1. INTENDED USE
The kit has been designed for the quantitative determination of Cancer Antigen 242(CA 242) in human serum. The method can be used for samples over the range of 0-200IU/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
The tumor marker Cancer Antigen 242(CA242) is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant has been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated lewis(CA199). Thus, CA242 is related, but not identical to the epitope of CA199. Serum levels of CA242 are low in healthy subjects and subjects with benign diseases, while elevated levels are commonly found in serum from patients with gastro-intestinal cancer. The CA242 marker may be used as an aid in the diagnosis and management of patients with known or suspected gastro-intestinal carcinomas. The CA242 should not be used as a substitute for any established clinical examination of malignancy, but may be used as a complement to existing clinical and laboratory methods.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay;
Use an anti-CA242 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 CA242 present in controls or samples.

4. KIT COMPONENTS
4.1 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</td>
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<tr>
<td>Calibrator high</td>
</tr>
<tr>
<td>ABEI Label: anti-CA242 monoclonal antibody labeled ABEI, contains BSA, 0.2%NaNO3</td>
</tr>
<tr>
<td>FITC Label: anti-CA242 monoclonal antibody labeled FITC, contains BSA, 0.2%NaNO3</td>
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</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.
- Keep upright for storage.
- Keep away from direct sunlight.

5. Origin of Calibrators
Calibrators in the Reagent Kit are from Biodesign. Biological root: Human Colorectal Carcinoma Phage. No HBSAg, anti-HCV, and anti-HIV is found.

6. Calibration

6.1 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.

6.2 Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new integral is used (recommendation).
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.

7. Sample Collection, Material and Storage

- Collect samples using standard procedures.
- Sample material: serum.
- Store at 2-8°C. 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- "Vacuum tubes"
  (a) Blank tubes are recommended type for collecting samples.
  (b) If plasma sample is needed, EDTA tube is confirmed has no effect on the results RLU.
  (c) Liquaemin Sodium tube is found to increase the sample RLU and cause test results deviation.
- Please ask SNIBE for advice if special additive must be used in the sample blood.

8. WARNING AND PRECAUTIONS FOR USERS

- For use in vitro diagnostic procedures only.

- Do not interchange reagents from different lots. Do not use kit reagents from different lots. Do not use kit 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 (USP 24,2000,p.2143). 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.

- 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.

9. Test Procedure

To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto Analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

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 CA242 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 IU/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results

- Reference values: < 15 IU/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 CA242 concentrations up to 4000 IU/ml.

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, repeated 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 of the assay defined as the concentration of CA242 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 0.5 IU/ml.

13.4 Specificity

The specificity of the CA242 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When Mitomycin-C=1000 ng/ml, the detection results of CA242 <2 IU/ml. When Doxorubicin =1000 ng/ml, the detection results of CA242 <2 IU/ml. When Fluorouracil=1000 ng/ml, the detection results of CA242 <2 IU/ml.

13.5 Linearity

Conduct a logarithmic transform to the RLU value and concentration value of 6 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References

5. Reiter W.; Combined Assays of Three Tumor Markers, CA125 and CA242 in Lung Cancer;modern tumor medicine–2005/2.
6. Hasholzner U.; Significance of serum CA242 in the differential diagnosis between benign and malignant lung diseases; cancer magazine–2002/1,297-305.
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