MAGLUMI HCV IgG (CLIA)

INTENDED USE
The kit has been designed for the qualitative determination of Hepatitis C virus IgG (HCV IgG) in human serum.

The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (including Maglumi 600,Maglumi 1000,Maglumi 1000 Plus, Maglumi 2000,Maglumi 2000 Plus,Maglumi 3000 and Maglumi 4000).

SUMMARY AND EXPLANATION OF THE TEST
Hepatitis C virus (HCV) is a small (55-65 nm in size), enveloped, positive sense single-stranded RNA virus of the family Flaviviridae. Hepatitis C virus is the cause of hepatitis C in humans. Hepatitis C virus has a positive sense single-stranded RNA genome. The genome consists of a single open reading frame that is 9600 nucleotide bases long. This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins. At the 5' and 3' ends of the RNA are the UTR, which are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (IRES, internal ribosome entry site) that starts the translation of a very long protein containing about 3,000 amino acids. This large pre-protein is later cut by cellular and viral proteases into the 10 smaller proteins that allow viral replication within the host cell, or assemble into the mature viral particles.

HCV mainly spread through blood, sexual transmission, mother to child transmission, etc. The major route of the HCV infection is through blood transmission. The current HCV detection and mainly anti-HCV HCV RNA testing, testing for anti-HCV screening. HCV RNA testing confirmed as indicators of HCV infection.

PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay:
Use recombinant protein HCV-Core+NS3+NS5 to label magnetic microbeads and use Mouse anti-human IgG monoclonal antibody to label ABEI. Sample, Calibrator or Control with HCV-Core+NS3+NS5 labeled magnetic microbeads and buffer 1 are mixed thoroughly and incubated at 37°C and cycle washing for 3 times. Then add ABEI Label, buffer 2 incubate and form a sandwich, and then washing for the 2nd time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of HCV IgG present in samples.

KIT COMPONENTS
Material Supplies

<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2% (W/V), 0.2%NaNO₃, coated with recombinant protein HCV-Core+NS3+NS5.</td>
</tr>
<tr>
<td>Calibrator Low: bovine serum, 0.2%NaNO₃</td>
</tr>
<tr>
<td>Calibrator High: bovine serum, 0.2%NaNO₃</td>
</tr>
<tr>
<td>ABEI Label: Mouse anti-human IgG monoclonal antibody labeled ABEI, contains BSA, 0.2%NaNO₃</td>
</tr>
<tr>
<td>Buffer 1: TRIS-HCl buffer, Goat anti-Human IgA , Goat anti-Human IgM, 0.2%NaNO₃</td>
</tr>
<tr>
<td>Buffer 2: TRIS-HCl buffer , contains BSA, 0.2%NaNO₃</td>
</tr>
</tbody>
</table>
Reagent Vials in kit box

| Internal Quality Control: containing BSA, 0.2% NaN₃; (target value refer to Quality Control Information date sheet) | 2.0ml |

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own standards and knowledge.

**Accessories Required But Not Provided**

| MAGLUMI Reaction Module REF: 630003 |
| MAGLUMI Starter 1+2 REF: 130299004M |
| MAGLUMI Wash Concentrate REF: 130299005M |
| MAGLUMI Light Check REF: 130299006M |

Please order accessories from SNIBE or our representative.

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**Preparation of the Reagent Integral**

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!). Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the refrigerator if it is not going to be used on board during the next 12 hours.

- Keep upright for storage.
- Keep away from sunlight.

**CALIBRATION AND TRACEABILITY**

1) Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the 4th WHO International Standard 06/102

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lots (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI.
- If controls are beyond the expected range.
- The room temperature has changed more than 5°C (recommendation).

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**SPECIMEN COLLECTION AND PREPARATION**

**Sample material:** serum
Collect 5.0ml venous blood into Blood Collection Tube (Tube without anticoagulant or coagulant, Anticoagulation tube with EDTA-K₂ or EDTA-Na₂ can be used. Anticoagulation tube with heparin sodium is not recommended).

Standing at room temperature, centrifuging, separating serum part. The serum sample is stable for up to 12 hours at 2-8°C. If preserved for more than 12 hours, please packed, -20°C can be stored for 30 days.

Avoid repeated freezing and thawing, the serum sample can be only frozen and thawed two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer).

Please ask local representative of SNIBE for more details if you have any doubt.

**Vacuum Tubes**

(a) Blank tubes are recommended type for collecting samples.
(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

**Specimen Conditions**

- Do not use specimens with the following conditions:
  - (a) heat-inactivated specimens;
  - (b) Cadaver specimens or body fluids other than human serum;
  - (c) Obvious microbial contamination.

- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.

- Serum specimens should be free of fibrin, red blood cells or other particulate matter.

- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

**Preparation for Analysis**

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.

- Specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.

- 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 discussion of onboard sample storage constraints.

**Storage**

- If testing will be delayed for more than 8 hours, remove serum from the separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C.

- Specimens can be stored up to 30 days frozen at -20°C or colder.

**Shipping**

Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in
compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

- For use in IN-VITRO diagnostic procedures only.
- Package insert instructions must be carefully followed. 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.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach. A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads require mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral 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 kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUM. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUM Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit. Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin < 0.4mg/ml, haemoglobin < 10mg/ml or triglycerides < 20mg/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the HCV IgG 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 U/ml. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUM Operating Instructions.

2) Interpretation of Results

Results obtained with the MAGLUMI HCV IgG assay can be interpreted as follows:
Non-reactive: A result less than 20U/ml (< 20U/ml) is considered to be negative.
Reactive: A result greater than or equal to 20U/ml (≥20U/ml) is considered to be positive.

PERFORMANCE CHARACTERISTICS

1) Precision
Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean(U/ml)</th>
<th>SD(U/ml)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>3.03</td>
<td>0.11</td>
<td>3.72%</td>
</tr>
<tr>
<td>Level 2</td>
<td>12.74</td>
<td>4.70</td>
<td>3.83%</td>
</tr>
<tr>
<td>Level 3</td>
<td>400.11</td>
<td>16.08</td>
<td>4.02%</td>
</tr>
</tbody>
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Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

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<th>Mean(U/ml)</th>
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<tbody>
<tr>
<td>Level 1</td>
<td>3.17</td>
<td>0.13</td>
<td>4.10%</td>
</tr>
<tr>
<td>Level 2</td>
<td>126.64</td>
<td>5.48</td>
<td>4.33%</td>
</tr>
<tr>
<td>Level 3</td>
<td>410.61</td>
<td>17.74</td>
<td>4.32%</td>
</tr>
</tbody>
</table>

2) Analytical Sensitivity
The sensitivity is defined as the concentration of HCV IgG equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 2 U/ml.

3) Specificity
The specificity of the HCV IgG assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. No cross reaction with IgG or IgM antibody of HAV, HBV, HIV, Syphilis. The ELISA diagnosed RF or ANA positive, which is non HBV infected sample, this reagent's determination results show negative. When HBeAb =282.843 index/ml, the HCV IgG detects results show negative.

4) Recovery
Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90%-110%.

<table>
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<td>212.7 U/ml</td>
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REFERENCES