1. INTENDED USE
The kit has been designed for the quantitative determination of Squamous Cell Carcinoma Antigen (SCCA) in human serum. The method can be used for samples over the range of 0-100ng/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST
Squamous cell carcinoma antigen (SCC) is a group of glycoproteins with molecular weight ~45 kDa, belonging to the family of serine/cysteine protease inhibitors. The protein was originally isolated by Kato and co-workers from human squamous cell carcinoma tissue and shown to consist of at least 10 subfractions differing in isoelectric point. More recent studies have shown that SCC antigen is composed of two distinct but highly homologous gene products, SCCA1 and SCCA2 with different inhibitor specificities.

SCC antigen is a serological marker of squamous cell carcinomas of the uterine cervix, vulva, lung, head & neck, and oesophagus. In squamous cell carcinoma of the uterine cervix, pre-treatment serum SCC antigen may be used as an early stage prognostic factor and the use of pre-treatment SCC antigen have been suggested in order to select high-risk patients for adjuvant therapy. Further, for patients with elevated levels of SCC antigen before start of treatment, the profile of SCC antigen correlates with the response to radio- and chemo-therapy and measurement of SCC antigen may thus be used to monitor the effect of therapy and for early detection of recurrent disease.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay;
Use an anti-SCCA monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator, or Control, 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 SCCA present in controls or samples.

4. KIT COMPONENTS
4.1 Material supplies
<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
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</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN₃, coated with sheep anti- FITC polyclonal antibody.</td>
</tr>
<tr>
<td>Calibrator low:</td>
</tr>
<tr>
<td>Calibrator high:</td>
</tr>
<tr>
<td>ABEI Label: anti-SCCA monoclonal antibody labeled ABEI contains BSA, 0.2%NaN₃.</td>
</tr>
<tr>
<td>FITC Label: anti-SCCA monoclonal antibody labeled FITC, contains BSA, 0.2%NaN₃.</td>
</tr>
</tbody>
</table>

All reagents are provided ready-to-use.

4.2 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 reagent area and let it stand there for 30 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange Nano Magnetic Microbeads from different reagents!

4.3 Storage of the Reagents Integral
- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
13. Performance Characteristics

13.1 Accuracy

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluent, 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%.

13.2 Precision

Intra-assay coefficient of variation was evaluated on Calibrator High repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should <10%. Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should <15%.

13.3 Sensitivity

The sensitivity is defined as the concentration of SCCA equivalent to the mean RLUs of 10 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.15ng/ml.

13.4 Specificity

The specificity of the SCCA assay system was assessed by measuring the effect on the results RLU of various potentially cross reactive analytes. When milrinonin=C=1000ng/ml, the detection results of SCCA <0.5ng/ml; When doxorubicin=C=10ng/ml, the detection results of SCCA <0.5ng/ml; When fluorouracil=C=1000ng/ml, the detection results of SCCA <0.5ng/ml.

13.5 Linearity

Conduct a logarithmic transform to the RLU value and concentration value of 8 Calibrators. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References


3. Reiter W; Combined assays of tumor marker CEA, CA153, SCCA and CA211 for differential diagnosis of pleural effusion; cancer2002/4

4. Nisson O; Combined Assays of Serum CEA,CA153, SCCA and CA211 in Lung Cancer.Clinic medicine--2002/23

5. Glimmelius B, Diagnostic significance of multiple tumor marker detection in hydropeuritics.clinic tumor--2004/6


<table>
<thead>
<tr>
<th>Sample, calibrator or controls</th>
<th>80µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>+40µl</td>
<td>ABEI Label</td>
</tr>
<tr>
<td>+40µl</td>
<td>FITC Label</td>
</tr>
<tr>
<td>+20µl</td>
<td>Nano magnetic microbeads</td>
</tr>
<tr>
<td>15 min</td>
<td>Incubation</td>
</tr>
<tr>
<td>40µl each time</td>
<td>Cycle washing</td>
</tr>
<tr>
<td>3 sec</td>
<td>Measurement</td>
</tr>
</tbody>
</table>

10. Quality Control

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

11 Results

11.1 Calculation of Results

The analyzer automatically calculates the SCCA 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 ng/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results

Reference values: < 2.5ng/ml.

Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

12 Limitations of the procedure

12.1 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.

12.2 HAMA

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

12.3 High-Dose Hook

No high-dose hook effect was seen for SCCA concentrations up to 2,000 ng/ml.