Snibe Diagnostic

CE 0123 REF

130219010M:100 tests/kit
130619010M: 50 tests/kit
130719010M: 30 tests/kit

MAGLUMI® *Chlamydia pneumoniae* IgG (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of *Chlamydia pneumoniae* 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 *Chlamydia pneumoniae* infection.

SUMMARY

Chlamydiae are obligate intracellular parasites that are classified as bacteria because of the composition of their cell wall and their growth by binary division. They have a unique biphasic life cycle with a smaller extracellular form, the elementary body (EB), and a larger replicating intracellular form, the reticulate body[1]. *Chlamydia pneumoniae* (*C. pneumoniae*) is a recognized third species of the genus *Chlamydia* which was first isolated in 1965 from the conjunctiva of a Taiwanese child participating in a trachoma vaccine trial [1]. *C. pneumoniae*, an obligate intracellular gram negative bacterium, which is a human pathogen that can cause pneumonia, bronchitis, upper respiratory tract infection, and may also trigger acute episodes of wheezing in children with asthma[2,3]. It disseminates via respiratory secretion, causing about 10 % of community-acquired pneumonia cases and 5% of bronchitis cases. In adults, infections caused by this agent may be of long duration and chronic infections[2,3]. *C.pneumoniae* is one of the most disseminated of human pathogens, with worldwide seroprevalence values of 40-60%[4]. Roughly half of *C. pneumoniae* infections are asymptomatic or of moderate sore throat, other *C. pneumoniae* infections are mainly manifested as sustained dry cough, headache, and fever[5]. *C. pneumoniae* chronic infection is more common in adults. More than 50% of adults are infected with *C. pneumoniae*, producing anti-*Chlamydia* antibody[5]. Laboratory methods for the diagnosis of *C. pneumoniae* infection include culture, antigen detection, serology, and PCR[6].

Infection with *C. pneumoniae* induces serum IgM, IgA, and IgG responses[1]. *C. pneumoniae* IgM antibody appears later, about 3 weeks after the onset of illness, and then IgG antibody may not appears until 6 to 8 weeks after onset[1]. In reinfection, The IgG antibody titer rises quickly, often in 1 to 2 weeks. And IgG persists and may be detected for over 3 years after acute infection[1]. Whereas fourfold rises in antibody titer in the IgM or IgG serum fraction are clearly related to current *C. pneumoniae* infection, a high titer of antibody in a single specimen may suggest current infection or persistence after a recent or past infection[1]. *Chlamydia*-specific IgG, IgM and IgA antibodies may currently be one of sensitive serological tool for diagnosing recent respiratory *chlamydia* infections[7].

TEST PRINCIPLE

Indirect chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with *Chlamydia pneumoniae* antigen are mixed thoroughly and incubated, performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with anti-human IgG monoclonal antibody are then added and incubated, reacting to form immuno-complexes. 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 *Chlamydia pneumoniae* IgG present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with *Chlamydia pneumoniae* antigen (~6.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of *Chlamydia pneumoniae* IgG in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of *Chlamydia pneumoniae* IgG in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	BSA, NaN_3 (<0.1%).	22.5 mL	12.0 mL	7.8 mL
ABEI Label	ABEI labeled with anti-human IgG monoclonal antibody (~12.5 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	22.5 mL	12.0 mL	7.8 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control	A high concentration of *Chlamydia pneumoniae* IgG (4.00 AU/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
All reagents are provided ready-to-use.				

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	6 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, Na2-EDTA , Li-heparin or Na-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 heat-inactivated samples or 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 are 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 8 hours at 10-30°C or 7 days at 2-8°C, or 12 months frozen at -20°C. Frozen specimens subjected to up to 5 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.

PROCEDURE

Materials Provided

Chlamydia pneumoniae 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 X3, MAGLUMI X6, MAGLUMI X8, 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[8].

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 *Chlamydia pneumoniae* 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 systems 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 *Chlamydia pneumoniae* IgG (CLIA) Controls (REF: 160201191MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the *Chlamydia pneumoniae* 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 AU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected range for the *Chlamydia pneumoniae* IgG assay was obtained by testing 428 *Chlamydia pneumoniae* IgG positive patients and 414 *Chlamydia pneumoniae* IgG negative people in China, gave the following expected value by ROC curve:

- Non-reactive: A result less than 1.00 AU/mL (<1.00 AU/mL) is considered to be negative.
- Reactive: A result greater than or equal to 1.00 AU/mL (≥1.00 AU/mL) is 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

