MAGLUMI free Estriol (CLIA)

REF 130202008M

Σ 100

EC REP

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CE

FOR PROFESSIONAL USE ONLY
Store at 2-8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

en

SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in the European community
	Manufacturer
	Consult instructions for use
CONT	Contents of kit
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
8°C 2°C	Temperature limitation (store at 2-8 °C)
Σ	Sufficient for
	Keep away from sunlight
	Keep upright for storage

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 0.13-80 ng/ml.
The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600,Maglumi 1000,Maglumi 1000 Plus, Maglumi 2000,Maglumi 2000 Plus,Maglumi 3000 and Maglumi 4000).

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 urinary 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.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay:
Use a purified FE3 antigen to label ABEI, and use an anti-FE3 polyclonal antibody to coat nano magnetic microbeads. Sample, Calibrator or Control, with ABEI Label, Displacing Reagent and magnetic microbeads coated with anti-FE3 are mixed thoroughly and incubated at 37℃, forming a antibody-antigen complexes; 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 FE3 present in samples.

CONT KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN_3, coated with sheep anti-FE3 polyclonal antibody.	2.5ml
Calibrator Low : bovine serum, 0.2%NaN_3	2.5ml
Calibrator High : bovine serum, 0.2%NaN_3	2.5ml
Displacing reagents	2.0ml
ABEI Label: purified FE3 antigen labeled ABEI, containing BSA, 0.2%NaN_3.	10.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own standards and knowledge.

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Please order accessories from SNIBE or our representative.

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 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
-  Keep upright for storage.
-  Keep away from sunlight.

CALIBRATION AND TRACEABILITY

1)Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the USP Estriol Reference Material.

2) 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.

3) Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every 2 week and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI..
- If controls are beyond the expected range.
- The room temperature has changed more than 5 ℃ (recommendation).

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect 5.0ml venous blood into Blood Collection Tube. Standing at room temperature，centrifuging, separating serum part.

The serum sample is stable for up to 12 hours at 2-8℃. More than 12 hours, please packed, -20 ℃ can be stored for 30 days.

Avoid repeated freezing and thawing, the serum sample can be only frozen and thawed two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer).

Please ask local representative of SNIBE for more details if you have any doubt.

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.

(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum from the serum separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C.

- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

IVD

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- 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.
- 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. A minimum of one hour at 121℃ is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens 13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

40μl +80μl +10μl +20μl	Sample, calibrator ABEI Label Displacing reagent Nano magnetic microbeads
15 min	Incubation
400μl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.
Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

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.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<0.06mg/ml, haemoglobin<16mg/dl or triglycerides< 12.5mg/ml.

3) 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.

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the free Estriol 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 Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

2) Interpretation of Results

- Reference values：

  Pregnant females:

14-20weeks 0.28-3.14ng/ml
20-31weeks 2.75-10.90ng/ml
31-37weeks 3.62-14.60ng/ml
37-40weeks 6.20-22.40ng/ml

- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	2.45	0.15	6.12
Level 2	3.75	0.22	5.97
Level 3	7.89	0.46	5.88

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	2.65	0.15	5.70
Level 2	3.64	0.34	9.21
Level 3	6.94	0.62	8.87

2) Analytical Sensitivity

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

3) Specificity

The specificity of the free Estriol assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
TEST	17ng/ml	5.9%
PROG	40ng/ml	2.5%
E2	3000pg/ml	10%

4) Recovery

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%.

Expected	Mean Measuring	Recovery
21.2 ng/ml	21.5 ng/ml	101%

5) Linearity

Use free Estriol calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration ng/ml	Absolute linear correlation coefficient (r)
A	0	
B	0.5	r=0.9925
C	2.0	
D	7.0	
E	25	
F	80	

(6)Method comparison

A comparison of MAGLUMI free Estriol(y) with a commercially available free Estriol(x) using clinical samples gave the following correlations(ng/ml):

Linear regression
y=0.9218x+0.0967
r=0.993

Number of samples measured:100
The sample concentrations were between 0.01-27.03ng/ml.

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