Snibe Diagnostic

CE 0123 REF 130210015M:100 tests/kit
130610015M: 50 tests/kit
130710015M: 30 tests/kit

MAGLUMI® Anti-HCV (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of Hepatitis C virus antibody (Anti-HCV) 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 diagnosis of HCV infection and for screening of blood donations.

SUMMARY

Hepatitis C virus (HCV) is a small, positive-sense, 55–65 nm single-stranded enveloped RNA virus belonging to family Flaviviridae. The genome, 9600 nucleotides long, encodes a large polypeptide of 3000 amino acids that is processed to produce smaller active proteins. HCV has a positive strand RNA genome encoding a single polyprotein that is cleaved by cellular and viral proteases into 10 different proteins: core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. The non-structural proteins NS3 to NS5B are involved in the replication of the viral genome, whereas the structural proteins (core, E1 and E2) are the components of the viral particle. The remaining proteins, p7 and NS2, are dispensable for RNA replication and there is no evidence that they are part of the viral particle[1-4].

Both core and NS proteins are used in the serological diagnosis of HCV. Based on genetic differences, HCV is divided into seven genotypes with several subtypes that exhibit inter-group divergence of nearly 30%. Subtypes are further broken down into quasi species or swarms of closely related but different viruses. Infection with one genotype does not confer immunity against others and concurrent infection with two strains is possible. Approximately 60% of infected people worldwide belong to subtypes 1a and 1b[5-7].

HCV infection is a major public health problem and a leading cause of chronic liver disease. The worldwide prevalence of HCV infection is estimated to be approximately 3%, corresponding to 170 million people. It is expected that the mortality associated with HCV infection will increase in the near future. The incubation period for HCV varies widely from 2–26 weeks. Only few HCV patients can resolve their infection. Approximately 75–85% of afflicted individuals with acute HCV infection will progress to chronic hepatitis, with 20–30% chronic carriers progressing to cirrhosis of the liver[7,8]. HCV is found in the serum of patients during acute and chronic infection. It is transmitted by direct percutaneous inoculation of blood or blood products and also by close physical contact with carriers of the virus, presumably by the passage of bodily fluids through cutaneous breaks or through oral and genital membranes. Serological tests like enzyme immunoassay (EIA), ELISA, and chemiluminescence immunoassays that detect specific antibody to HCV (anti-HCV) are used for the detection of HCV infection[7].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, the Mixed Antigens (containing FITC-labeled recombinant HCV antigen and biotinylated recombinant HCV antigen) reacting to form sandwich complexes and incubating. After adding magnetic microbeads coated with streptavidin and ABEI labeled with sheep anti-FITC polyclonal antibody are mixed thoroughly and incubating. The sandwich complex reacts with ABEI labeled with sheep anti-FITC polyclonal antibody on the one hand, and becomes bound to the magnetic microbeads via interaction of biotin and streptavidin on the other hand. 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-HCV present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with streptavidin (~8.00 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-HCV in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Calibrator High	A high concentration of Anti-HCV in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	2.0 mL
Mixed Antigens	Biotinylated recombinant HCV antigen (3.20 μg/mL) and FITC-labeled recombinant HCV antigen (1.67 μg/mL) in Tris buffer, NaN_3 (<0.1%).	7.5 mL	4.5 mL	3.5 mL
ABEI Label	ABEI labeled with sheep anti-FITC polyclonal antibody (~0.125 μg/mL) in PBS buffer, NaN_3 (<0.1%).	12.5 mL	7.5 mL	5.5 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control	A high concentration of Anti-HCV (10.0 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	6 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/accessory, or tubes containing clot activator or clot activator with gel
Plasma	Sodium citrate (1:9), K2-EDTA, K3-EDTA, Li-Heparin, Na-Heparin, 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 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 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 8 hours at 10-30°C or 7 days at 2-8°C, or 3 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.

PROCEDURE

Materials Provided

Anti-HCV (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 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 28 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 Anti-HCV 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-HCV (CLIA) Controls (REF: 160201173MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Anti-HCV 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 Anti-HCV assay was obtained by testing 367 reference individuals (194 Anti-HCV negative people and 173 Anti-HCV positive patients) in China, gave the following expected value:

- 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 Anti-HCV 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.
- The frozen and thawed heparin plasma samples may be partially coagulated, which could lead slightly higher results. So fresh sample is recommended when testing heparin plasma. If a low positive result was get when measuring the heparin plasma sample, repeated test should be conducted after centrifuged especial severely or using additional test to confirm the result.
- 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 or heat inactivation of the specimens may affect the test results.
- Due to a long time period from infection to seroconversion, negative Anti-HCV test results may occur during early infection. If acute hepatitis C infection is suspected, measuring of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) may give evidence of HCV infection.
- The intended testing population are individuals with suspected or confirmed HCV infection and blood donors.
- Results from patients taking biotin supplements or receiving high-dose biotin therapy should be interpreted with caution. Specimens that contain biotin at a concentration of 40 ng/mL demonstrate no significant effect on the assay.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision for Anti-HCV assay was determined as described in the CLSI EP5-A2. 2 controls and 3 human serum pools containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The result is summarized in the following table:

