en

Immunoassay

REF **CMF0401/CMF0402/CMF0403/CMF0404/CMF0405**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Testosterone CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Testosterone concentration in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact the local dealers for all product related questions in your local language

Introduction

Testosterone is a steroid hormone from the androgen group. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair.[1]

Generally, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone. In females, it is needed for hormonal balance and to help women's bodies to function normally, if a woman's body is producing too much testosterone, she may have more body hair than average, have abnormal or no menstrual periods, or be infertile. There are many factors that can affect the testosterone levels for an individual.

Testosterone testing is used to diagnose several conditions in men, women, and boys. The conditions include testicular tumors in men; hypothalamus or pituitary disorders; hirsutism and virilization in girls and women. A number of methods for detecting testosterone use by athletes have been employed, most based on a urine test. In some testing programs, an individual's own historical results may serve as a reference interval for interpretation of a suspicious finding.[2] Another approach being investigated is the detection of the administered form of testosterone.[3,4]

Measurement Principle

This assay is based upon the one-step competitive method. The sample, goat polyclonal anti-mouse IgG antibodies coated microparticles, Antibody Solution and enzyme labeled testosterone are combined. During the incubation, enzyme labeled testosterone and testosterone present in the sample compete for binding to the antibodies in Antibody Solution, then the reaction mixture bind to the anti-mouse IgG antibodies coated on microparticles. After washing, a complex is generated between the solid phase, antibodies in Antibody Solution, testosterone in the sample and enzyme-linked testosterone by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the amount of testosterone in the sample.

Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F with corresponding approximate testosterone concentrations. The matrix is PBS (phosphate buffered saline) buffer containing hormone-free human serum. Contains a selection of preservatives..

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2
Antibody Solution	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Goat polyclonal anti-mouse IgG antibodies coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives..

- Enzyme Conjugate

Horseradish-peroxidase labeled testosterone in MES buffer containing BSA. Contains a selection of preservatives.

- Antibody Solution

Mouse monoclonal anti-testosterone in Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand (analyte) in the Assay Analyzer Testosterone Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.

13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators to 2-8 ℃, under which conditions the stability will be retained for 2 months, for longer use, store opened calibrators in aliquots and freeze at -20 ℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 50 μL of samples for each test. But considering the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Antibody Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of testosterone in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual

Measurement Results

The sample test results are determined automatically by the system software. The amount of testosterone in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored results.

The default unit for this assay is ng/mL.
Conversion formula: 1ng/mL × 3.47 = 1nmol/L

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. 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. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 0.1- 15 ng/mL.

Biological Reference Interval

The suggested normal range (central 95% interval) was obtained by testing serum samples from 200 apparently healthy males and 200 apparently healthy, non-pregnant females. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

	N	Mean Value (ng/mL)	Reference Interval (ng/mL)
Males	200	4.77	2.3-8.58
Females	200	0.46	＜0.1-0.9

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	1.48	5.28	7.65
2	80	6.10	4.61	6.93
3	80	10.16	3.76	5.57

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank=0.1ng/mL
Limit of Detection=0.25ng/mL
Limit of Quantitation= 0.4ng/mL with a coefficient of variation of ≤ 20%.

3. Analytical Specificity

Cross reaction: this assay is designed to have an analytical specificity of less than 100 ng/mL cross reactivity with the substances listed below, at the concentration levels listed, in hormone-free human serum and the following cross-reactivities (%) were found:

Substances	Concentration (ng/mL)	Cross reactivity %
Cortisol	100000	≤0.025%
Danazol	1000	≤0.025%
Dehydroepiandrosterone	1000	≤0.2%
Estradiol	50000	≤0.2%
Progesterone	1000	≤0.2%
17α-Hydrodypregnenolone	10000	≤0.2%
Prednisone	10000	≤0.2%
Dexamethasone	10000	≤0.2%
21-Hydroxyprogesterone	10000	≤0.2%
Corticosterone	10000	≤0.2%
17α-Hydroxyprogesterone	10000	≤0.2%
Cortisone	10000	≤0.2%
Estrone	50000	≤0.2%
Dehydroepiandrosterone-S	100000	≤0.2%
Norgestrel	1000	≤0.2%
Androstenedione	100	≤4%
Mesterolone	100	≤4%
5α－Dihydrotestosterone	100	≤4%

Interference: this assay is designed to have no interference with the substances listed below, at the concentration levels listed, in serum samples.

Interferent	Concentration
Bilirubin	20 mg/dL
Hemoglobin	3000 mg/dL
Triglyceride	3000 mg/dL

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a testosterone assay which was already CE marked. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	93	0.1311	1.0119	0.9893

Literature References

1. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. *Endocr. Rev.* 1987;8(1):1-28.

2. Strahm E, Emery C, Saugy M, Dvorak J, Saudan C. Detection of testos-

terone administration based on the carbon isotope ratio profiling of endogenous steroids: international reference populations of professional soccer players. *Br J Sports Med*. 2009;43(13):1041-1044.

3. Kicman AT, Cowan DA. Subject-based profiling for the detection of testosterone administration in sport. *Drug Test Anal*. 2009;1(1):22-24.

4. Pozo OJ, Deventer K, Van Eenoo P, Rubens R, Delbeke FT. Quantification of testosterone undecanoate in human hair by liquid chromatography-tandem mass spectrometry. *Biomed. Chromatogr.* 2009;23(8): 873-880.