

MAGLUMI[®] Anti-HBc IgM (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of Anti-HBc IgM in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used as an aid in the diagnosis of acute or recent hepatitis B virus infection.

SUMMARY

Hepatitis B is caused by hepatitis B virus (HBV) infection and is a global infectious disease that seriously threatens human health. After infected with HBV, its antigens stimulated the immune system to produce an immune response, and antibodies appear in the blood, among which Anti-HBc IgM appears earlier. Subsequently, the titers of Anti-HBc IgM decreased, and Anti-HBc IgG rised rapidly¹⁻³. Research show that high levels of anti-HBc IgM have been detected in patients with acute hepatitis B viral infection, and hepatitis B virus surface antigen (HBsAg) generally also existed. In the convalescent phase, anti-HBc IgM will persist after the disappearance of HBsAg and decrease slowly over time³⁻⁶. Anti-HBc IgM may also be present in patients with chronic hepatitis B viral infection. The concentrations are generally lower than those associated with acute infections and may rise and fall with the progression of the disease⁷⁻¹¹.

TEST PRINCIPLE

Capture chemiluminescence immunoassay.

The prediluted sample, buffer, magnetic microbeads coated with anti-human IgM monoclonal antibody are mixed thoroughly and incubated, performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with hepatitis B virus core antigen 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 Anti-HBc IgM present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-human IgM monoclonal antibody (~10.0 µg/mL) in PBS buffer, NaN ₃ (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of Anti-HBc IgM in Tris-HCl buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of Anti-HBc IgM in Tris-HCl buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	PBS buffer, NaN ₃ (<0.1%).	13.5 mL	8.0 mL	4.8 mL
ABEI Label	ABEI labeled with Hepatitis B virus core antigen (~31.0 ng/mL) in PBS buffer, NaN ₃ (<0.1%).	23.5 mL	13.0 mL	7.8 mL
Diluent	0.9% NaCl, NaN ₃ (<0.1%).	23.5 mL	13.5 mL	8.0 mL
Negative Control	Tris-HCl buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control	Anti-HBc IgM (4.00 AU/mL) in Tris-HCl 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	20 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	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, K3-EDTA, Li-heparin, Na-heparin, Sodium Citrate(1:9), ACD-B, CPD, CPDA and K-Oxalate/NaF

- 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 at $\geq 10,000 \times g$ for 10 minutes 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 μ L.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 10 hours at 10-30°C, or 7 days at 2-8°C, or 12 months frozen at -20°C or colder. 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.

Specimen Dilution

- Samples cannot be diluted for the MAGLUMI Anti-HBc IgM assay.

■ PROCEDURE

Materials Provided

Anti-HBc IgM (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, register 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 Paul-Ehrlich-Institute, Langen, Germany, HBc Referenzserum IgM 84 (IgM anti-HBc).

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 guidelines¹².

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 Anti-HBc IgM 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. 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 Anti-HBc IgM (CLIA) Controls (REF: 160201155MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Anti-HBc IgM 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

Results obtained with the Anti-HBc IgM assay can be interpreted as follows:

- Non-reactive: A result less than 1.0 AU/mL (<1.0 AU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 1.0 AU/mL (≥ 1.0 AU/mL) considered to be reactive.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Anti-HBc IgM 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^{13,14}. 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 observed¹⁵.
- 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	0.607	N/A	N/A	N/A	N/A	N/A	N/A
Serum Pool 2	1.528	0.056	3.66	0.029	1.90	0.073	4.78
Serum Pool 3	7.536	0.187	2.48	0.101	1.34	0.287	3.81
Plasma Pool 1	0.607	N/A	N/A	N/A	N/A	N/A	N/A
Plasma Pool 2	1.515	0.040	2.64	0.003	0.20	0.058	3.83
Plasma Pool 3	7.479	0.107	1.43	0.069	0.92	0.180	2.41
NQC	<0.050	N/A	N/A	N/A	N/A	N/A	N/A
PQC	4.036	0.098	2.43	0.049	1.21	0.142	3.52

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 $\pm 10\%$. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Acetyl cysteine	15.0 mg/dL	Ibuprofen	50.0 mg/dL
Ampicillin-Na	100 mg/dL	Theophylline	10.0 mg/dL
Ascorbic acid	30.0 mg/dL	Lamivudine	30.0 mg/dL
Cyclosporine	0.5 mg/dL	Entecavir	0.5 mg/dL
Cefoxitin	250 mg/dL	telbivudine	60.0 mg/dL
Levodopa	2.0 mg/dL	Adefovir	1.0 mg/dL
Metronidazole	20.0 mg/dL	Bilirubin	40 mg/dL
Tetracycline	5.0 mg/ dL	Intralipid	1000 mg/dL
Acetylsalicylic acid	100 mg/dL	Hemoglobin	2000 mg/dL
Rifampicin	6.0 mg/dL	Rheumatoid factor	1500 IU/mL
Acetaminophen	20.0 mg/dL	HAMA	30 ng/mL

