BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Testosterone
Testosterone, Total

REF 33560

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

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.

Measurement of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

SUMMARY AND EXPLANATION

The Access Testosterone assay can be used in males as an aid in the differential diagnosis of hypogonadism, hypopituitarism and hyperprolactinemia, as well as monitoring of anti-androgen and testosterone replacement therapy.[1] In females, the assay may be used in the evaluation of hyperandrogenism, congenital adrenal hyperplasia and other disorders of the hypothalamic-pituitary-ovarian axis.[2]

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. High total testosterone values in males can be caused by exogeneous testosterone use, congenital adrenal hyperplasia or disorders of the hypothalamic-pituitary-testicular axis.[2]

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.

METHODOLOGY

Assay type: one-step, competitive

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, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate 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 analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and lithium heparin plasma are the recommended samples. EDTA plasma is not recommended.
2. Observe the following recommendations for handling, processing, and storing blood samples:[3,4]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - 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.

REAGENTS

CONTENTS

Access Testosterone Reagent Pack

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

The same reagent formulation is used on all Access Immunoassay Systems.

- All antisera are polyclonal unless otherwise indicated.

Well	Contents	Ingredients
R1a:	3.25 mL	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:	3.1 mL	Sample Treatment Solution, < 0.1% sodium azide.
R1c:	3.1 mL	Monoclonal anti-testosterone (mouse), protein (BSA, mouse, goat), < 0.1% sodium azide, 0.1% ProClin 300.

*ProClin is a trademark of LANXESS Corp.

WARNING 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.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Particle/Conjugate (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Sample Treatment Solution (Compartment R1b)	DANGER	
		
	H315	Causes skin irritation.
	H318	Causes serious eye damage.

	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Acetic Acid < 5%
Antibody (Compartment R1c)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

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).
   Ref. No. 33565
2. Quality Control (QC) materials: commercial control material.
3. Lumi-Phos PRO
   Ref. No. B96000
4. UniCel DxI Wash Buffer II
   Ref. No. A16793

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	28 days

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

CALIBRATION

CALIBRATION INFORMATION

An active calibration is required for all tests. Calibration is required every 14 days. See calibrator Instructions For Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials are essential for monitoring the system performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[5] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

TESTING PROCEDURE(S)

PROCEDURE

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. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.
3. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
4. Use 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.
5. **The system default unit of measure for sample results is ng/mL.** To change sample reporting units to ng/dL or 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. To manually convert concentrations to ng/dL, multiply ng/mL by multiplication factor 100.

LIMITATIONS

1. 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 human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[6,7] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

2. Other potential interferences in the sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, fibrin, endogenous alkaline phosphatase, exogenous alkaline phosphatase (e.g. asfotase alfa, Strensiq), and proteins capable of binding to alkaline phosphatase. Carefully evaluate results if the sample is suspected of having these types of interferences.[8,9]
3. The 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.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. 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.

REPORTING RESULTS

MEASURING INTERVAL

Approximately 0.4 - 16.0 ng/mL (1.39 - 55.5 nmol/L)

Samples can be accurately measured within the measuring interval defined as the lower Limit of Quantitation (LoQ) and the highest calibrator value.

1. If a sample contains less than the lower limit for the assay, report the result as less than that value (i.e. <0.4 ng/mL [1.39 nmol/L]).
2. If a sample contains more than the stated value of the highest calibrator, report the result as greater than that value. Alternatively, the sample may be diluted to obtain a result.
    - For manual dilutions, dilute one volume of sample with one volumes of Access Testosterone Calibrator S0. 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.

EXPECTED VALUES

1. Each laboratory should validate or establish its own reference intervals 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 in various age groups using the Access Testosterone assay. The observed ranges of testosterone concentrations are shown below for each population represented.
3. As recommended by Endocrine Society Guidelines, the diagnosis of androgen deficiency should be made only in men with consistent symptoms and signs and unequivocally low serum testosterone levels. The threshold testosterone level below which symptoms of androgen deficiency and adverse health outcomes occur and testosterone administration improves outcomes in the general population is not known. For most symptoms, the average testosterone threshold corresponded to the lower limit of the normal range for young men.[10] Test results from different manufacturers will vary. 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.
4. **Serum and plasma values should not be used interchangeably.** Refer to the appropriate section of the table below.
5. **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	0.30	< 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	3.86	1.68 - 7.58
	Females	240	43	21 - 73	0.41	< 0.1 - 0.90

Additional analysis on the data from the male reference group provided age-stratified expected values:

Sample Type	Reference Group	N	Median Age (years)	Age Range (years)	Median Concentration (ng/mL)	95% Reference Intervals (ng/mL)
Serum	Males	47	26	18 - 30	4.50	2.59 - 8.16
		116	39	31 - 44	3.86	1.98 - 6.79
		77	51	45 - 66	3.34	1.50 - 6.84

Sample Type	Reference Group	N	Median Age (years)	Age Range (years)	Median Concentration (ng/mL)	95% Reference Intervals (ng/mL)
Plasma (heparinized)	Males	47	26	18 - 30	4.44	2.44 - 8.24
		116	39	31 - 44	3.78	1.86 - 7.16
		77	51	45 - 66	3.35	1.55 - 7.21

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

METHODS COMPARISON

A study based on CLSI EP09c, 3rd Edition[11] using Weighted Deming regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (ng/mL)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R^2
108	0.48 - 14	0.95	0.93 - 0.98	0.028	-0.015 - 0.071	0.98

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[12] performed on the DxI 9000 Access Immunoassay Analyzer determined the assay demonstrated linearity across the measuring interval.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- ≤ 0.14 ng/mL (0.49 nmol/L) SD at concentrations ≤ 1.4 ng/mL (4.9 nmol/L)
- ≤ 10.0% CV at concentrations > 1.4 ng/mL (4.9 nmol/L)

A study based on CLSI EP05-A3[13] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

Concentration (ng/mL)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	88	0.71	0.03	3.6	0.02	3.4	0.02	3.4	0.04	6.0
Sample 2	88	2.0	0.05	2.6	0.05	2.6	0.05	2.3	0.09	4.4
Sample 3	88	4.8	0.09	1.9	0.08	1.7	0.14	3.0	0.19	3.9
Sample 4	88	7.1	0.13	1.8	0.09	1.2	0.29	4.0	0.33	4.6
Sample 5	88	8.6	0.18	2.1	0.25	2.9	0.40	4.7	0.51	5.9
Sample 6	88	14	0.3	2.2	0.2	1.6	1.0	7.3	1.1	7.8

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, 1,000 mg/dL (10 g/L) hemoglobin, the equivalent of 1,800 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

Substance	Analyte Added (ng/mL)	Cross-reactivity (%)
Androstenediol	100	0.6
Androstenedione	100	0.7
DHEA	1,000	0.0
DHEA-sulfate	1,000	0.0
Androsterone	100	0.2
Corticosterone	1,000	0.0
Cortisol	1,000	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	1,000	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	1,000	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

DETECTION CAPABILITY

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted on the DxI 9000 Access Immunoassay Analyzer following CLSI guideline EP17-A2.[14] The LoB study included multiple reagent lots and 3 instruments over a minimum of 3 days. The LoD and LoQ studies included multiple reagent lots and 3 instruments over a minimum of 5 days.

	ng/mL	nmol/L
Limit of Blank (LoB)	0.2	0.7
Limit of Detection (LoD)	0.4	1.39
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.4	1.39

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

Revision B

Updated ProClin trademark statement.

Revision C

Updated "Reporting Results" section.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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