1. INTENDED USE
The kit has been designed for the quantitative determination of Cholylglycine (CG) in human serum. The method can be used for samples over the range of 0-40 μg/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
A bile salt that is usually used as the sodium salt. It is the glycine conjugate of CHOLIC ACID. It acts as a detergent to solubilize fats for absorption and is itself absorbed. It is used as a cholagogue and choleretic.

3. PRINCIPLE OF THE TEST
Competitive immunoluminometric assay;
Use purified CG antigen to label ABEI, and use an anti-CG polyclonal antibody to label FITC. Sample, Calibrator, or Control, ABEI Label, FITC Label and nano magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated for 15 minutes at 37°C, forming complexes; 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 CG present in controls or samples.

4. KIT COMPONENTS

4.1 Material supplies
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<th>Reagent Integral for 100 determinations</th>
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| Nano magnetic microbeads: TRIS buffer, 7.2%(W/V), coated with sheep anti-FITC polyclonal antibody.
| Calibrator low: 2.5ml
| Calibrator high: 2.5ml
| ABEI Label: purified CG antigen labeled ABEI, contains BSA, 0.2%Na3N.
| FITC Label: anti-CG polyclonal antibody labeled FITC, contains BSA, 0.2%Na3N.

All reagents are provided ready-to-use.

Accessories required but not provided:
- Maglumi Reaction module
- Maglumi Starter kit 1+2
- Maglumi Light check
- Maglumi Wash/System Liquid

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 Sigma.
Biological root: synthetic materials, processed by TLC purification, with a purity ≥97%. 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.
  (d) Please ask SNIBE for advice if special additive must be used in the sample blood.

8. WARNING AND PRECAUTIONS FOR USERS
- For use in IN-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- 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 (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.

| 50µl | Sample, calibrator or controls |
| +50µl | ABEI Label |
| +50µl | FITC Label |
| +20µl | Nano magnetic microbeads |
| 15 min | Incubation |
| 400µl each time | Cycle washing |
| 3 s | Measurement |

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

11.2 Interpretation of Results
- Reference values: <2.7 µg/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 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.

13. Performance Characteristics
13.1 Accuracy
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%.

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 CG 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 0.065µg/ml.

13.4 Specificity
When Progesterone=400µg/ml, the detection results of CG<40µg/ml.
When Testosterone=170µg/ml, the detection results of CG <17µg/ml.

13.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of each standard. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References