- If the *Chlamydia pneumoniae* IgG results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- The assay is mainly used to aid in the diagnosis of individuals with suspected or confirmed *Chlamydia pneumoniae* infection.
- 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[9,10]. 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[11].
- Early in infection, antibodies may not be produced or may be produced at levels below the minimum detection limit, resulting in a negative result. A negative test does not rule out acute infection, and etiological testing is recommended for suspicious samples, or retesting at a minimum interval of 7 days.
- Evaluation of serological test results requires comprehensive consideration of the clinical course of disease, basic condition, age and other factors of patients. For example, people with low immune function and deficiency, and infants with low antibody production ability may not produce or produce low titer antibodies, and the reference value of serological antibody detection is limited.
- People who have been treated with blood transfusion or other blood products in the last few months should be carefully analyzed for positive test results.
- *Chlamydia pneumoniae* IgG persists in partially cured patients, and patients who are re-infected within a short period of time should be confirmed by dynamic antibody titer test or nucleic acid quantitative test of *Chlamydia pneumoniae*.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

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 (AU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
Serum Pool 1	0.608	NA	NA	NA	NA	NA	NA
Serum Pool 2	1.156	0.038	3.29	0.028	2.42	0.060	5.19
Serum Pool 3	6.192	0.173	2.79	0.096	1.55	0.235	3.80
Plasma Pool 1	0.726	NA	NA	NA	NA	NA	NA
Plasma Pool 2	1.209	0.044	3.64	0.022	1.82	0.064	5.29
Plasma Pool 3	6.638	0.185	2.79	0.104	1.57	0.307	4.62
Negative Control	0.205	NA	NA	NA	NA	NA	NA
Positive Control	3.965	0.144	3.63	0.080	2.02	0.189	4.77

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	Moxifloxacin	24 mg/dL
Hemoglobin	800 mg/dL	Gemifloxacin	12 mg/dL
Intralipid	3000 mg/dL	Azithromycin	60 mg/dL
HAMA	30 ng/mL	Erythrocin	120 mg/dL
Rheumatoid factor	2000 IU/mL	Clarithromycin	60 mg/dL
ANA	398 AU/mL	Roxithromycin	18 mg/dL
K2-EDTA	22.75 µmol/mL	Sulfasomizole	120 mg/dL
Na2-EDTA	22.75 µmol/mL	Doxycycline	12 mg/dL
Heparin lithium salt	80 IU/mL	Minocyline	42 mg/dL
Heparin sodium salt	80 IU/mL	Tigecycline	6 mg/dL
Total IgG	4086 mg/dL	Metacortandracin	42 mg/dL
Total IgM	364 mg/dL	Ibuprofen	144 mg/dL
Levofloxacin	24 mg/dL		

Cross-Reactivity

The assay is highly specific for *Chlamydia pneumoniae* IgG antibodies, with no observed cross-reactivity to TOXO IgG, Rubella IgG, CMV IgG, *Mycoplasma pneumoniae* IgG, *Chlamydia pneumoniae* IgM, EBV VCA IgG, EBV EA IgG, Anti-*Treponema pallidum*, *Chlamydia trachomatis* IgG, *Chlamydia psittaci* IgG, *Ureaplasma urealyticum* IgG, *Mycoplasma genitalium* IgG, *Mycoplasma hominis* IgG, *Legionella pneumophila* IgG, *Streptococcus pneumoniae* IgG, *Haemophilus influenzae* IgG, *Klebsiella pneumoniae* IgG, *E. coli* IgG, *Staphylococcus aureus* IgG, *Mycobacterium tuberculosis* IgG, *Pseudomonas aeruginosa* IgG, *Bordetella pertussis* IgG, *Acinetobacter baumannii* IgG, Group A *Streptococcus* IgG, Influenza B virus IgG, Human *parainfluenza* virus IgG, *Adenovirus* IgG, *Respiratory syncytial* virus IgG, *Rhinovirus* IgG, Human *metapneumovirus* IgG and Anti-HIV .

High-Dose Hook

No high-dose hook effect was seen for *Chlamydia pneumoniae* IgG concentrations up to 2000 AU/mL.

Clinical Sensitivity

The clinical sensitivity of the *Chlamydia pneumoniae* IgG assay was determined in China by testing 433 samples collected from expected positive population with commercial assay confirmation of *Chlamydia pneumoniae* IgG positive result.

N of samples	Reactive	Sensitivity	95% CI
433	430	99.31%	98.53%-100.00%

Clinical Specificity

The clinical specificity of the *Chlamydia pneumoniae* IgG assay was determined in China by testing 567 samples collected from expected negative population with commercial assay confirmation of *Chlamydia pneumoniae* IgG negative result.

N of samples	Non-reactive	Specificity	95% CI
567	562	99.12%	98.35%-99.89%

REFERENCES

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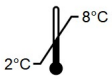
10. Primus F J, Kelley E A, Hansen H J, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy[J]. Clinical Chemistry, 1988, 34(2):261-264.
11. Boscato L M , Stuart M C . Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34(1):27-33.

SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	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		

MAGLUMI® and **Biolumi**® are trademarks of Snibe. All other product names and trademarks are the property of their respective owners.

Summary of safety and performance is available at Eudamed.

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax:+86-755-28292740

EC REP

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726