Snibe Diagnostic

CE 0123 REF

130201521M:100 tests/kit
130601521M: 50 tests/kit
130701521M: 30 tests/kit

MAGLUMI® H. pylori IgG (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of H. pylori IgG in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of H. pylori infection in persons.

SUMMARY

Helicobacter pylori (*H.pylori*) is a gram negative, curved, spiral shaped rod (0.5-1.0 μm in width by 3.0-4.0 μm in length). *H. pylori* colonization is found in the deep portions of the mucous gel layer that coats the gastric mucosa, and between the mucous gel layer and apical surface of the gastric mucosal epithelial cells[1-3]. In some patients infected by *H. pylori*, *H. pylori* may also be located in the regions of the junctions between adjacent mucosal epithelial cells. It produces three enzymes in large amounts; urease, superoxide dismutase and catalases. Urease splits urea to produce ammonia, which provides conditions needed for the multiplication and sustenance of the organism in the gastric environment[3]. The colonization may induce the host's local and systemic immune response and may cause clinical signs and symptoms including neutrophil infiltration and the production of specific antibodies[4]**.** *H. pylori* is now accepted as a cause of gastritis, and *H. pylori* infections have also been associated with duodenal ulcer, gastric ulcer and nonulcer dyspepsia[5]**.**

The presence of *H. pylori* has been detected by using both invasive and non-invasive methods. Invasive methods include culture, histology, and the rapid urease test done on biopsy samples[6]. Many serological tests, mainly immune-globulin G (IgG) based, have been validated against invasive methods. The IgG antibody level to *H. pylori* is usually increased and may, in assays using specific antigens, be a marker for *H. pylori* infection[7]. The test for IgG antibody is a good and reliable test for the detection of antibodies to *H. pylori* and as an indication of *H. pylori* infection. The determination of IgA antibodies may be used as a test that complements the IgG antibody assay[8]**.**

TEST PRINCIPLE

Indirect chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with H.pylori antigen are mixed thoroughly, incubating and performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with anti-human IgG antibody are then added, reacting to form sandwich complexes and incubating. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of H. pylori IgG present in the sample.

REAGENTS

Kit Contents

Component	Contents	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with H.pylori antigen (~6.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of H. pylori IgG in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Calibrator High	A high concentration of H. pylori IgG in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Buffer	BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL	4.8 mL
ABEI Label	ABEI labeled with anti-human IgG antibody (~25.0 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	12.5 mL	7.5 mL	4.8 mL
Diluent	0.9% NaCl.	15.0 mL	10.0 mL	5.0 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
Positive Control 1	A low concentration of H. pylori IgG (50.0 EIU) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
Positive Control 2	A high concentration of H. pylori IgG (100 EIU) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
All reagents are provided ready-to-use.				

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA, lithium heparin

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 10 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 15 hours at 10-30°C, or 78 hours at 2-8°C, or 4 months frozen at -20°C or colder. Frozen specimens subjected to up to 4 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, H. pylori IgG concentrations above the analytical measuring interval, can be diluted with Diluent either automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:5. The concentration of the diluted sample must be >40 EIU.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

H. pylori IgG (CLIA) assay. control barcode labels

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the Snibe internal reference standard.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 14 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[9].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the H. pylori IgG assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order H. pylori IgG (CLIA) Controls (REF: 160201430MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the H. pylori IgG concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in EIU. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected results for the H. pylori IgG assay was obtained by testing 432 H. pylori IgG positive patients and 426 H. Pylori IgG negative people in China, gave the following expected value by ROC curve:

- Non-reactive: A result less than 30 EIU (<30 EIU) is considered to be negative.
- Reactive: A result greater than or equal to 30 EIU is (≥30 EIU) considered to be positive.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- The assay is mainly used to aid in the diagnosis of individuals with suspected or confirmed H.pylori infection.
- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the H. pylori IgG results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[10,11]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[12].
- Bacterial contamination of the specimens may affect the test results.
- The assay cannot be used as a basis for early diagnosis of malignant tumors, and is not suitable for disease monitoring of tumor patients.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (EIU) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (EIU)	%CV	SD (EIU)	%CV	SD (EIU)	%CV
Serum Pool 1	18.178	NA	NA	NA	NA	NA	NA
Serum Pool 2	38.932	1.034	2.66	0.781	2.01	2.023	5.20
Serum Pool 3	60.357	1.436	2.38	1.009	1.67	2.022	3.35
Plasma Pool 1	19.112	NA	NA	NA	NA	NA	NA
Plasma Pool 2	38.534	1.170	3.04	0.387	1.00	1.657	4.60
Plasma Pool 3	61.921	1.473	2.38	0.534	0.86	2.285	3.69
Positive Control 1	49.781	1.376	2.76	1.079	2.17	2.544	5.11
Positive Control 2	100.528	2.461	2.45	0.910	0.91	3.395	3.38
Negative Control	<2.000	NA	NA	NA	NA	NA	NA

Measuring Range

2.00-200 EIU (defined by the Limit of Blank and the maximum of the master curve).

Reportable Interval

2.00-1000 EIU (defined by the Limit of Blank and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =2.00 EIU.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	20 mg/dL	Human anti Sm Antigen	400 AU/mL
Hemoglobin	800 mg/dL	Human anti Human RNP Antigen	400 AU/mL
Intralipid	1000 mg/dL	Biotin	50 μg/mL
HAMA	30 ng/mL	Aspirin	65.16 mg/dL
Rheumatoid factor	1500 IU/mL	Ibuprofen	49.96 mg/dL
ANA	6 (S/CO) strong positive	Omeprazole	0.6003 mg/dL
Total IgG	4086 mg/dL	Lansoprazole	0.6421 mg/dL
Total IgM	364 mg/dL	Salazosulfapyridine	30.01 mg/dL
Total cholesterol	503.1 mg/dL	Amoxicillin	7.52 mg/dL

Cross-Reactivity

The assay is highly specific for H. pylori IgG antibodies, with no observed crossreactivity to *Bacillus cereus, E. coli, Enterobacter cloacae subsp. Cloacae, Proteus vulgaris, Candida albicans, Pseudomonas aeruginosa, Clostridium sporogenes, Staphylococcus aureus, Campylobacter jejuni* and *Enterococcus faecalis.*

High-Dose Hook

No high-dose hook effect was seen for H. pylori IgG concentrations up to 10000 EIU.

Clinical Sensitivity

The clinical sensitivity of the H. pylori IgG assay was determined in China by testing 440 samples confirmed H. pylori infected specimens with UBT confirmation of positive result.

N of samples	Reactive	Sensitivity	95% CI
440	427	97.05%	95.46%-98.63%

Clinical Specificity

The clinical specificity of the H. pylori IgG assay was determined in China by testing 671 samples collected from healthy individuals with UBT confirmation of negative result.

N of samples	Non-reactive	Specificity	95% CI
671	659	98.21%	97.21%-99.21%

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE 0123	CE marking with notified body ID number		

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