Snibe Diagnostic

CE 2934 REF

130210023M:100 tests/kit
130610023M: 50 tests/kit
130710023M: 30 tests/kit

MAGLUMI® Anti-HBc (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of Anti-HBc 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 and for screening of blood donations.

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%[1]. The transmission route of HBV is primarily through blood and bodily fluids and includes perinatal and early infant transmission as well as sexual and parenteral modes[2].

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[3]. Persistent HBV infection has a wide spectrum of clinical manifestations, including inactive carrier state, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC)[4-6].

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[3,4,7]. Hepatitis B core antibody (anti-HBc) is considered the most sensitive serological marker for history of hepatitis B virus (HBV) infection[8]. Despite the fact that HBcAg is an internal component of the virion, high titers of HBc antibody (anti-HBc) are produced in virtually all patients who have been exposed to HBV and usually persist, irrespective of ongoing liver disease or clearance of the virus[9]. During the acute phase of infection, anti-HBc of the IgM class predominates. As the infection evolves, anti-HBc IgM levels gradually decline and anti-HBc IgG can persist with slowly decreasing titers for many years. For this reason, antibodies to the core of hepatitis B virus are considered to be the most reliable serological marker of HBV infection[9].

TEST PRINCIPLE

Competitive chemiluminescence immunoassay.

The sample, Buffer, ABEI labeled with monoclonal anti-HBc, FITC labeled with recombinant HBcAg and magnetic microbeads coated with anti-FITC polyclonal antibody are mixed thoroughly and incubated, forming 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 RLU which is inversely proportional to the concentration of anti-HBc present in sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-FITC polyclonal antibody (~50.0 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.5 mL
Calibrator Low	A low concentration of monoclonal Anti-HBc in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.5 mL
Calibrator High	A high concentration of monoclonal Anti-HBc in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.5 mL
FITC Label	FITC labeled with recombinant HBcAg (~1.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	6.5 mL	4.5 mL	3.5 mL
ABEI Label	ABEI labeled with monoclonal anti-HBc (~0.50 μg/mL) in PBS buffer, NaN_3 (<0.1%).	6.5 mL	4.5 mL	3.5 mL
Buffer	PBS buffer, NaN_3 (<0.1%).	6.5 mL	4.5 mL	3.5 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control	Monoclonal anti-HBc (2.00 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	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

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	Sodium Citrate, K2-EDTA, K3-EDTA, Li- heparin, 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 at ≥ 10,000×*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.

PROCEDURE

Materials Provided

Anti-HBc (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: 95/522; WHO 1st International Standard for anti-Hepatitis B core antigen (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 guidelines[10].

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 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-HBc (CLIA) Controls (REF: 160201453MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Anti-HBc 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.

Conversion factor: AU/mL×1=IU/mL

Interpretation of Results

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

- Non-reactive: A result less than 0.35 AU/mL (<0.35 AU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 0.35 AU/mL (≥0.35 AU/mL) is 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 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[11,12]. 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[13].
- 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
Ps1	2.004	0.092	4.59	0.065	3.24	0.125	6.24
Ps2	9.977	0.344	3.45	0.126	1.26	0.508	5.09
Ps3	31.487	0.947	3.01	0.202	0.64	1.194	3.79
Pp1	2.037	0.092	4.52	0.051	2.50	0.137	6.73
Pp2	9.990	0.352	3.52	0.180	1.80	0.46	4.82
Pp3	31.805	0.777	2.44	0.562	1.77	1.130	3.55
NQC	<0.100	/	/	/	/	/	/
PQC	1.985	0.057	2.87	0.042	2.12	0.086	4.33

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	Metronidazole	20 mg/dL
Hemoglobin	500 mg/dL	tetracycline	5 mg/dL
Intralipid	2000 mg/dL	Aspirin	100 mg/dL
HAMA	40 ng/mL	Rifampicin	6 mg/dL
Rheumatoid factor	1500 IU/mL	Acetaminophen	20 mg/dL
Acetylcysteine	15 mg/dL	Ibuprofen	50 mg/dL
Ampicillin Sodium	100 mg/dL	Theophylline	10 mg/dL
Ascorbic Acid	30 mg/dL	Lamivudine	30 mg/dL
Cyclosporine	0.5 mg/dL	Entecavir	0.5 mg/L
Cefoxitin	250 mg/dL	Telbivudine	60 mg/dL
Levodopa	2 mg/dL	Adefovir	1 mg/dL

Cross-Reactivity

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

Condition	Number of samples tested	Number of Anti-HBc reactive
Rheumatoid factor	5	0
c-ANCA	9	0
Pregnant women	9	0
Pregnant women, multipara	10	0
Hyper IgG/IgM	9	0
Dialysis patients	6	0
Influenza vaccine patients	9	0
Covid vaccinated patients	5	0
Anti-HIV	3	0
Anti-Syphilis	1	0

Anti-HSV1/2	5	0
Anti-VZV	4	0
Anti-EBV	5	1
Anti-CMV	4	0
Anti-HAV	5	0
Anti-HEV	5	0
Anti-HCV	5	0
HBs/HBc	2	1
Total	**101**	**2**

Clinical Sensitivity

A total of 410 samples were tested, including 10 samples from patients with an acute infection, 10 samples from patients with a chronic infection, 10 samples from patients with a recovered infection. The diagnostic sensitivity of the Anti-HBc assay was found to be 100%(410/410).

Group	Number of samples tested	Number of Anti-HBc reactive
Anti-HBc positive from acute infection	10	10
Anti-HBc positive from chronic infection	10	10
Anti-HBc positive from recovered infection	10	10
Anti-HBc positive	380	380
Total	410	410

Clinical specificity

A total of 5368 samples were tested, including 5040 samples from blood donors, 227 samples from hospitalized patients and 101 potentially cross-reacting samples. The diagnostic specificity of the Anti-HBc assay was found to be 99.7%(5353/5368).

Group	Number of samples tested	Reactive	Non-reactive
Blood donors	5040	13	5027
Hospitalized patients	227	0	227
Potentially cross-reacting samples	101	2	99
Total	5368	15	5353

Seroconversion sensitivity

Seroconversion sensitivity of the Anti-HBc assay has been evaluated by testing 3 commercial seroconversion panels, which have been tested by commercially available CE-marked Anti-HBc assays. The Anti-HBc assay showed equivalent performance compared to the results from other commercially available assays.

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 2934	CE marking with notified body ID number		

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