Snibe Diagnostic

CE 0123 REF 130210015M: 100 tests
130610015M: 50 tests

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 or plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

SUMMARY AND EXPLANATION OF THE TEST
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[5,6,8].
HCV infection is a major public health problem and a leading cause of chronic liver disease. The world wide 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[8].

PRINCIPLE OF THE TEST
The Anti-HCV assay is a sandwich chemiluminescence immunoassay.
1st incubation: The sample (or calibrator/control, if applicable), and the Mixed Antigens (containing FITC-labeled recombinant HCV antigen and biotinylated recombinant HCV antigen) react to form a sandwich complex.
2nd incubation: After adding ABEI-labeled sheep anti-FITC polyclonal antibody and streptavidin-coated magnetic microbeads, the sandwich complex reacts with ABEI-labeled 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 (or calibrator/control, if applicable).

KIT COMPONENTS
Material Provided

Component	Contents	100 tests	50 tests
Magnetic Microbeads	Magnetic microbeads coated with streptavidin (1.0 mg/mL), PBS buffer, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	PBS buffer, containing low concentration (4.729 AU/mL) of anti-HCV and BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	PBS buffer, containing high concentration (229.932 AU/mL) of anti-HCV and BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Mixed Antigens	Biotinylated recombinant HCV antigen (3.2 µg/mL) and FITC-labeled recombinant HCV antigen (1.67 µg/mL), Tris buffer, containing NaN_3 (<0.1%).	7.5 mL	4.5 mL
ABEI Label	Sheep anti-FITC polyclonal antibody labeled with ABEI (0.125 µg/mL), PBS buffer, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Negative Control	PBS buffer, containing BSA, NaN_3 (<0.1%).	1.0 mL	1.0 mL
Positive Control	PBS buffer, containing BSA and anti-HCV (10.0 AU/mL), NaN_3 (<0.1%).	1.0 mL	1.0 mL
All reagents are provided ready-to-use.			

Internal quality control is only applicable with MAGLUMI system. For instructions for use and target value refer to ***Anti-HCV Quality Control Information***. User needs to judge results with their own standards and knowledge.

Materials Required (But Not Provided)
MAGLUMI Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101
Maglumi 600	REF: 23020018
Maglumi 800	REF: 23020003
Maglumi 2000	REF: 23020006
Maglumi 2000 Plus	REF: 23020007
Maglumi 4000 Plus	REF: 23020037
Maglumi 1000	REF: 23020009
MAGLUMI X8	REF: 010101008801
MAGLUMI X3	REF: 010101003301

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.

CALIBRATION
Traceability: This method has been standardized against the SNIBE internal reference substance.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:
- After each exchange of lots (Reagent or Starter 1+2).

- Every week and/or each time a new reagent kit is used.
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
For details about entering quality control values, refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.
To monitor system performance, quality control materials (positive control and negative control) are required. Treat all quality control samples with the same level of care as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, measurement of the quality control should be repeated. If the quality control results still do not fall within the range, do not report results 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 instruction for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Human serum or plasma samples may be used with the Anti-HCV (CLIA) assay. Serum including samples collected using standard sampling tubes or tubes containing separating gel. For plasma samples, the anticoagulants including Na-Citrate, K2-EDTA, K3-EDTA, Lithium Heparin, Sodium Heparin, ACD-B, CPD, CPDA and K-Oxalate/NaF, have been tested and may be used with this assay.
- Do not use heat-inactivated or grossly hemolyzed specimens.
- 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.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed instruction of onboard sample storage constraints.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- If testing will be delayed for more than 8 hours, remove serum or plasma from the separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored 7 days at 2-8°C.
- Specimens can be stored 3 months frozen at -20°C or colder. The sample can be frozen and thawed for only two times. Avoid repeated freezing and thawing. Frozen specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at ≥ 10,000RCF (Relative Centrifugal Force) for 15 minutes. 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.
- Before shipping specimens, it is recommended that specimens be removed from the separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen (dry ice).
- The sample volume required for a single determination is 50 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and container must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets, which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- 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 Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Store at 2-8°C. Do not freeze.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

Stability of the reagent	
unopened at 2-8°C	until the stated expiration date
opened at 2-8°C	4 weeks
On-board	4 weeks

TEST PROCEDURE

Preparation of the Reagent

- Take the reagent kit out of the box and observe the sealing film and other parts of the reagent kit to see if there is any leakage. In case of leakage, please contact your local agent immediately. And then tear off the kit 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 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

- Click **<Calibration>** or **<Batch Calibration>** button to execute calibration operation; For specific information on ordering calibrations, refer to the

Calibration Section of the Operating Instructions.
- Execute recalibration according to the calibration interval required in this manual.

Quality Control
- In order to avoid manually error in entry of QC information, the provided barcode labels of quality control in the kit can be used attached on the test tubes.
- If users do not use the provided barcode labels for positive and negative controls contained within the packaging, then quality controls should be ordered manually.
- For specific information on ordering quality controls, refer to the Quality Control Section of the Operating Instructions.

Sample Testing
- Order the samples in the Sample Area of the software and click the **<Start>** button to execute testing. For specific information on ordering patient specimens, refer to the Sample Ordering Section of the Operating Instructions.

To ensure proper test performance, strictly adhere to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually with serum from donors who have not infected HCV. The recommand dilution factor is 50 times (1:50). After manual dilution, multiply the result by the dilution factor.

High-Dose Hook

High-dose hook effect was evaluated by sequentially diluting three high positive anti-HCV samples with anti-HCV negative serum. No false negative result due to high-dose hook effect was found with the Anti-HCV assay.

LIMITATIONS

- This test is suitable only for investigating single samples, not for pooled samples or heat-inactivated specimens.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.
- 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.
- Assay results should be utilized in conjunction with other clinical and laboratory methods to assist the clinician in making individual patient diagnostic decisions.
- If the Anti-HCV results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- False positive results can be expected with any test kit. The proportion of these falsely reactive specimens is dependent upon the specificity of the test kit, specimen integrity, and on the prevalence of HCV antibodies in the population being screened.
- Heterophilic antibodies in test samples may cause interference in immunoassays.
- 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.

RESULTS

Calculation of Results

The analyzer automatically calculates the 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 operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results

Results obtained with the Anti-HCV assay can be interpreted as follows:
- Non-reactive: A result less than 1.00 AU/mL (<1.00 AU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 1.00 AU/mL (≥1.00 AU/mL) is considered to be reactive.

PERFORMANCE CHARACTERISTICS

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

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. The results of the interferences are listed in the following table:

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

Drug interference

Three serum samples (one negative sample, one low positive and one high positive sample) were spiked with potential endogenous 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
Phenylbutazone	200 µg/mL
Aspirin	1000 µg/mL
Acetaminopthen	400 µg/mL
Ibuprofen	500 µg/mL
Sodium salicylate	500 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	3	0
Syphilis positive	7	0
Anti-HIV positive	7	0
HBsAg positive	6	0
Anti-EBV positive	5	0
Anti-CMV positive	5	0
Anti-VZV positive	4	1
Anti-HSV positive	5	0
Anti-HAV positive	6	0
Anti-HBs/HBc positive	7	0
Anti-HEV positive	7	0
Total	83	1

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.

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		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code
CE 0123	CE mark		