Snibe Diagnostic

CE 2934 REF

130210011M:100 tests/kit
130610011M: 50 tests/kit
130710011M: 30 tests/kit

MAGLUMI® HBeAg (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of hepatitis B e antigen (HBeAg) 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

It is estimated that worldwide well over 350 million individuals are chronically infected with hepatitis B virus (HBV) and these patients are at increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC)[1-4]. 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[5].

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]. HBeAg is a soluble protein derived from its precore/core precursor following proteolytic processing. It is therefore a non-structural protein, is not essential for viral replication, is used as a marker of infectivity and has tolerogenic and immune modulating activity that plays a significant role in viral persistence[1,4,6].

Presence of HBeAg for more than 10 weeks indicates a high likelihood of transition to persistent infection. People with HBeAg-positive chronic infection usually have high levels of hepatitis B virus DNA, whereas serum concentrations are lower in patients with HBeAg-negative infection[4]. Chronic hepatitis B is characterized by an early replicative phase (HBeAg positive chronic hepatitis) and a late low or non-replication phase with HBeAg seroconversion and liver disease remission (inactive carrier state). Most patients become inactive carriers after spontaneous HBeAg seroconversion with good prognosis, but progression to HBeAg negative chronic hepatitis due to HBV variants not expressing HBeAg occurs at a rate of 1–3 per 100 person years following HBeAg seroconversion. The incidence of cirrhosis appears to be about 2-fold higher in HBeAg negative compared to HBeAg positive chronic hepatitis[7]. After HBeAg seroconversion, 1–4% patients have HBeAg seropositive hepatitis again (HBeAg reversion), whereas a greater proportion of patients develop HBeAg-negative chronic hepatitis B because of reactivation of the hepatitis B virus with pre-core or core promoter mutations that abolish or down regulate HBeAg production[4].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with monoclonal anti-HBe are mixed thoroughly and incubated, performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with another monoclonal anti-HBe are then added 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 HBeAg present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with monoclonal anti-HBe (~10.0 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of recombinant HBeAg in Tris-HCl buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Calibrator High	A high concentration of recombinant HBeAg in Tris-HCl buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Buffer	Tris-HCl buffer, NaN_3 (<0.1%).	12.5 mL	7.5 mL	4.8 mL
ABEI Label	ABEI labeled with monoclonal anti-HBe (~0.167 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	12.5 mL	7.5 mL	4.8 mL
Diluent	PBS buffer, NaN_3 (<0.1%).	25.0 mL	15.0 mL	10.0 mL
Negative Control	Tris-HCl buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
Positive Control 1	A low concentration of recombinant HBeAg (0.500 IU/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
Positive Control 2	A high concentration of recombinant HBeAg (50.0 IU/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 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	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 ≥ 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 50 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 HBeAg 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 1:200. The concentration of the diluted sample must be >1 IU/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

HBeAg (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 PEI standard (Code number: 129097/12; WHO 1st International Standard for HBeAg).
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 HBeAg 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 HBeAg (CLIA) Controls (REF: 160201136MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the HBeAg 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 IU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

Results obtained with the HBeAg assay can be interpreted as follows:

- Non-reactive: A result less than 0.10 IU/mL (<0.10 IU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 0.10 IU/mL (≥0.10 IU/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 HBeAg 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[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].
- 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 (IU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV
Ps1	0.487	0.022	4.52	0.014	2.87	0.032	6.57
Ps2	50.141	1.770	3.53	0.829	1.65	3.081	6.14
Ps3	157.978	3.708	2.35	2.152	1.36	6.823	4.32
Pp1	0.505	0.021	4.16	0.007	1.39	0.028	5.54
Pp2	49.229	1.832	3.72	0.882	1.79	2.38	5.60
Pp3	158.942	4.258	2.68	2.524	1.59	5.716	3.60
NQC	<0.010	/	/	/	/	/	/
PQC1	0.503	0.020	3.98	0.015	2.98	0.032	6.36
PQC2	50.030	2.508	5.01	0.703	1.41	3.568	7.13

Linear Range

0.100-200 IU/mL based on a study performed with guidance from CLSI EP6-A.

Reportable Interval

0.100-40000 IU/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.010 IU/mL.

Limit of Detection (LoD) =0.100 IU/mL.

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 HBeAg assay. The results were summarized in

the following table:

Condition	Number of samples tested	Number of HBeAg reactive
Autoimmune	5	0
Rheumatoid factor	3	0
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	1
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**

High-Dose Hook

No high-dose hook effect was seen for HBeAg concentrations up to 5000 IU/mL.

Clinical Sensitivity

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

Group	Number of samples tested	Number of HBeAg reactive	Clinical Sensitivity
HBeAg positive patients	205	205	100%

Clinical Specificity

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

Group	Number of samples tested	Number of HBeAg Nonreactive	Clinical Specificity
Unselected donors	204	204	100%
Hospitalized patients	203	203	100%
Total	**407**	**407**	100%

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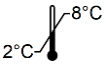
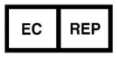
SYMBOLS EXPLANATIONS

	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
	*In vitro* diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking with notified body ID number		

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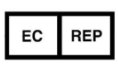

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