BECKMAN COULTER

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ACCESS
Immunoassay Systems

Instructions For Use

Access Testosterone
Testosterone, Total

REF 33560

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

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.

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]

METHODOLOGY

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 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.

SPECIMEN

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:[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

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.

*ProClin™ is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

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.
	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.
	P261	Avoid breathing vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	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 techdocs.beckmancoulter.com

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).
   Cat. No. 33565
2. Quality Control (QC) materials: commercial control material
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1	Access Testosterone Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

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.[5] 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.

TESTING PROCEDURE(S)

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.

RESULTS INTERPRETATION

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.

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should 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 established when there are symptoms and signs consistent with androgen deficiency and/or serum testosterone levels that are below the lower limit of the 95% reference interval. Although the threshold testosterone level for androgen deficiency has not been established for the Access Testosterone assay, the cited guidelines recommend using the lower limit of the normal range established in your laboratory[6] to evaluate testosterone levels. Testosterone 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	0.29	< 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	3.86	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

PROCEDURAL NOTES

LIMITATIONS

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.

PERFORMANCE CHARACTERISTICS

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, 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
Androstenediol	100	0.6
Androstenedione	100	0.7
DHEA	1,000	0.0
DHEA-sulfate	1,000	0.0
Androsterone	100	0.2

Substance	Analyte Added (ng/mL)	Cross-reactivity (%)
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

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.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision U

IFU updated to add Dutch, Finnish, Macedonian, Traditional Chinese, and Estonian

SYMBOLS KEY

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

REFERENCES

1. Wilson, JD, Foster, DW (Eds) Williams Textbook of Endocrinology, 8th Edition, W.B. Saunders, Philadelphia, Pennsylvania 19106, 1992, page 822-832.

2. Brutis, CA, Ashwood, ER (Eds) Tietz Fundamentals of Clinical Chemistry, 4th Edition W.B. Saunders, Philadelphia, Pennsylvania 19106, 1996, page 671 - 672.

3. Cowan BD, and Seifer, DB (Eds) Clinical Reproductive Medicine, Lippincott-Raven, Philadelphia, Pennsylvania 19106, page 98-100.

4. Approved Guideline - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, GP44-A4. 2010. Clinical and Laboratory Standards Institute.

5. Cembrowski GS, Carey RN. Laboratory quality management: QC ⇌ QA. ASCP Press, Chicago, IL, 1989.

6. Bhasin S., Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM. Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology and Metabolism. June 2010, Vol. 95(6):2536-2559.

7. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.

8. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613 - 621.

9. Approved Guideline - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, EP06-A. April 2003. Clinical and Laboratory Standards Institute.

10. Approved Guideline - Evaluation of precision performance of clinical chemistry devices, EP5-A. 1999. National Committee for Clinical Laboratory Standards, 19: No. 2.

11. Krouwer, JS, Rabinowitz, R. How to improve estimates of imprecision. Clinical Chemistry 1984; 30: 290-292.

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