Sample	Mean(AU/mL)	Within-Run		Between-Run		Total	
	(N=80)	SD(AU/mL)	%CV	SD(AU/mL)	%CV	SD(AU/mL)	%CV
Negative serum	0.508	0.032	6.30	0.043	8.46	0.053	10.43
Low positive serum	3.009	0.100	3.32	0.150	4.99	0.181	6.02
High positive serum	100.069	2.628	2.63	3.002	3.00	3.990	3.99
Negative control	0.204	0.018	8.82	0.020	9.80	0.027	13.24
Positive control	10.040	0.295	2.94	0.304	3.03	0.424	4.22

Analytical Sensitivity

Limit of Blank (LoB) =0.300 AU/mL.

Limit of Detection (LoD) =1.200 AU/mL.

Endogenous interference

Three serum samples (one negative sample, one low positive and one high positive sample) were spiked with potential endogenous interference including hemoglobin, bilirubin, triglycerides, biotin, rheumatoid factor and HAMA. For negative samples, the test results of interference substances are negative. For the positive samples, the measurement deviation of Biotin is within ±20%, and the measurement deviation of other interference substances are within ±10%. The results of the interferences are listed in the following table:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1000 mg/dL	Biotin	40 ng/mL
Bilirubin	20 mg/dL	Rheumatoid Factor	1500 IU/mL
Intralipid	2000 mg/dL	HAMA	40 ng/mL

Drug interference

Three serum samples (one negative sample, one low positive and one high positive sample) were spiked with potential exogenous interference including phenylbutazone, aspirin, acetaminophen, ibuprofen and sodium salicylate. The results of the interferences are listed in the following table:

Interference	No interference up to	Interference	No interference up to
Phenylbutazone	200 μg/mL	Ibuprofen	500 μg/mL
Aspirin	1000 μg/mL	Sodium salicylate	500 μg/mL
Acetaminopthen	400 μg/mL	-	-

Analytical specificity

Clinical interference samples, which contain the following potential cross-reactants, were used to evaluate the cross-reactivity of Anti-HCV assay. Of all the potential cross-reactants, one sample was found to be false positive in the Anti-HCV assay. The results were summarized in the following table:

Condition	MAGLUMI® Anti-HCV (CLIA) test kit	
	Number of Anti-HCV non-reactive	Number of Anti-HCV reactive
Autoimmune diseases	10	0
Hyper IgG/IgM	2	0
Pregnant women (Multipara)	3	0
Influenza vaccine recipients	3	0
Dialysis patients	3	0
Rheumatoid Factor positive	11	0
Syphilis positive	17	0
Anti-HIV positive	17	0
HBsAg positive	16	0
Anti-EBV positive	15	0
Anti-CMV positive	15	0
Anti-VZV positive	4	1
Anti-HSV positive	15	0
Anti-HAV positive	14	0
Anti-HBs/HBc positive	17	0
Anti-HEV positive	7	0
HAMA	8	0
ANA	8	0
Total	185	1

High-Dose Hook

No high-dose hook effect was seen for Anti-HCV concentrations up to 30000 AU/mL.

Clinical Sensitivity

400 samples from HCV infected patients with different stages of disease and infected with different HCV genotypes (type1, 2, 3, 4, 5 and 6), all samples were found to be reactive with the Anti-HCV assay. The diagnostic sensitivity of the Anti-HCV assay was found to be 100%.

Group	Number of samples tested	Number of Anti-HCV reactive
Unspecified Anti-HCV positive	274	274
HCV genotypes type 1	21	21
HCV genotypes type 2	21	21
HCV genotypes type 3	21	21
HCV genotypes type 4	21	21
HCV genotypes type 5	21	21
HCV genotypes type 6	21	21

Clinical specificity

In a group of randomly selected blood donors and hospitalized patients, the diagnostic specificity of the Anti-HCV assay was found to be greater than 99.8%.

Group	N	Reactive	Non-reactive
Unselected donors	5053	10	5043
Hospitalized patients	202	0	202
Total	5255	10	5245

Seroconversion sensitivity

Seroconversion sensitivity of the Anti-HCV assay has been evaluated by testing 30 commercial seroconversion panels, which have been tested by commercially available CE-marked anti-HCV assays. The Anti-HCV assay showed equivalent performance compared to the results from other commercially available assays.

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SYMBOLS EXPLANATIONS

	Consult instructions for use	REF	Catalogue number	EC REP	Authorized representative in the European Community
2°C 8°C	Temperature limit (Store at 2-8°C)		Manufacturer	CONTENTS	Kit component
Σ	Contains sufficient for<n> tests		Use-by date	LOT	Batch code
	This way up		Keep away from sunlight	CE 0123	CE marking with notified body ID number
IVD	*In vitro* diagnostic medical device				

Summary of safety and performance is available at Eudamed.

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