Access
Immunoassay Systems

TESTOSTERONE

REF 33560

Intended Use

The Access Testosterone assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total testosterone levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Testosterone in males is secreted by adult Leydig cells and is controlled principally by lutenizing hormone (LH). The majority of serum testosterone is bound to sex hormone binding globulin (SHBG), but it also exists loosely bound to albumin and in the free state. An abnormally low total testosterone level in males can be indicative of hypogonadism, hypopituitarism, hyperprolactinemia, renal failure, hepatic cirrhosis, or Kleinfelter's syndrome. High total testosterone values in males can be caused by adrenal and testicular tumors, congenital adrenal hyperplasia or abnormalities of the hypothalamic-pituitary-testicular axis.[1]

In females, testosterone is produced in the ovaries, adrenal gland, and peripheral fatty tissues and has a serum concentration that is approximately 10-fold less than in males. As with males, the majority of serum testosterone in females is bound to SHBG and albumin with a small amount in the free state.[2] Increased female total testosterone levels may indicate polycystic ovary syndrome (PCOS), stromal hyperthecosis, ovarian and adrenal tumors, congenital adrenal hyperplasia and other disorders of the hypothalamic-pituitary-ovarian axis.[3]

Principles of the Procedure

The Access Testosterone assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel along with Sample Treatment Solution, mouse monoclonal anti-testosterone antibody, testosterone alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse polyclonal antibody. Testosterone in the sample is released from the carrier proteins by the Sample Treatment Solution and competes with the testosterone alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-testosterone monoclonal antibody. The resulting antigen-antibody complexes are then bound to the solid phase by the capture antibody. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of testosterone in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access Testosterone Reagent Pack

Cat. No. 33560: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-mouse IgG; testosterone alkaline phosphatase conjugate with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	Sample Treatment Solution, < 0.1% sodium azide.
R1c:	Monoclonal anti-testosterone (mouse), protein (BSA, mouse, goat), < 0.1% sodium azide, 0.1% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[4]
- Xi. Irritant: 0.1% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin) are the recommended samples. EDTA plasma is not recommended.
2. Observe the following recommendations for handling, processing, and storing blood samples:[5]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Samples may be thawed and frozen up to two times.

Materials Provided

R1 Access Testosterone Reagent Packs

Materials Required But Not Provided

1. Access Testosterone Calibrators
   Provided at zero and approximately 0.5, 1.5, 4.0, 8.0 and 16.0 ng/mL (1.7, 5.2, 13.9, 27.8 and 55.5 nmol/L).
   Cat. No. 33565
2. Quality Control (QC) materials: commercial control material
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use twenty (20) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 3.47.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access Testosterone assay, calibration is required every 14 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[6] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.1–16 ng/mL [0.35–55.5 nmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the result as less than that value (i.e., < 0.1 ng/mL [< 0.35 nmol/L]).
   - If a sample contains more than the stated value of the highest Access Testosterone Calibrator (S5), report the result as greater than that value (i.e., > 16 ng/mL [> 55.5 nmol/L]). Alternatively, dilute one volume of sample with one volume of Access Testosterone Calibrator S0 (zero). Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[7,8]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Testosterone results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Testosterone was measured in human serum and heparinized plasma samples from apparently healthy male and female subjects using the Access Testosterone assay. The observed ranges of testosterone concentrations are shown below for each population represented.
3. **Serum and plasma values should not be used interchangeably.** Refer to the appropriate section of the table below.
4. **EDTA plasma has been shown to give erroneous results. Do not use EDTA plasma.**

Sample Type	Reference Group	n	Median Age (years)	Age Range	Median Concentration (ng/mL)	95% Reference Interval (ng/mL)
Serum	Males	240	41	18–66	3.84	1.75–7.81
	Females	240	43	21–73	0.29	< 0.1–0.75

Sample Type	Reference Group	n	Median Age (years)	Age Range	Median Concentration (ng/mL)	95% Reference Interval (ng/mL)
Plasma (heparinized)	Males	240	41	18–66	3.84	1.68–7.58
	Females	240	43	21–73	0.41	< 0.1–0.90

Specific Performance Characteristics

Methods Comparison

A comparison of 343 serum testosterone values using the Access Testosterone assay on the Access Immunoassay system and a commercially available radioimmunoassay kit gave the following statistical data using Deming calculations:

n	Range of Observations (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
343	0.11–14.81	0.18	0.95	0.98

Linearity

Based on CLSI EP6-A,[9] one high sample (≥ 16 ng/mL) and one low sample (≤ 0.1 ng/mL) were mixed to make seven evenly distributed sample concentrations. Four replicates of the seven mixed samples, 8 replicates of the low sample, and 2 replicates of the high sample were run on a single Access 2 and a single DxI 800 system. Using weighted cubic regression, the Access Testosterone assay was linear, with a maximum deviation from linearity of 9.1% for samples > 1.4 ng/mL. Samples ≤ 1.4 ng/mL demonstrated a maximum deviation from linearity of 0.15 ng/mL.

