11. Assay Procedure Continued…

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**.

9. Add 100µl of Stop Solution **(Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Quantitative Results

Plot the OD of each standard against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve. Values below 6.25 U/ml are considered normal. Values between 6.25 and 7.00 U/ml are indeterminate. Values above 7.00 U/ml are regarded as positive. Values above 100 U/ml should be re-assayed at a higher dilution e.g. 1:600.

Qualitative Results

ODs below that of the 6.25 U/ml standard are considered normal. Samples giving ODs greater than the 6.25 U/ml standard +10% are regarded as positive. Samples with ODs between the OD of the 6.25 U/ml and 6.25U/ml OD +10% are indeterminate.

Interpretation ranges are given as a guide and laboratories can establish their own reference ranges.

14. Limitations of the Procedure

1. For diagnostic purposes, *H.pylori* IgG ELISA results should be used in conjunction with other test results and overall clinical presentation.
2. Although the presence of antibodies to *H.pylori* is indicative of *H.pylori* infection in symptomatic individuals, antibody levels may remain high for some time following eradication of the organism.
3. A positive *H.pylori* antibody test result does not distinguish between active and inactive infection. Positive results should be confirmed by other diagnostic testing procedures.

15. Performance Characteristics

Comparative Study

The Genesis Diagnostics kit was compared with a commercially available ELISA assay. The results are summarised below.

Comparative Study (n=132)		Reference ELISA Kit	
		+	-
Genesis Diagnostics Kit	+	69	3
	-	1	59

Sensitivity = 99%, Specificity = 95%, Accuracy = 97%

16. Reproducibility

Precision

	n	Mean Conc.	SD Within Assay	CV% Within Assay	SD Between Assay	CV% Between Assay
Sample 1	40	85.2	4.40	5.16	8.43	9.90
Sample 2	40	14.3	0.93	6.50	1.69	11.81
Sample 3	40	25.2	1.30	5.16	2.26	8.95

17. Method Summary

- Dilute sera 1:200 with Sample Diluent **(Reagent 1)**
- Dispense Standards, the Positive and Negative Controls and the diluted sample into the microplate wells
- Incubate for **30 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30 minutes**
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10 minutes**
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

18. Further Reading

Warren J R & Marshall B J, *Lancet* 1983, I , 1273-1275
Goodwin C S *et al*, *Int. J. Syst. Bacteriol.* 1989, 39, 397 - 405
Rathbone B J *et al*, *Gut* 1986, 27, 635-641
Wyatt J L *et al*, Scand. *J. Gastroenterology* 1989, 24 (Sup 160), 27-34
von Wuffen H & Grote H J, *Euro. J. Clinical. Microbiol. Infect. Dis.* 1988, 1, 559-565
Loffield R J L F *et al*, *Lancet* 1989i: 1182-1185
B J et al, *Gut* 1986, 27, 1132-1137
Goodwin C S, *Lancet* 1988ii 1467-1469
Forman D et al, *Int J Cancer* 1990, 46, 608-611
Seppala K M et al, *Scand J Gastroenterol* 1992, 27, 973-976
Nomura A et al, *N Engl J Med* 1991; 325, 1132-1136
Kosunen T U et al, *Lancet* 1992, 339, 893-895

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G•E•N•E•S•I•S Diagnostics

H. pylori IgG ELISA Kit

Quantitative / Qualitative Assay for IgG Antibodies to *Helicobacter pylori*

[REF] GD001/10

CE [IVD]

1. Materials Included in the Kit

- **[MTP] (x10):** 96 wells in 12 X 8 break-apart strips, pre-coated with partially purified *H.pylori* antigens, with holder in a foil bag with desiccant
- **[DIL] (x1)** 150mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 120ml, (blue), **concentrate** (x15)
- **[WB] (x8)** 100mM Tris-buffered saline with detergent, pH 7.2, 150 ml, **concentrate** (x10)
- **[CONJ] (x1)** rabbit anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 144 ml, (red), ready to use
- **[SUBS] (x1)** aqueous solution of TMB and hydrogen peroxide, 144 ml, ready to use
- **[STOP] (x1)** 0.25M sulphuric acid, 144 ml, ready to use
- **[STD[1..6] (x4):** 0, 6.25, 12.5, 25, 50 & 100 U/ml, 3ml of 10mM Tris-buffered saline containing human serum IgG antibodies to *H.pylori*, ready to use
- **[PC] (x4):** 3ml of 10mM Tris-buffered saline containing human serum antibodies to *H.pylori*, ready to use
- **[NC] (x4):** 3ml of 10mM Tris-buffered saline containing normal human serum, ready to use
- **Instructions for Use**

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water •

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absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The *H.pylori* IgG kit is a rapid ELISA method for the detection of IgG antibodies to *Helicobacter pylori*. It is intended for use in clinical laboratories as an aid to diagnosis of *H.pylori* infections. The components of the kit are for *in vitro* diagnostic use only.

4. Explanation of the Test

H. pylori infection is a major causative factor in gastritis, gastric and duodenal ulcers, and gastric cancer. The organism can be demonstrated by culture or by histological staining in 90% of affected patients. The distribution of the organism within the gut lining is patchy and can be missed during endoscopy.

Detection of serum antibodies to *H. pylori* is a reliable indicator of *H. pylori* infection. The measurement of IgG antibodies provides a rapid, simple and non-invasive method for diagnosing *H. pylori* infection. Although the initial concentration of anti-*H. pylori* antibodies does not correlate with disease severity, several studies have confirmed that successful treatment can be monitored by studying the fall in serum levels of IgG antibodies over a period of up to one year after the start of therapy. A decrease in anti-*H.pylori* IgG antibodies after three months is strong evidence of successful treatment.

The kit uses an antigen preparation that has been partially purified to yield principally high molecular weight *H.pylori* membrane proteins.

5. Principle of the Test

Diluted serum samples are incubated with partially purified *H.pylori* antigens immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, controls and samples are measured using a microplate reader at 450nm.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and controls for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the TMB Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 - 8˚C after use.
3. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 - 8˚C.
4. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
5. Strictly observe the indicated incubation times and temperature.
6. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
7. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
8. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
9. Do not allow microwells to dry between incubation steps.
10. Strictly follow the described wash procedure. Insufficient washing may cause high background signal
11. Avoid direct sunlight and exposure to heat sources during all incubation steps.
12. Replace colour-coded caps on their correct vials to avoid cross-contamination
13. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer and Sample Diluent (see Technical Precautions) have a shelf life of 3 months if stored in a closed bottle at 2 - 8°C.

9. Specimen Collection and Storage

Serum or plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

1. Dilute the Sample Diluent **(Reagent 1)** 1:14 in distilled water to make sufficient buffer for the assay run e.g. add 10ml sample diluent concentrate to 140 ml water.

2. Dilute the Wash Buffer **(Reagent 2)** 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

11. Assay Procedure

1. Dilute patient samples 1:200 in diluted Sample Diluent (e.g. 5µl serum in 1ml diluent).

2. Assemble the number of strips required for the assay.

3. For quantitative assays, dispense 100 µl of each Standard, the Negative and Positive Controls and the diluted patient samples into appropriate wells. For qualitative assays, dispense only the 6.25 U/ml Standard together with controls and samples.

4. Incubate for **30 minutes** at room temperature.

5. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

6. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **30 minutes** at room temperature.

7. After 30 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.