Snibe Diagnostic

CE 0123 REF 130210029M:100 tests/kit
130610029M: 50 tests/kit

MAGLUMI® HEV IgM (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of HEV 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 for an aid in the diagnosis of individuals with suspected or confirmed HEV infection.

SUMMARY

Hepatitis E is an important public health disease in many developing countries of Asia and Africa. Sporadic cases of hepatitis E have also been reported in many industrialized countries. A unique feature of hepatitis E is the relatively high mortality rate reported during pregnancy. Hepatitis E virus (HEV), the causative agent of hepatitis E, belongs to the family Hepeviridae[1]. The virion of HEV is a nonenveloped sphere of 27 to 34 nm diameter, and its genome is a single-stranded, positive-sense RNA molecule approximately 7.5 kb in length[2]. The HEV genome includes 2 short, noncoding regions that surround 3 open reading frames (ORFs 1–3)[3]. HEV has four reported genotypes. Genotypes 1 and 2 exclusively infect humans. Genotypes 3 and 4 infect humans, pigs, and other animal species[4]. Furthermore, Hepatitis E virus is known to exist as a single serotype. In conventional serological assays, HEV particles and HEV-specific recombinant antigens react equally well with sera from convalescent patients irrespective of geographical origin of either viruses or sera[5].

HEV infection is transmitted via the faecal-oral route. Under natural conditions, faecally contaminated drinking water seems to be the principal vehicle of HEV transmission. There are a few reports pointing to the possibility of an alternative, non-faecal-oral, route of HEV transmission such as blood transfusion, vertical and sexual[5]. In most patients, hepatitis E causes a self-limiting illness which lasts a few weeks. Following an incubation period of 2 to 6 weeks, symptoms of hepatitis develop, with fever and nausea followed by abdominal pain, vomiting, anorexia, malaise, and hepatomegaly. Jaundice occurs in about 40% of patients. Excess mortality is seen in pregnant females and individuals with underlying chronic liver disease[6].

In humans, infection with HEV induces the production of specific antibodies belonging to various isotypes, including IgM, IgG[7]. IgM anti-HEV appears during the early acute phase of illness and may be detected as early as 4 days after the onset of jaundice and lasts for up to five months. IgM anti-HEV, therefore, is a marker eminently suitable for the diagnosis of acute infection[8].

TEST PRINCIPLE

Capture chemiluminescence immunoassay.

The 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 HEV 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 HEV IgM present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-human IgM monoclonal antibody (~20.0 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL
Calibrator Low	A low concentration of HEV IgM in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL
Calibrator High	A high concentration of HEV IgM in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL
Buffer	Tris-HCl buffer, NaN_3 (<0.1%).	22.5 mL	12.0 mL
ABEI Label	ABEI labeled with HEV antigen (~0.167 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	22.5 mL	12.0 mL
Diluent	0.9% NaCl.	5.5 mL	3.5 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL
Positive Control	A high concentration of HEV IgM (4.00 AU/mL) in PBS buffer, NaN_3 (<0.1%).	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.
- The safety data sheet is available 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.
- Do not transfer opened reagents between instruments.
- 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	14 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 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, or tubes containing clot activator or clot activator with gel.
Plasma	K2-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 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.
- For reliable results, specimens should be free of fibrin, red blood cells, or other particulate matter. Specimens 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 12 hours at 10-30°C or 7 days at 2-8°C, or 6 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.

Specimen Dilution

- Samples, with HEV IgM 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 1:3(sample volume:total volume). The concentration of the diluted sample must be >33 AU/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

HEV 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.
- For Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip and Reaction Cup, please refer to corresponding Analyzer Operating Instructions for catalogue number.
- 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 registrating 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 registrating 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 registrating samples, refer to the sample 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[9].

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 HEV IgM assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

The first seven digits of the lot number on the controls must match those of the corresponding reagent kit. 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 HEV IgM (CLIA) Controls (REF: 160201215MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the HEV 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

The expected range for the HEV IgM assay was obtained by testing 206 HEV IgM positive patients and 198 HEV IgM negative people, 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

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the HEV 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[10,11]. 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[12].
- Bacterial contamination 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-A2,EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 20 days at three different sites using three lots of reagent kits (n = 720). The following results were obtained:

Sample	Mean (AU/mL) (n=720)	Within-Run		Between-Run		Reproducibility	
		SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
Serum Pool 1	0.683	NA	NA	NA	NA	NA	NA
Serum Pool 2	1.299	0.038	2.91	0.038	2.91	0.131	10.11
Serum Pool 3	5.952	0.171	2.87	0.151	2.53	0.527	8.85
Plasma Pool 1	0.707	NA	NA	NA	NA	NA	NA
Plasma Pool 2	1.320	0.048	3.62	0.039	2.99	0.115	8.73
Plasma Pool 3	5.714	0.166	2.91	0.153	2.67	0.491	8.60
Negative Control	0.081	NA	NA	NA	NA	NA	NA
Positive Control	3.862	0.114	2.95	0.113	2.93	0.430	11.14

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
Hemoglobin	2000 mg/dL	Rheumatoid factor	1500 IU/mL
Intralipid	1000 mg/dL	ANA	398 AU/mL
Bilirubin	40 mg/dL	Biotin	50 μg/mL
HAMA	40 ng/mL	Total IgG	8 g/dL
K2-EDTA	22.75 μmol/mL	Total IgM	1 g/dL
Ribavirin	2 mg/mL	Cyclosporine A	0.5 mg/mL
Mycophenolate Mofetil	1.2 mg/mL	Tacrolimus	10 ug/mL
Rapamycin	1.2 μg/mL	Interferon-alpha	20 μg/mL
Everolimus	6 mg/mL	Heparin lithium salt	80 IU/mL
Sofosbuvir	2 μg/mL	Heparin sodium salt	80 IU/mL
HEV IgG	1:32 (Titer)	Pegylated Interferon-alpha	20 μg/mL

Cross-Reactivity

The assay is highly specific for HEV IgM antibodies, with no observed cross-reactivity to Anti-HAV, Anti-HBs, HBsAg, HBeAg, Anti-HBe, Anti-HBc, Anti-HCV, HEV IgG, CMV IgG, CMV IgM, EBV VCA IgG, EBV VCA IgM, EBV EA IgG, EBV EA IgM, EBV NA IgG,Toxo IgG, Toxo IgM, Rubella IgG, Rubella IgM, HSV-1/2 IgG, HSV-1/2 IgM, Anti-*Treponema pallidum*, Anti-HIV, Anti-*E. coli*.

High-Dose Hook

No high-dose hook effect was seen for HEV IgM concentrations up to 300 AU/mL.

Relative Sensitivity

The relative sensitivity of the HEV IgM assay was determined by testing 122 samples collected from expected positive population with commercial assay confirmation of HEV IgM positive result.

N of samples	Reactive	Relative **Sensitivity**	95% CI
122	121	99.18%	95.50%-99.86%

Relative Specificity

The relative specificity of the HEV IgM assay was determined by testing 265 samples collected from expected negative population with commercial assay confirmation of HEV IgM negative result.

N of samples	Non-reactive	Relative **Specificity**	95% CI
265	263	99.25%	97.29%-99.79%

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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 0123	CE marking with notified body ID number		

Summary of safety and performance is available at Eudamed.

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