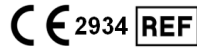


MAGLUMI® Anti-HBs (CLIA)



130210010M: 100 tests/kit
130610010M: 50 tests/kit
130710010M: 30 tests/kit

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Hepatitis B surface antibody (Anti-HBs) 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 HBV infection.

SUMMARY

Hepatitis B virus (HBV) infection is the most common chronic viral infection in the world. Globally, in 2015, an estimated 257 million people were living with chronic HBV infection. The global prevalence of HBV infection in the general population was 3.5%. The transmission route of HBV is primarily through blood and bodily fluids and includes perinatal and early infant transmission (mother to child transmission) as well as sexual and parenteral modes.

HBV infection leads to a wide spectrum of liver disease ranging from acute (including fulminant hepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Acute HBV infection can be either asymptomatic or present with symptomatic acute hepatitis. Most adults infected with the virus recover, but 5%-10% are unable to clear the virus and become chronically infected. Persistent HBV infection has a wide spectrum of clinical manifestations, including inactive carrier state, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).¹⁻⁶

Hepatitis B virus is a double-stranded DNA virus of the *hepadnaviridae* family. The virus is enveloped, and contains a viral DNA genome of about 3200 bps within its core.^{4,7} Hepatitis B virus is a worldwide leading cause of acute and chronic liver disease including cirrhosis and hepatocellular carcinoma. Effective vaccines had been available since the early '80s and vaccination has proved highly successful in reducing the disease burden, the development of the carrier state and the HB-related morbidity and mortality in the countries where vaccination has been implemented.^{8,9} The presence of anti-HBs represents immunity to HBV infection. It is the only HBV marker detected in people who have acquired immunity through vaccination, and is present in association with anti-HBc IgG in those who have recovered from previous HBV infection.^{5,10}

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, magnetic microbeads coated with recombinant HBsAg, ABEI labeled with another recombinant HBsAg and buffer are mixed thoroughly and incubated, reacting to form sandwich 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-HBs present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with recombinant HBsAg (~4.00 µg/mL) in PBS buffer, NaNa ₃ (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of polyclonal Anti-HBs in PBS buffer, NaNa ₃ (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Calibrator High	A high concentration of polyclonal Anti-HBs in PBS buffer, NaNa ₃ (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Buffer	PBS buffer, NaNa ₃ (<0.1%).	6.5 mL	4.5 mL	2.7 mL
ABEI Label	ABEI labeled with recombinant HBsAg (~0.50 µg/mL) in PBS buffer, NaNa ₃ (<0.1%).	6.5 mL	4.5 mL	3.0 mL
Diluent	Containing bovine serum, NaNa ₃ (<0.1%).	25.0 mL	15.0 mL	10.0 mL
Negative Control	PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control 1	A low concentration of polyclonal Anti-HBs (30.0 mIU/mL) in PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control 2	A high concentration of polyclonal Anti-HBs (140 mIU/mL) in PBS buffer, NaNa 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 handling 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
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Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	24 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

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 and Sodium Citrate (1:9)

- 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 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 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 40 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 24 hours at 10-30°C, or 14 days at 2-8°C, or 6 months frozen at -20°C or colder. Frozen specimens subjected to up to 6 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 with Anti-HBs concentrations above the analytical measuring interval, can be diluted with Diluent either by following automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:100. The concentration of the diluted sample must be >10 mIU/mL.
- 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

Anti-HBs (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 WHO NIBSC standard (Code number: 07/164; WHO 2nd International Standard for anti-hepatitis B surface antigen (anti-HBs) immunoglobulin).

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 guidelines¹¹.

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-HBs 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 Anti-HBs (CLIA) Controls (REF: 160201124MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Anti-HBs 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 mIU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

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

- Non-reactive: A result less than 10.0 mIU/mL (<10.0 mIU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 10.0 mIU/mL is (≥10.0 mIU/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-HBs results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- 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 observed¹².
- 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 (mIU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (mIU/mL)	%CV	SD (mIU/mL)	%CV	SD (mIU/mL)	%CV
Ps1	29.838	1.332	4.46	0.867	2.91	2.060	6.90
Ps2	140.951	4.371	3.10	2.344	1.66	6.689	4.75
Ps3	794.934	19.844	2.50	8.132	1.02	39.258	4.94
Pp1	29.875	1.221	4.09	1.037	3.47	1.833	6.14
Pp2	137.602	4.526	3.29	3.744	2.72	6.97	6.41
Pp3	798.663	19.696	2.47	16.012	2.00	32.754	4.10
NQC	<0.200	/	/	/	/	/	/
PQC1	29.614	1.490	5.03	1.009	3.41	1.904	6.43
PQC2	140.932	5.735	4.07	3.722	2.64	8.573	6.08

Linear Range

0.500-1000 mIU/mL (based on a study performed with guidance from CLSI EP6-A).

Reportable Interval

0.500-100,000 mIU/mL (defined by the Limit of Detection and the maximum of the master curve*Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.200 mIU/mL.

Limit of Detection (LoD) =0.500 mIU/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferences 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	30 mg/dL	Lamivudine	30 mg/dL
Hemoglobin	500 mg/dL	Adefovir	1 mg/dL
Intralipid	2000 mg/dL	Entecavir	1 mg/dL
HAMA	40 ng/mL	Tenofovir	60 mg/mL
Rheumatoid factor	1500 IU/mL	Telbivudine	24.5 mg/dL

Cross-Reactivity

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

Condition	Number of samples tested	Number of Anti-HBs reactive
Rheumatoid Factor (RF)	5	0
Pregnant women	3	0
Pregnant women, multipara	1	0
Hyper IgM/IgG	5	0
Dialysis patients	4	0
Influenza vaccine recipients	5	0
Anti-HIV 1/2	3	0

Anti-Syphilis	1	0
Anti-HSV 1/2	4	0
Anti-VZV	3	0
Anti-EBV	2	0
Anti-CMV	3	0
Anti-HAV	5	0
Anti-HEV	4	0
Anti-HCV	4	0
Total	52	0

High-Dose Hook

No high-dose hook effect was seen for Anti-HBs concentrations up to 30,000 mIU/mL.

Clinical Sensitivity

A total of 211 samples were tested, including 105 samples from HBV vaccinated persons and 106 samples from naturally infected persons. The diagnostic sensitivity of the Anti-HBs assay was found to be 98% (206/211).

Group	Number of samples tested	Number of Anti-HBs reactive
HBV vaccinated persons	105	104
Naturally infected persons	106	102
Total	211	206

Clinical specificity

A total of 606 samples were tested, including 331 samples from blood donors, 223 samples from hospitalized patients and 52 potentially cross-reacting samples. The diagnostic specificity of the Anti-HBs assay was found to be 98% (591/606).

Group	Number of samples tested	Reactive	Non-reactive
Blood donors	331	12	319
Hospitalized patients	223	3	220
Potentially cross-reacting samples	52	0	52
Total	606	15	591

Seroconversion sensitivity

Seroconversion sensitivity of the Anti-HBs assay has been evaluated by testing 12 commercial seroconversion panels, which have been tested by commercially available CE-marked Anti-HBs assays. The Anti-HBs assay showed equivalent performance compared to the results from other commercially available assays.

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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
	<i>In vitro</i> 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.

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