Imprecision

This assay exhibits total imprecision of ≤ 20% at 0.5 ng/mL and < 10% from 2–10 ng/mL of testosterone. One study, using frozen human serum samples, generating a total of 20 assays, 2 replicates per assay, over 10 days provided the following data, analyzed via analysis of variance (ANOVA).[10,11]

Sample	Grand Mean (n=40) (ng/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
1	0.35	3.93	7.08	8.10
2	0.83	2.89	4.51	5.36
3	2.25	1.67	4.78	5.07
4	5.38	1.99	4.22	4.67
5	8.31	2.14	4.91	5.36
6†	12.88	2.71	5.65	6.26

†Serum sample spiked with testosterone.

Analytical Specificity/Interferences

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, 1000 mg/dL (10 g/L) hemoglobin, the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides (Triolein), or between 5.5–8.5 g/dL total protein (human serum albumin) do not significantly affect the concentration of total testosterone assayed.

The following table describes the cross-reactivity of the assay with substances that are similar in structure to testosterone. Potential cross-reactors were spiked into a testosterone sample of approximately 1.5 ng/mL.

Substance	Analyte Added (ng/mL)	Cross-Reactivity (%)
Compounds Present in Human Serum		
Testosterone-glucoronide	100	0.4
Testosterone-sulfate	100	0.3
5-alpha-DHT	100	2.0
Androstanediol	100	0.4
Androstenediol	100	0.6
Androstenedione	100	0.7
DHEA	1000	0.0
DHEA-sulfate	1000	0.0
Androsterone	100	0.2
Corticosterone	1000	0.0
Cortisol	1000	0.0
Estradiol	100	0.0
Estradiol-sulfate	100	0.0
Estriol	100	0.2
Estrone	100	0.4
Estrone-glucuronide	100	0.0
Estrone-sulfate	100	0.0
Progesterone	100	0.4
11-Deoxycortisol	1000	0.0
17-alpha-Hydroxyprogesterone	100	0.1
19-Hydroxytestosterone	100	0.5
2-Hydroxyestradiol	100	0.0
Birth Control		
Ethinylestradiol	100	0.3
Mestranol	100	0.0
Norethindrone	100	0.05
Norgestrel	100	0.3
Drugs		
Danazol	100	0.3
Mesterolone	100	1.5
Dexamethasone	1000	0.0
19-Nortestosterone	100	1.6
Ethinyltestosterone	100	0.07
Structurally Related Compounds		
19-Norethisterone Acetate	100	0.02
11B-Hydroxytestosterone	100	4.1
11-Ketotestosterone	100	6.7
17-alpha-Methyltestosterone	100	0.2

Analytical Sensitivity

The lowest detectable level of testosterone distinguishable from zero (Access Testosterone Calibrator S0) with 95% confidence is 0.1 ng/mL (0.35 nmol/L). This value is determined by processing a complete six-point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is calculated from the curve at the point that is two standard deviations from the fitted zero calibrator signal.

Access
Immunoassay Systems

TESTOSTERONE CALIBRATORS

REF 33565

Intended Use

The Access Testosterone Calibrators are intended to calibrate the Access Testosterone assay for the quantitative determination of total testosterone levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access Testosterone Calibrators is traceable to USP reference material. 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 Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access Testosterone Calibrators

Cat. No. 33565: S0–S5, 2.5 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

S0:	Buffered bovine serum albumin (BSA) matrix, 0.5% ProClin** 300 and < 0.1% sodium azide.
S1, S2, S3, S4, S5:	BSA matrix with 0.5% ProClin 300 and < 0.1% sodium azide and testosterone at 0.5, 1.5, 4.0, 8.0, or 16.0 ng/mL (1.7, 5.2, 13.9, 27.8, and 55.5 nmol/L).
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[4]
- Xi. Irritant: 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access Testosterone Calibrators are provided at six levels - zero and approximately 0.5, 1.5, 4.0, 8.0 and 16.0 ng/mL. The calibrators are prepared gravimetrically from testosterone and a buffered BSA matrix. Assay calibration data are valid up to 14 days.

Calibrators run in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

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