



MAGLUMI® Testosterone (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Testosterone (TEST) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis and treatment of individuals with suspected or confirmed disorders of sex hormones.

SUMMARY

Testosterone, the most abundant androgen in men, is a steroid hormone that is synthesized predominantly by the Leydig cells of the testes, while in women, minor amounts are synthesized in the ovaries and placenta^{1,2}. Androgen precursors are also produced and secreted from the adrenal glands in both sexes, where they undergo peripheral conversion to testosterone. Circulating concentrations are approximately 15-25 times higher in adult men compared to women¹. Testosterone circulates in blood bound to three proteins. In both sexes the greater proportion is bound to sex hormone binding globulin (SHBG); about 60% in non-pregnant women and 80% in men. Most of the remaining testosterone is bound to albumin with a very small amount bound to cortisol-binding globulin^{1,3}. Hypogonadism is a clinical syndrome characterized by low and/or below normal testosterone concentration, low sperm count, and clinical signs and symptoms of hypogonadism. As testosterone decreases with age, the incidence of hypogonadism increases⁴. Patients with primary hypogonadism have low testosterone. Patients with secondary hypogonadism have low testosterone⁵. Testosterone assays play an important role in the clinical evaluation of a number of very common endocrine disorders. In males, testosterone assays are used primarily to confirm the diagnosis of hypogonadism, and also to evaluate boys with delayed or precocious puberty and monitor the adequacy of testosterone therapy. Because the clinical manifestations of androgen deficiency are nonspecific, the presence of low serum testosterone levels in men with symptoms and findings consistent with androgen deficiency confirms the diagnosis of hypogonadism. In females, testosterone assays are used in the evaluation of hyperandrogenism (e.g. idiopathic hirsutism, congenital adrenal hyperplasia, polycystic ovarian syndrome, and androgen-secreting ovarian or adrenal tumors), and more recently, to diagnose androgen deficiency⁶.

TEST PRINCIPLE

Competitive chemiluminescence immunoassay.

The sample, buffer, ABEI labeled with anti-TEST antibody are mixed thoroughly and incubated, then magnetic microbeads coated with TEST antigen and buffer are added and incubated. TEST present in the sample compete with TEST antigen immobilized on the magnetic microbeads for binding anti-TEST antibody labeled with ABEI, and form immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of TEST present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with TEST antigen (~14.5 µg/mL) in PBS buffer, NaN ₃ (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of TEST antigen in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of TEST antigen in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	PBS buffer, NaN ₃ (<0.1%).	7.5 mL	4.5 mL	3.3 mL
ABEI Label	ABEI labeled with anti-TEST antibody (~50.0 ng/mL) in Tris-HCl buffer, NaN ₃ (<0.1%).	7.5 mL	4.5 mL	3.3 mL
Control 1	A low concentration of TEST antigen (2.00 ng/mL) in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of TEST antigen (6.00 ng/mL) in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL

All reagents are provided ready-to-use.

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skilful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks



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130252010M: 100 tests/kit
130652010M: 50 tests/kit
130752010M: 30 tests/kit

On-board	4 weeks
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Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 20 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 10-30°C, or 7 days at 2-8°C, or 3 months frozen at -20°C. Frozen specimens subjected to up to 2 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, TEST concentrations above the analytical measuring interval, can be diluted with manual dilution procedure. The recommended dilution ratio is 1:5 (sample volume:total volume). The concentration of the diluted sample must be >340 ng/mL.
- For manual dilution, multiply the result by the dilution factor.
- Please choose applicable diluents or ask Snibe for advice before manual dilution.

PROCEDURE

Materials Provided

TEST (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the USP reference standard (Catalog number: 1646009).

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines*.

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the TEST assay:

- Whenever the kit is calibrated
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Testosterone (CLIA) Controls (REF: 160201255MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

- The analyzer automatically calculates the TEST 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 Analyzer Operating Instructions.

- Conversion factors:
ng/mL x 3.47 = nmol/L
nmol/L x 0.288 = ng/mL

Interpretation of Results

The expected range for the TEST assay was obtained by testing 570 apparently healthy individuals in China, gave the following expected value:

Test subjects	N	Mean (ng/mL)	2.5 th percentile (ng/mL)	97.5 th percentile (ng/mL)
Males	285	4.797	1.71	7.89

