MAGLUMI Free Estriol (CLIA)

1. INTENDED USE
The kit has been designed for the quantitative determination of Free Estriol in human serum.

The method can be used for samples over the range of 0-80 ng/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
Most of the estriol circulating or excreted during the third trimester of pregnancy is the fetus by the adrenal glands, and transformed by the fetal liver and the joint product of fetus and placenta, originating from a precursor synthesized in placenta into estriol. On traversing the placenta, this is rapidly metabolized, primarily in the maternal liver, to conjugated forms: the estriol sulfates and glucuronides. As a result, "free" estriol, the unconjugated form, accounts for barely nine percent of the total estriol in circulation; the estriol sulfates, which are relatively long-lived, account for roughly half. Urinary estriol consists entirely of conjugated forms since only free estriol enters the maternal circulation while only the conjugated forms are excreted. Normally, as the fetus develops, estriol production increases, resulting in a nearly three-fold rise in circulating estriol levels during the final trimester, and a corresponding increase in urine levels. There is typically a surge at about the 36th week. According to the literature, free and total estriol concentrations reach approximately 15 and 250 ng/ml, at term, while the urinary output climbs to approximately 45 mg/day. After 40 weeks, estriol levels gradually subside, declining by roughly 12 percent per week.

There is considerable patient-to-patient variability: the reference range for a given gestational age may encompass estriol levels from 50 to 200 percent of the median for that age. Hence the pattern generated by serial determinations is ordinarily of greater significance than the results of isolated measurements. Persistently low or rapidly falling estriol levels suggest fetal distress. However, because estriol concentrations are subject to diurnal and episodic variation, it is common practice to refer serum measurements to a baseline, defined for the patient as either the average or the highest of her three most recent estriol results. A drop of 40 percent or more relative to this baseline is likely to be significant.

In combination with other techniques for fetal surveillance, serial determinations have been used in the management of pregnancies complicate by diabetes, hypertension, prolonged gestation and uncertain dates. These clinical applications have been recently reviewed.

3. PRINCIPLE OF THE TEST
Competitive immunoluminometric assay:

Use a purified FE3 antigen to label ABEI, and use an anti-FE3 polyclonal antibody.

Sample, Calibrator or Control, ABEI Label, Displacing Reagent and magnetic microbeads coated with antibody to coat nano magnetic microbeads. Sample, Calibrator or Control, and antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing it for 1 times. 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 FE3 present in controls or samples.

4. KIT COMPONENTS

4.1 Material supplies

<table>
<thead>
<tr>
<th>Material supplies</th>
<th>Volume (ml)</th>
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</thead>
<tbody>
<tr>
<td>Reagent integral for 100 determinations</td>
<td></td>
</tr>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO3, coated with sheep anti- FE3 polyclonal antibody.</td>
<td>2.5ml</td>
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<tr>
<td>Calibrator low</td>
<td></td>
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<tr>
<td>Calibrator high</td>
<td></td>
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<tr>
<td>Displacing reagents</td>
<td></td>
</tr>
<tr>
<td>ABEI Label: purified FE3 antigen labeled ABEI, containing BSA, 0.2%NaNO3</td>
<td>10.5ml</td>
</tr>
</tbody>
</table>

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 ≥ 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 2 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).
- Vacuum tubes
  - (a) Blank tubes are recommended type for collecting samples.
  - (b) If plasma sample is needed, EDTA tube is conformed has no effect on the results RLU.
  - (c) Liquaemin Sodium tube is found to increase the sample RLU and effect on the results RLUs.
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  - (c) Liquaemin Sodium tube is found to increase the sample RLU and effect on the results RLUs.

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.

| 15 min | Incubation |
| 40 μl each time Cycle washing |
| 3 s | Measurement |

+20 μl Nano magnetic microbeads
+10 μl Displacing reagent

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 FE3 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:
  - Pregnant females: 14-20 weeks 0.28-3.14 ng/ml 20-31 weeks 2.75-10.90 ng/ml 31-37 weeks 3.62-14.60 ng/ml 37-40 weeks 6.20-22.40 ng/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.

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.

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 Sensitivity

The sensitivity is defined as the concentration of FE3 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 0.13 ng/ml.

13.3 Specificity

The specificity of the FE3 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When TEST=17 ng/ml, the detection results of FE3 <1 ng/ml; When PROG =40 ng/ml, the detection results of FE3 <1 ng/ml; When E2 =3000 ng/ml, the detection results of FE3 <0.3 ng/ml.

13.4 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