MAGLUMI CA 72-4 (CLIA)

INTENDED USE
The kit has been designed for the quantitative determination of Cancer Antigen 72-4 (CA 72-4) in human serum.

The method can be used for samples over the range of 2.0-500.0 U/ml.

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
Tumor-associated glycoprotein-72 (TAG-72) is a high molecular weight, mucin-like, glycoprotein complex (220-400 kDa) that was originally defined by B72.3 antibody reactivity. The Cancer Antigen 72-4 (CA 72-4) antigenic determinant is located on TAG-72. CA 72-4 is expressed in a wide range of human carcinomas but has little or no staining in lymphomas, mesotheliomas, neural tumors, sarcomas, or benign tumors. CA 72-4 is generally not found in normal adult tissue, with the exception of secretory endometrium, but was detected in total colon, esophagus, and stomach2, 3. By fine needle on breast masses of volunteer, B72 is found. Antibody was shown to react with 96% of malignant cases (26/27) and only 4% benign cases (1/23). It has also been reported that 77% of primary ovarian cancers (40/55) and 71% of metastatic ovarian cancers (22/31) stained positive with B72.3 in at least 5% of cells, while only 4% of benign ovarian tumors did (1/21). No staining was seen in normal ovarian tissue. In another study, B72.3 antibody showed reactivity in 92% of adenocarcinomas (38/39) and no reactivity in mesotheliomas (1/6).

PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay;
Use an anti-CA72-4 monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control, with ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 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 CA72-4 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 sheep anti-FITC polyclonal antibody.</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>FITC Label: anti-CA72-4 monoclonal antibody labeled FITC contains BSA, 0.2%NaNO₃</td>
</tr>
<tr>
<td>ABEI Label: anti-CA72-4 monoclonal antibody labeled ABEI contains BSA, 0.2%NaNO₃</td>
</tr>
</tbody>
</table>

All reagents are provided ready-to-use.
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 micro-beads compartment to and fro, until the color of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic micro-beads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability
- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's 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 SNIBE internal reference substance. Calibrators in the Reagent Kit are from Fitzgerald.

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).
- After every 2 weeks 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.
- If the room temperature has changed more than 5°C (recommendation)

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum
Collect 5.0ml venous blood into Blood Collection Tube. Standing at room temperature, centrifuging, separating serum part. The serum sample is stable for up to 12 hours at 2-8°C. 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).

If sediments appeared in the specimens, it should be centrifugate before analysis.

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

- If testing will be delayed for more than 8 hours, remove serum from the separator gel, cells or clot. Patient specimens with a cloudy or turbid appearance must be 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 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

IVD

• 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.

• Results of the kits are only for clinical reference. For the patient’s clinical diagnosis and treatment should be performed by the doctor. The symptoms and signs should be combined with the medication history, family and personal medical history and then take them into consideration compositely.
• It may have different results in using different manufacturers’ reagents for the same sample to detect tumor marker. Because of the methodology, specificity of the antibody and so on. To avoid the wrong medicine interpretation, in the process of monitoring tumor, the different reagent testing results should not be directly compared with each other. Suggest the laboratories give test reports to the clinical doctor indicating the reagent characteristics. When the reagent type changed in the series of monitoring, it should be has extra continuity testing and compare with the original reagent results parallelly to determine the baseline value again.
• 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, even they have passed the tests of HBs-Ag, HIV1/2-Ag, HCV-Ag and so on; 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 infectious. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agency/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 Blood borne Pathogens. Biosafety Level 2 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 requires mixing to re-suspend microbeads that have settled during shipment.
• For micro-beads 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.
• Pay attention to the silicon film still exists on the surface of the kits.
• 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 MAGLUMI. 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 MAGLUMI Operating Instructions.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>80μl</td>
<td>Sample, calibrator</td>
</tr>
<tr>
<td>+80μl</td>
<td>ABE Label</td>
</tr>
<tr>
<td>+80μl</td>
<td>FITC Label</td>
</tr>
<tr>
<td>+20μl</td>
<td>Nano magnetic microbeads</td>
</tr>
<tr>
<td>30 min</td>
<td>Incubation</td>
</tr>
<tr>
<td>400μl</td>
<td>Cycle washing</td>
</tr>
<tr>
<td>3 s</td>
<td>Measurement</td>
</tr>
</tbody>
</table>

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!

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.

2) 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.

3) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<66mg/dl, haemoglobin<2.2g/dl. Triglycerides<1500mg/dL. RF<1500U/ml.

4) High-Dose Hook
RESULTS

1) Calculation of Results

The analyzer automatically calculates the CA72-4 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 MAGLUMI Operating Instructions.

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were < 5 U/ml.
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should state its own reference range.

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>Calibrator</th>
<th>Concentration (U/ml)</th>
<th>Absolute linear correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>r=0.9904</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

6) Method comparison

A comparison of MAGLUMI CA72-4(y) with a commercially available CA72-4 test (x) using clinical samples gave the following correlations (U/ml):

Linear regression

\[ y = 0.9015x + 0.3039 \]

\[ r = 0.9905 \]

Number of samples measured: 100

The sample concentrations were between 8.23 and 35.17 U/ml.

REFERENCES

8. Hasholzner U; Significance of serum CA72-4 in the differential diagnosis between benign and malignant lung diseases; cancer magazine—2002/1.
9. Stieber P; Expression of Serum TPS and CA72-4 in Patients with Breast Cancer and Its Clinical Significance; college paper--2004/2.
11. Tokoo M; Clinical value of serum CA72-4 assay in the patients with recurrent breast carcinoma; tumor clinic--2003/6.