H.Pylori IgG

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Helicobactor Pylori (ELISA)

REF: DS177780 Σ 96

Intended use
The DiaSino H.Pylori IgG assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of H.Pylori antibody of IgG type in human serum or plasma.

Summary
Helicobacter pylori is one of the most common bacterial infections in humans, affecting nearly 50% of the global population.[1] H.pylori has been associated with the development of serious upper gastrointestinal (GI) conditions including chronic gastritis, peptic ulcer disease, gastric cancer,[2] and mucosa-associated lymphoid tissue (MALT).
A serological test has been the first choice for the detection of H. pylori infection because it is easy to perform compared to the more invasive diagnostic tests. A positive serologic test indicates the presence of H. pylori antibodies that confirms both a possibility for past infection or potential current infection.

Test principle
Indirect method, total duration of assay: **70 minutes.**
The DiaSino H.Pylori IgG ELISA employs solid phase, indirect ELISA method for detection of antibodies to H.Pylori IgG in two-step incubation procedure.
Polystyrene microwell strips are pre-coated with highly immunoreactive human H.Pylori antigens. During the first incubation step, anti-H.Pylori IgG specific antibodies, if present, will be bound to the solid phase pre-coated H.Pylori antigens. The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody(IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

Reagents
Materials provided
- **Coated Microplate - symbol** HP PLATE , 8 x 12 strips, 96 wells. Pre-coated with human TG antigen.
- **Calibrators - symbol** HP CAL A-F , 6 vials, 1 mL each, ready to use; Concentrations: 0(A), 10(B), 25(C), 50(D), 100(E) and 150(F) U/mL*
  *DiaSino's reference value
- **Enzyme Conjugate - symbol** HP CONJ , 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled rabbit anti-human IgG antibodies (anti-IgG) in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **Sample Diluent - symbol** HP DILUT 1 vial, 11mL**. Ready to use.** Containing buffer salts and a dye
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU:** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Storage and stability
- Store at 2-8℃.
- Don't freeze.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Test procedure
Ensure the patients' samples and reagents are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplate wells for each calibrator and sample to be assayed.
- Add **100 µL** of **calibrators** to each well.
- Add **100 µL** of **Sample Diluent (Green color)** to each well **Except** the Calibrator-wells.
- Add **10 µL** of **Sample** to each Sample Diluent well *(NOTE: Reagents in Wells will turn Blue color from Green), then shake 30 seconds.*
- Cover the plate with a plate lid and incubate at **37 °C** for **30 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **enzyme conjugate**,
- Cover the plate with a plate lid and incubate at **37 °C for 30 minutes**.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **Substrate** to each well. Cover and incubate at **ambient temperature (18-25℃)** in the dark for reaction for **10 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in U/mL for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay

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Sample	Value (U/mL)	Absorbance
Calibrator A	0	0.023
Calibrator B	10	0.246
Calibrator C	25	0.687
Calibrator D	50	1.336
Calibrator E	100	2.019
Calibrator F	150	2.846
Control 1	17.72	0.473
Control 2	88.51	1.862
Sample	109.43	2.175

Limitations - interference

- Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Antimicrobials, proton pump inhibitors and bismuth preparations are known to suppress H. pylori and if ingested may give a false negative result.
- The DiaSino® H.Pylori IgG assay has not been evaluated in a pediatric population.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

0.2-150 U/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.2 U/mL. Values above the measuring range are reported as > 150 U/mL.

Lower limits detection

0.2 U/mL

The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n=21).

Expected values

Studies conducted with the DiaSino H.Pylori IgG assay covering a total of 493 healthy subjects confirmed the currently used threshold value of **20 U/mL**; this value corresponds to the 94th percentile. Therefore, values in excess of **20 U/mL** are considered positive for the presence of H.Pylori IgG antibody.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean (U/mL)	SD (U/mL)	CV%	SD (U/mL)	CV%
Human Serum 1	5.58	0.41	7.43	0.46	8.28
Human Serum 2	63.27	4.25	6.71	5.15	8.14
Human Serum 3	121.75	6.72	5.52	7.63	6.27
PC Universal 1	18.63	1.09	5.58	1.32	7.11
PC Universal 2	92.71	4.88	5.26	5.40	5.82

*Repeatability = within-run precision

Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the '0 U/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:
The DiaSino Anti-H. Pylori IgG ELISA test system has a sensitivity of **0.2 U/mL**

Method comparison

A total of 877 serum samples, prospectively collected from subjects sent to laboratory for H. pylori IgG testing, were tested by the DiaSino® H. pylori IgG assay and a reference H. pylori IgG assay. Results are summarized in the table below:

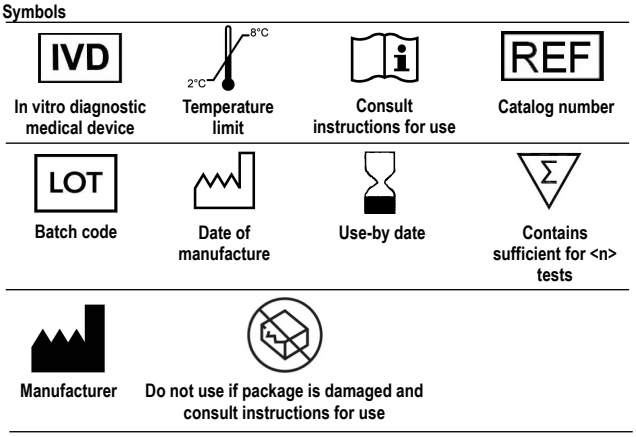
Optical agreement with Reference assay				
DiaSino H.Pylori IgG	Reference assay			Total
	Positive	Equivocal	Negative	
Positive	361	9	5	375
Equivocal	2	4	3	9
Negative	8	6	479	493
Total	371	19	487	877

Positive agreement	(361/371) 97.3%	95% CI (95.8-98.4)
Negative agreement	(479/487) 98.4%	95% CI (96.6-99.1)
Overall agreement	(844/877) 96.2%	95% CI (94.3-97.7)

References

1. Goh K-L, Chan W-K, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori Infection and Public Health Implications. Helicobacter 2011;16(Suppl. 1):1–9.
2. Malfertheiner P, M_egraud F, O'Morain C, Kuipers E, Bazzoli F, EL-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, the European Helicobacter Study Group. Current concepts in the management of Helicobacter pylori infection. The Maastricht-32005 Consensus Report. Gut 2007;56:772–81.

Symbols



In vitro diagnostic medical device | Temperature limit | Consult instructions for use | Catalog number

Batch code | Date of manufacture | Use-by date | Contains sufficient for <n> tests

Manufacturer | Do not use if package is damaged and consult instructions for use

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