MAGLUMI FSH (CLIA)

1. INTENDED USE
The kit has been designed for the quantitative determination of follicular-stimulating hormone (FSH) in human serum. The method can be used for samples over the range of 0-400mIU/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 gonadotrophins LH and FSH glycoproteins with a molecular weight of approx. 30,000 Dalton are secreted by the basophilic cells of the pituitary gland. Their pulsatile secretion is regulated by the hypothalamic gonadotrophin releasing hormone (GnRH, LHHRH). In the female, the gonadotrophins stimulate the growth of ovarian follicles during the follicular phase. During the luteal phase, their secretion is inhibited by the influence of progesterone and estradiol. In menopausal women, there is a striking rise in FSH serum concentrations, while LH does not exceed the usual level of the mid-cycle peak. High postmenopausal LH and FSH levels are due to the absence of the progesterone/estradiol response.

The secretion of LH is regulated by a negative feedback mechanism of testosterone; the regulation of FSH is subject to the influence of inhibin. During childhood, LH and FSH levels are normally too low to be detectable. With the beginning of puberty, FSH is the first gonadotrophin to reach detectable values.

The determination of LH and FSH plays an important role in the detection of dysfunctions of the pituitary-ovarian axis, clinically manifested by amenorrhoea, oligomenorrhoea, anovulatoric cycles and menorrhagia. For differential diagnosis between hypophysial and pituitary disturbances, the GnRH and the clomiphene tests are widely used. Determination of LH serum concentrations is also used as an indicator of ovulation in in vitro fertilisation.

In men, determination of gonadotrophins is mainly used for the differentiation between primary and secondary hypogonadism. In children and juveniles, LH and FSH determinations are indicated in case of either precocious or delayed puberty.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay:
Use an anti-FSH 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 it 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 FSH 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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<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with sheep anti-FITC polyclonal antibody.</td>
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<tr>
<td>Calibrator low</td>
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<td>Calibrator high</td>
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<tr>
<td>ABEI Label: anti-FSH monoclonal antibody labeled ABEI containing BSA, 0.2%NaNO₃</td>
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<tr>
<td>FITC Label: anti-FSH monoclonal antibody labeled FITC, containing BSA, 0.2%NaNO₃</td>
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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 HPLC purification, with a purity >98%. No HSAg, 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).
- After 4 weeks 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).

8. Interfering Substances
No interference with test results is seen by concentrations of bilirubin <0.125mg/mL, haemoglobin <16g/dl or triglycerides <12.5mg/mL.

9. 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 dates.
- 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 decontaminated.
- 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.

10. Test Procedure
To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto analyzer. Each test parameter is calculated via a barcode on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11. Quality Control
- Be familiar with quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

12. Results
12.1 Calculation of Results
- The analyzer automatically calculates the FSH 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 mIU/mL. For further information please refer to the Maglumi Fully Auto Operator's Manual.
- 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 Interpretation of Results
- Reference values:
  - Men: 1.5-11.8mIU/mL
  - Women:
    - follicular phase: 3.2-15.5mIU/mL
    - ovulatory phase: 7.5-20mIU/mL
    - luteal phase: 1.3-11mIU/mL
    - postmenopause: 36-138mIU/mL
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

13. Limitations of the procedure
13.1 FSH assay values may only be interpreted in context with the clinical picture and other diagnostic procedures.

13.2 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.

13.3 High-Dose Hook
No high-dose hook effect was seen for FSH concentrations up to 3,000 mIU/mL.

14. Performance Characteristics
14.1 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%.

14.2 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 be <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 be <15%. Sensitivity
The sensitivity is defined as the concentration of FSH 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 0.5mIU/mL.

14.4 Specificity
The specificity of the FSH assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When LH=200mIU/mL, the detection results of FSH <2.0mIU/mL; When HCG =500mIU/mL, the detection results of FSH <5.0mIU/mL.

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

15. References