Cross-Reactivity

Clinical interference samples, which contain potential cross-reactants were used to evaluate the cross-reactivity of Anti-HBc IgM assay. The results were summarized in the following table:

Condition	Number of samples tested	Number of Anti-HBc IgM reactive
Autoimmune	5	0
Rheumatoid factor	3	1
CMV IgM	5	0
EBV IgM	3	0
Syphilis	3	0
Anti-HEV	3	0
VZV IgG	3	0
Anti-HAV	3	0
Anti-HCV	4	0
Influenza	4	0

Pregnant women Multipara	3	0
HSV 1/2 IgG	3	0
Dialysis patients	3	0
Pregnant women	4	0
Hyper IgG/IgM	6	0
HIV Ag/Ab	3	0
Total	58	1

Clinical Sensitivity

In a group of samples from Anti-HBc IgM positive patients, the diagnostic sensitivity of Anti-HBc IgM assay was found to be 100%.

Group	Number of samples tested	Number of Anti-HBc IgM reactive	Clinical Sensitivity
Anti-HBc IgM positive patients	223	223	100%

Clinical Specificity

In a group of randomly selected blood donors and hospitalized patients, the diagnostic specificity of the Anti-HBc IgM assay was found to be 100%.

Group	Number of samples tested	Number of Anti-HBc IgM Nonreactive	Clinical Specificity
Unselected donors	229	229	100%
Hospitalized patients	205	205	100%
Total	434	434	100%

REFERENCES

- Liang T J. Hepatitis B: the virus and disease[J]. Hepatology, 2009, 49(S5): S13-S21.
- Hwang E W, Cheung R. Global epidemiology of hepatitis B virus (HBV) infection[J]. North American Journal of Medicine and Science, 2011, 4(1).
- Chau K H, Hargie M P, Decker R H, et al. Serodiagnosis of recent hepatitis B infection by IgM class anti - HBc[J]. Hepatology, 1983, 3(2): 142-149.
- Lindsay K L, Nizze J A, Koretz R, et al. Diagnostic usefulness of testing for anti - HBc IgM in acute hepatitis B[J]. Hepatology, 1986, 6(6): 1325-1328.
- Eble K, Clemens J, Krenc C, et al. Differential diagnosis of acute viral hepatitis using rapid, fully automated immunoassays[J]. Journal of medical virology, 1991, 33(3): 139-150.
- Gerlich W H, Uy A, Lambrecht F, et al. Cutoff levels of immunoglobulin M antibody against viral core antigen for differentiation of acute, chronic, and past hepatitis B virus infections[J]. Journal of clinical microbiology, 1986, 24(2): 288-293.
- Galli C, Orlandini E, Penzo L, et al. What is the role of serology for the study of chronic hepatitis B virus infection in the age of molecular biology?[J]. Journal of medical virology, 2008, 80(6): 974-979.
- Colloredo G, Bellati G, Leandro G, et al. Quantitative analysis of IgM anti-HBc in chronic hepatitis B patients using a new "gray-zone" for the evaluation of "borderline" values[J]. Journal of hepatology, 1996, 25(5): 644-648.
- Banninger P, Altorfer J, Frösner G G, et al. Prevalence and significance of anti - HBc IgM (radioimmunoassay) in acute and chronic hepatitis B and in blood donors[J]. Hepatology, 1983, 3(3): 337-342.
- Kiyosawa K, Sodeyama T, Franca S T M, et al. Serial assay for IGM anti - HBc in patients with anti - HBe - positive chronic hepatitis and its significance for long - term prognosis[J]. Journal of medical virology, 1988, 24(3): 241-250.
- Mels G C, Bellati G, Leandro G, et al. Fluctuations in viremia, aminotransferases and IgM antibody to hepatitis B core antigen in chronic hepatitis B patients with disease exacerbations[J]. Liver, 1994, 14(4): 175-181.
- CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
- Robert W. Schroff, Kenneth A. Foon, Shannon M. Beatty, et al. Human Anti-Murine Immunoglobulin Responses in Patients Receiving Monoclonal Antibody Therapy [J]. Cancer Research, 1985, 45(2):879-885.
- 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.
- Boscato L M, Stuart M C. Heterophilic antibodies: a problem for all immunoassays [J]. Clinical Chemistry, 1988,34(1):27-33.

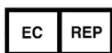
SYMBOLS EXPLANATIONS

	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		Authorized representative in the European Community
	In vitro diagnostic medical device		Kit component
	Catalogue number		Batch code
	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.



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



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