Test subjects	N	Mean (ng/mL)	95 th percentile (ng/mL)
Females	285	0.472	0.92

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the TEST results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed*.
- Bacterial contamination of the specimens may affect the test results.
- A strong interaction with 19-nortestosterone (Nandrolone) was found. Do not use samples from patients receiving Nandrolone treatment.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (ng/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Serum Pool 1	1.483	0.057	3.84	0.043	2.90	0.093	6.27
Serum Pool 2	7.765	0.283	3.64	0.156	2.01	0.414	5.33
Serum Pool 3	14.912	0.444	2.98	0.248	1.66	0.728	4.88
Plasma Pool 1	1.470	0.050	3.40	0.038	2.59	0.073	4.97
Plasma Pool 2	8.155	0.298	3.65	0.174	2.13	0.384	4.71
Plasma Pool 3	14.816	0.442	2.98	0.331	2.23	0.657	4.43
Control 1	1.987	0.077	3.88	0.057	2.87	0.116	5.84
Control 2	6.108	0.218	3.57	0.081	1.33	0.335	5.48

Based on internal testing on the MAGLUMI and Biolumi systems, the overall repeatability (% CV) is estimated to be ≤ 5% for samples tested, the overall reproducibility (%CV) is estimated to be ≤ 10%. Performance of the assay at individual laboratories may vary.

Trueness

Trueness (bias) was determined using the assay and samples in a protocol (EP15-A3) of the CLSI (Clinical and Laboratory Standards Institute). The observed mean is within the verification interval.

Linear Range

0.250-17.0 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.100-85.0 ng/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.010 ng/mL.
Limit of Detection (LoD) =0.100 ng/mL.
Limit of Quantitation (LoQ) =0.250 ng/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	10 mg/dL	Rheumatoid factor	1500 IU/mL
Hemoglobin	1000 mg/dL	ANA	398 AU/mL
Intralipid	1800 mg/dL	Biotin	0.5 mg/dL
Testosterone Glucuronide	100 ng/mL	Estrone-glucuronic acid	100 ng/mL
Testosterone-sulfate	100 ng/mL	Estrone sulfate	100 ng/mL
5α-dihydro-testosterone	100 ng/mL	11-Deoxycortisol	100 ng/mL
5α-androstane-3β, 17β-diol	100 ng/mL	17-α-hydroxyprogesterone	100 ng/mL
Androstenediol	100 ng/mL	19-hydroxytestosterone	100 ng/mL
Androstenedione	100 ng/mL	2-hydroxyestradiol	100 ng/mL
Dehydroepiandrosterone	1000 ng/mL	Ethinylestradiol	1000 ng/mL
Dehydroepiandrosterone sulfate	1000 ng/mL	Mestranol	1000 ng/mL
Ketosteroid	100 ng/mL	Norethindrone	1000 ng/mL
Corticosterone	1000 ng/mL	Norgestrel	1000 ng/mL
Cortisol	1000 ng/mL	Danazol	100 ng/mL
Estradiol	100 ng/mL	Adanon	100 ng/mL
Estradiol sulfate	100 ng/mL	Dexamethasone	1000 ng/mL
Estrinol	100 ng/mL	Aethisteron	100 ng/mL
Oestrone	100 ng/mL	Progesterone	100 ng/mL

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
19-norethindrone acetate	100 ng/mL	11-Ketotestosterone	100 ng/mL
11β-hydroxy testosterone	100 ng/mL	Methyltestosterone	100 ng/mL

Method Comparison

A comparison of the TEST assay with a commercially available immunoassay, gave the following correlations (ng/mL):

Number of samples measured: 113

Passing-Bablok: y=1.0045x+0.0103, r=0.974.

The clinical specimen concentrations were between 0.23 and 16.74 ng/mL.

REFERENCES

1. Shea J L, Wong P Y, Chen Y. Free testosterone: clinical utility and important analytical aspects of measurement[M]//Advances in clinical chemistry. Elsevier, 2014, 63: 59-84.
2. Eisenegger C, Haushofer J, Fehr E. The role of testosterone in social interaction[J]. Trends in cognitive sciences, 2011, 15(6): 263-271.
3. Wheeler M J. The determination of bio-available testosterone[J]. Annals of clinical biochemistry, 1995, 32(4): 345-357.
4. Karakas S E, Surampudi P. New biomarkers to evaluate hyperandrogenemic women and hypogonadal men[M]//Advances in clinical chemistry. Elsevier, 2018, 86: 71-125.
5. Basaria S. Male hypogonadism[J]. The Lancet, 2014, 383(9924): 1250-1263.
6. Matsumoto A M, Bremner W J. Serum testosterone assays—accuracy matters[J]. The Journal of Clinical Endocrinology & Metabolism, 2004, 89(2): 520-524.
7. CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
8. Boscaro L M, Stuart M C. Heterophilic antibodies: a problem for all immunoassays [J]. Clinical Chemistry, 1988, 34(1):27-33.

SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	Contains sufficient for<n> tests		Keep away from sunlight
	This way up		Authorized representative in the European Community
	<i>In vitro</i> diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking with notified body ID number		

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