DXI TESTOSTERONE

☒ St. Joseph Medical Center Tacoma, WA ☐ St. Clare Hospital Lakewood, WA ☐ St. Elizabeth Hospital Enumclaw, WA
☐ St. Francis Hospital Federal Way, WA ☐ St. Anthony Hospital Gig Harbor, WA ☐ ENUM ☐ GIGL ☐ PSC

PURPOSE

To provide instruction for performing Testosterone testing on the DXI instrument.

BACKGROUND

Principle

The Testosterone reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of Testosterone concentration in human serum or plasma.

Clinical Significance

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.

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. 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.

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 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.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics

R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CG0824	DXI & Access Controls
J-F-CH0825	DXI Calibrators
R-F-CH2000	Access 2 and DXI Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma (heparin) are the preferred specimens.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed and in a vertical position at all times. It is recommended that the serum or plasma be physically separated from contact with cells within two hours of the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Serum	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months.

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. See also Related Documents: Specimen Rejection/Cancellation Protocol

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

1. R1: Access Testosterone Reagent Pack
   100 determinations, 2 packs, 50 tests/pack.

   Provided ready to use. Store upright and refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. When stored at 2 to 10°C, reagents remain stable until the expiration date stated on the label or 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.

2. Access Testosterone Calibrators
   S0–S5, 2.5 mL/vial

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

   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.

   - S0: Buffered bovine serum albumin (BSA) matrix, 0.5% ProClin 300 and < 0.1% sodium azide.
   - S1–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

3. Access Substrate
   4 x 130 mL

   Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability

Condition	Storage	Stability
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

   R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

   Refer to the appropriate system manuals and/or Help system for detailed instructions.

4. Access, Access 2, SYNCHRON LXI
   Access Wash Buffer II
   UniCel DXI;

   Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

   R3 Wash Buffer II: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin 300.

Refer to the appropriate system manuals and/or Help system for detailed instructions.

6. Access Immunoassay System and supplies

7. 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.
- 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.
- Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate

CALIBRATION

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.

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.

Calibrators run in duplicate.

QUALITY CONTROL

See Related Documents: DXI & Access Controls

PROCEDURE STEPS

1. Access Instrument
   Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. Assay 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

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.

PERFORMANCE CHARACTERISTICS

Reference Range

Male:

16-17 yrs	154 - 735 ng/dL
18-39 yrs	332 - 896
40-59 yrs	291 - 739
60 yrs +	291 - 598
Tanner Stage I	0 - 16
Tanner Stage II	2 - 124
Tanner Stage III	6 - 632
Tanner Stage IV	136 - 709
Tanner Stage V	161 - 650

Females, healthy males under the age of 16, and severely hypogonadal men should not be tested using this method, as it is not as sensitive or specific as LC/MS for very low levels of testosterone

Analytic Measurement Range (AMR)

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 10–1600 ng/dL [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. < 10 ng/dL [< 0.35 nmol/L]).
- If a sample contains more than the stated value of the highest Access Testosterone Calibrator (S5)(> 1600 ng/dL), 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.

LIMITATIONS

- 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.
- Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
- 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.

- 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 lowest detectable level of testosterone distinguishable from zero (Access Testosterone Calibrator S0) with 95% confidence is 10 ng/dL (0.35 nmol/L).
- 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 150 ng/dL.

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

PROCEDURAL NOTES

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/dL. 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/dL by multiplication factor 3.47.

REFERENCES

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