MAGLUMI Testosterone (CLIA)

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

The test can be used for samples over the range of 0.13-17 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
In males, testosterone is mainly synthesized in the interstitial Leydig cells of the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary, the male equivalent of ICSH. Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and axillary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, prostatic hypertrophy, estrogen therapy, Klinefelter's syndrome, hypogonadotropic and hepatic cirrhosis.

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women 50–60% of the daily testosterone production arises from peripheral metabolism of androgens, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

PRINCIPLE OF THE TEST
Competitive immunoluminometric assay.

Use an anti-TEST monoclonal antibody to label ABEI, and use a purified TEST antigen to label FITC. Sample, Calibrator or Control with ABEI Label, displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37 °C, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then wash it. 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 TEST present in samples.

CONT

KIT COMPONENTS

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 anti-FITC polyclonal antibody</td>
</tr>
<tr>
<td>Calibrator Low: bovine serum, 0.2%NaNO₃</td>
</tr>
</tbody>
</table>

FOR PROFESSIONAL USE ONLY

Store at 2-8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

SYMBOLS EXPLANATIONS

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

Shenzhen New Industries Biomedical Engineering Co., Ltd
4/F, Wearmes Tech Bldg, Science & Industry Park, Nanshan, Shenzhen, 518057CHINA
Tel. + 86-755-86028224 Fax. + 86-755-26654850
Lotus Global Co., Ltd
15 Alexandra Road, London, UK
NW8 0DP
Tel. + 44-20-75868010 Fax. + 44-20-79006187

016130729-V2.3-EN
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 reagents and turn the small wheel of the magnetic stirrer 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 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°C 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 is 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 Testosterone 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 weeks 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 °C (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°C. More than 12 hours, please packed, -20 °C 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 specimens 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 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 or plasma 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**

- 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°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.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens, 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 kit 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.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>40μl</td>
<td>Sample, calibrator</td>
</tr>
<tr>
<td>+20μl</td>
<td>Displacing Reagent</td>
</tr>
<tr>
<td>+40μl</td>
<td>ABEI Label</td>
</tr>
<tr>
<td>+40μl</td>
<td>FITC Label</td>
</tr>
<tr>
<td>+20μl</td>
<td>Nano magnetic microbeads</td>
</tr>
<tr>
<td>15 min</td>
<td>Incubation</td>
</tr>
<tr>
<td>400μl</td>
<td>Cycle washing</td>
</tr>
<tr>
<td>3 s</td>
<td>Measurement</td>
</tr>
</tbody>
</table>

**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 carefully. This 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<30mg/dl, haemoglobin<1800mg/dl, triglycerides<2000mg/dl.

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 occasionly influence results.

**RESULTS**

1) Calculation of Results

- The analyzer automatically calculates the Testosterone 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.
  - Conversion factor: 1ng/ml = 3.47nmol/L.

2) Interpretation of Results

- Normal range:
  - Male 2.2-10.5ng/ml
  - Female<1.0ng/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.

<table>
<thead>
<tr>
<th>Level</th>
<th>Control Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>2.15</td>
<td>0.15</td>
<td>8.11</td>
</tr>
<tr>
<td>Level 2</td>
<td>1.92</td>
<td>0.16</td>
<td>8.33</td>
</tr>
<tr>
<td>Level 3</td>
<td>2.25</td>
<td>0.73</td>
<td>8.84</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
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<th>Control Mean (ng/ml)</th>
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<td>0.73</td>
<td>8.84</td>
</tr>
</tbody>
</table>

2) Analytical Sensitivity
The sensitivity is defined as the concentration of Testosterone 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 Testosterone assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROG</td>
<td>100 ng/ml</td>
<td>2.5%</td>
</tr>
<tr>
<td>E2</td>
<td>3000pg/ml</td>
<td>4.3%</td>
</tr>
<tr>
<td>Cortisol</td>
<td>600ng/ml</td>
<td>0.33%</td>
</tr>
</tbody>
</table>

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%.

<table>
<thead>
<tr>
<th>Expected</th>
<th>Mean Measuring</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 ng/ml</td>
<td>5.6 ng/ml</td>
<td>102%</td>
</tr>
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</table>

5) Linearity
Use Testosterone 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.

<table>
<thead>
<tr>
<th>Calibrator Point</th>
<th>Concentration ng/ml</th>
<th>Absolute linear correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
<td>r=0.9952</td>
</tr>
<tr>
<td>C</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>17.0</td>
<td></td>
</tr>
</tbody>
</table>

(6) Method comparison
A comparison of MAGLUIMI Testosterone(y) with a commercially available Testosterone(x) using clinical samples gave the following correlations (ng/ml):

Linear regression
\[ y=1.1222x+0.18 \]

REFERENCES