	**10.86**

3. Intra-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean (OD's)	1.43	0.98	0.26
SD	0.096	0.067	0.019
CV%	**6.71**	**6.83**	**7.30**

REFERENCES

1. Marshall B.J. and Warren J.R.: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet i, 1311 (1984).
2. Jones D.M., Lessels A.M., Eldridge J.: Campylobacter-like organisms on the gastric mucosa: culture, histological and serological studies. J. Clin. Pathol. 37: 1002 (1984).
3. Blaser M.J.: H. pylori and the pathogenesis of gastroduodenal inflammation. J. Inf. Dis. 161: 626 (1990).
4. Valle J., Seppälä K., Sipponen P., Kasunen T.U.: Disappearance of gastritis after eradication of H. pylori: a morphometric study. Scand. J. Gastroenterology 26: 1057 (1991).
5. G.B. Wisdom: Enzyme-Immunoassay. Clin. Chem. 22: 1243 (1976).

Helicobacter pylori IgA Elisa
Catalog No. KAPRHLA09

IVD CE

DIAsource ImmunoAssays SA	Phone : + 32 67 88 99 99
Rue de l'Industrie 8	Fax : + 32 67 88 99 90
1400 Nivelles	Email : tech.support@diasource.be
Belgium	Website : www.diasource.be

INTENDED USE
The DIAsource KAPRHLA09 Helicobacter pylori IgA Elisa test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgA-class antibodies to *Helicobacter pylori* bacteria in human serum.This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION
In 1983, Warren and Marshall identified *Helicobacter pylori*, a new gram-negative bacterial pathogen, in patients suffering from gastritis, and this finding led to studies on the relationship between bacterial infection and chronic gastric disease. It has been demonstrated that in patients with gastritis, eradication of the bacteria led to healing of the anatomical lesion (4).
Diagnostic procedures for the detection of the organism generally involve invasive (gastroscopic) techniques for sample collection.
However, a specific immune response is seen in infected patients. There is an excellent correlation between the clinical presentation of gastritis, the presence of H.pylori in stomach and elevated serum H.pylori IgG and IgA antibodies.The serological test thus represents a useful alternative to the invasive bioptic technique. IgA levels rise with primary and chronic infection and remain shortly. The efficacy of antimicrobial therapy can therefore be monitored via changes in specific IgG antibody.

PRINCIPLE OF THE TEST
The KAPRHLA09 Helicobacter pylori IgA kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Helicobacter pylori antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgA antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-*Helicobacter pylori* IgA antibodies present.

REAGENTS
The DIAsource *Helicobacter pylori* IgA ELISA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED

H.pylori- Antigen -Coated Microtitration Strip		Quantity : 1 plate
Wash Concentrate	WASH SOLN CONC	Quantity : 1 bottle
Sample Diluent	DIL SPE	Quantity : 1 bottle
TMB-Substrate	CHROM TMB	Quantity : 1 bottle
Negative control	CONTROL -	Quantity : 1 vial
Cut off control	CONTROL CO	Quantity : 1 vial
Positive control	CONTROL +	Quantity : 1 vial
2nd Antibody Conjugate	Ab HRP	Quantity : 1 bottle
Stopping Solution	STOP SOLN	Quantity : 1 bottle

Sorbent A | SORBENT A | Quantity : 1 bottle

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl, and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator capable of maintaining temperature at 37 +/- 2°C

Helicobacter-Antigen-Coated Microtitration Strips

One stripholder containing 12x8 (96) microtitration wells coated with *Helicobacter pylori* antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent

One bottle, 100 ml, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

***Helicobacter pylori* IgA Controls**

Three vials, negative, cut off and positive , each 2 ml of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate

One bottle, 12 ml, containing anti-human IgA monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

Sorbent A

One Bottle, 4 ml, containing protein solution, in a phosphate buffer solution with 0.02% proclin. Store at 2°-8° C.

TMB-Substrate

One bottle, 12 ml, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution

One bottle, 15 ml, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose of all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product.Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS

Wash Solution

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Assay Procedure

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µl of serum into 1 ml of Sample Diluent.
3. Pipette 100 µl of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µl of the TMB-substrate. Add 30ul Sorbent A only in to the wells of diluted samples.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 ml of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µl of ready to use Enzyme-Labeled 2nd Antibody-conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µl of TMB Chromogen Solution to each well (including the blank) using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µl of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

	Consult instructions for use		Manufacturer
	Storage temperature		Contains sufficient for n tests
	Use by	IVD	In vitro diagnostic medical device
LOT	Batch code	REF	Catalogue number

Revision date : 2010-02-04