ARCHITECT
2nd Generation Testosterone

REF 2P13-28
REF 2P13-23

en

2nd Generation Testosterone
2P13
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Read Highlighted Changes: Revised November 2016.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

ARCHITECT 2nd Generation Testosterone

INTENDED USE

The ARCHITECT 2nd Generation Testosterone assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of testosterone in human serum and plasma. Measurements 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 OF THE TEST

Testosterone is regarded as the most important of the androgen steroids. In males, it is secreted by the Leydig and interstitial cells of the testes which are stimulated by luteinizing hormone (LH). Control of testosterone secretion is through a negative feedback loop to the hypothalamus where secretion of gonadotrophin-releasing hormone promotes synthesis and release of LH and follicle-stimulating hormone (FSH) from the anterior pituitary gland.

In females, testosterone is secreted by the follicular theca and interstitial cells of the ovaries and also produced by metabolism of adrenal androgens. The concentrations of testosterone are typically about 10-20 times lower for females than for males.

In the circulation, approximately 97% of testosterone is transported by proteins, most notably by binding to sex hormone-binding globulin (SHBG) with an affinity of approximately 10^9 $Lmol^{-1}$.[1] Testosterone is also weakly bound to albumin.

The ARCHITECT 2nd Generation Testosterone assay releases testosterone from binding proteins and measures total testosterone. Free testosterone can be calculated from the total testosterone, SHBG and albumin concentrations.[2] The Free Androgen Index (FAI) may also be calculated (FAI = [Total Testosterone] / [SHBG]) and provides an index of free testosterone status. This ratio correlates well with both measured and calculated values of free testosterone and helps to discriminate subjects with excessive androgen activity from normal individuals.[3-5]

The concentration of testosterone in an individual fluctuates over 24 hours.[6] The pulsatile release of LH in the night typically leads to a peak of testosterone concentration in the morning. Time of day, age, sex, puberty, pre- and post-menopause, and disease, all have an influence on testosterone concentration and should be considered in interpreting individual results.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT 2nd Generation Testosterone assay is a delayed one-step immunoassay for the quantitative determination of testosterone in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, assay specific diluent and anti-testosterone (sheep, monoclonal) coated paramagnetic microparticles are combined. The testosterone present in the sample binds to the anti-testosterone coated microparticles.
2. After incubation, testosterone acridinium-labeled conjugate is added to the reaction mixture.
3. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of testosterone in the sample and the RLUs detected by the ARCHITECT iSystem optics.

The concentration of testosterone is interpolated from a calibration curve established with calibrators of known testosterone concentration.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT 2nd Generation Testosterone 2P13

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	2P13-28	2P13-23
Σ	100	400
MICROPARTICLES	1 x 6.6 mL	4 x 6.6 mL
CONJUGATE	1 x 6.9 mL	4 x 6.9 mL
ASSAY SPECIFIC DILUENT	1 x 25.0 mL	4 x 25.0 mL
SPECIMEN DILUENT	1 x 12.2 mL	4 x 12.2 mL

MICROPARTICLES Anti-Testosterone (sheep, monoclonal) coated microparticles in BIS Tris buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solids. Preservative: ProClin 300.

CONJUGATE Testosterone acridinium-labeled conjugate in BIS Tris buffer with surfactant stabilizer. Minimum concentration: 6.5 nmol/L. Preservative: ProClin 300.

ASSAY SPECIFIC DILUENT Testosterone Assay Specific Diluent containing phosphate and glycine in citrate buffer. Preservative: ProClin 300.

SPECIMEN DILUENT Testosterone Specimen Diluent containing PBS buffer. Preservative: ProClin 300.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[7-10]

The following warnings and precautions apply to: **MICROPARTICLES**	
	
WARNING	Contains methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: **CONJUGATE** and **SPECIMEN DILUENT**	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: **ASSAY SPECIFIC DILUENT**	
	
WARNING	Contains hydrochloric acid and methylisothiazolones.
H317	May cause an allergic skin reaction.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P390	Absorb spillage to prevent material damage.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in

their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT 2nd Generation Testosterone assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

The default result unit for the ARCHITECT 2nd Generation Testosterone assay is nmol/L. Alternate result units, ng/dL or ng/mL, may be selected for reporting results by editing assay parameter "Result concentration units", to ng/dL or ng/mL. The conversion factor used by the system is 28.84 for the conversion to ng/dL, and 0.2884 for the conversion to ng/mL.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator tubes
Plasma	Sodium heparin
	Lithium heparin
	Dipotassium EDTA

- Lithium heparin plasma separator tubes (PST and PST-II) cannot be used with the ARCHITECT 2nd Generation Testosterone assay.
- Other specimen collection tube types have not been tested with this assay.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum and plasma.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥1,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	Room temperature	≤ 8 hours
	2-8°C	≤ 7 days
	-20°C or colder	- -

Remove the serum from clot or separator gel as soon as possible after complete clot formation, or plasma from red blood cells as soon as possible upon receipt. If testing will not be performed within 8 hours of draw, specimens may be stored either at 2-8°C for up to 7 days or frozen (-20°C or colder), prior to being tested.

Avoid more than 1 freeze/thaw cycle.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

2P13 ARCHITECT 2nd Generation Testosterone Reagent Kit

Materials Required but not Provided

- ARCHITECT 2nd Generation Testosterone Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 2P13-01 ARCHITECT 2nd Generation Testosterone Calibrators
- 2P13-10 ARCHITECT 2nd Generation Testosterone Controls (or other control material)
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.

- **Invert the microparticle bottle 30 times.**
- Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
- **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.

- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Select the appropriate assay protocol for control and specimen testing.
  - Default Assay Dilution Protocol

    The 1:3 dilution protocol is the default protocol for all patient samples and the medium and high controls. In this protocol, samples are diluted 1:3 with Specimen Diluent. If sample results are greater than 35 nmol/L, the instrument automatically orders a retest using the 1:4 dilution protocol. If sample results are less than 0.45 nmol/L, the instrument automatically orders a retest using the neat (undiluted) assay protocol. Laboratories may also choose to override the default for any sample with a result less than 1.0 nmol/L.
  - 1:4 Assay Dilution Protocol

    The 1:4 dilution protocol is an alternate dilution protocol on the instrument. In this protocol, samples are diluted 1:4 with Specimen Diluent. If sample results generated by the default protocol (1:3) are greater than 35 nmol/L, the instrument automatically orders a retest using this protocol. Laboratories may also choose to override the default for any sample with a result greater than 35 nmol/L.
  - Neat (Undiluted) Assay Protocol

    The neat protocol tests samples undiluted. This protocol is utilized for patient samples with results between 0.00 and 1.0 nmol/L, and the low control. Samples may be tested using this protocol initially or if the result produced is less than 0.45 nmol/L.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- For the default dilution protocol (1:3) / dilution protocol (1:4):
  - Priority: 100 µL / 88 µL for the first ARCHITECT 2nd Generation Testosterone test plus 50 µL / 38 µL for each additional ARCHITECT 2nd Generation Testosterone test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT 2nd Generation Testosterone test plus 50 µL for each additional ARCHITECT 2nd Generation Testosterone test from the same sample cup.
- For the neat (undiluted) protocol:
  - Priority: 200 µL for the first ARCHITECT 2nd Generation Testosterone test plus 150 µL for each additional ARCHITECT 2nd Generation Testosterone test from the same sample cup.
  - ≤ 3 hours on board: 200 µL for the first ARCHITECT 2nd Generation Testosterone test plus 150 µL for each additional ARCHITECT 2nd Generation Testosterone test from the same sample cup.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - The ARCHITECT 2nd Generation Testosterone Calibrators and Controls may be thawed at room temperature for 90 to 120 minutes or overnight at 2-8°C. After thawing, it is suggested to record the thaw date on the carton or the bottles as an aid in tracking to the expiration date.
  - Mix the ARCHITECT 2nd Generation Testosterone Calibrators and all Controls by gentle inversion (10 times) prior to use.
  - To obtain the recommended volume requirements for the ARCHITECT 2nd Generation Testosterone Calibrators and Controls, hold the bottles **vertically**, and dispense 10 drops of each calibrator or 10 drops of the low control, and 5 drops of the medium and high control into each respective sample cup.
  - Follow the manufacturer's instructions for preparation of commercially available control material.

  NOTE: It is very important to return the ARCHITECT 2nd Generation Testosterone Calibrators and Controls to the correct storage conditions immediately after use, as follows:
- **Return ARCHITECT 2nd Generation Testosterone Calibrators and Controls to their carton and store at 2-8°C.**
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Samples Dilution Procedures

Samples with testosterone serum or plasma value exceeding 35 nmol/L are flagged with the code " > 35" and may be diluted using the Automated Dilution Protocol.

Automated Dilution Protocol

The system performs a 1:4 dilution of the sample with the Specimen Diluent and automatically calculates the concentration of the specimen before dilution and reports the result.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  A single replicate of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0.0 - 30.0 nmol/L.
- Once an ARCHITECT 2nd Generation Testosterone calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT 2nd Generation Testosterone assay is that a single replicate of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT 2nd Generation Testosterone assay belongs to method group 6.

RESULTS

Calculation

The ARCHITECT 2nd Generation Testosterone assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measuring Interval (Reportable Range)

Measuring interval is defined as the range of values in nmol/L which meets the acceptable performance for both imprecision and bias across all available assay file dilutions. The range was 0.15 nmol/L (Limit of Quantitation - LoQ) to 64.57 nmol/L.

LIMITATIONS OF THE PROCEDURE

- Lithium heparin plasma separator tubes (PST and PST-II) cannot be used with the ARCHITECT 2nd Generation Testosterone assay.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the testosterone results are inconsistent with clinical evidence, additional testing is recommended.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT 2nd Generation Testosterone that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[11, 12]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[13]
- A strong interaction with 19-nortestosterone (Nandrolone) was found. Do not use samples from patients receiving Nandrolone treatment.

EXPECTED VALUES

The expected ranges for the ARCHITECT 2nd Generation Testosterone assay were obtained from testing a minimum of 120 samples from apparently healthy individuals in the following categories: normal males, 21-49 years of age with an intact reproductive system, and normal females 21-49 years of age. Additional samples were tested from apparently healthy males and females (≥ 50 years of age). The data are summarized in the following table.

Category		Age Range	Testosterone nmol/L (ng/dL)				
Apparently Healthy	n	(years)	Median	Min.	Max.	5th percentile	95th percentile
Males (21-49 years of age)	129	21-49	17.13 (494.03)	1.63 (47.01)	34.00 (980.56)	8.33 (240.24)	30.19 (870.68)
Males (≥50 years of age)	71	50-77	15.34 (442.41)	4.41 (127.18)	35.38 (1020.36)	7.66 (220.91)	24.82 (715.81)
Females (21-49 years of age)	129	21-49	0.86 (24.80)	0.25 (7.21)	2.75 (79.31)	0.48 (13.84)	1.85 (53.35)
Females (≥50 years of age)	52	50-82	0.82 (23.50)	0.30 (8.65)	1.28 (36.92)	0.43 (12.40)	1.24 (35.76)

Expected ranges for pediatric samples were obtained for the ARCHITECT 2nd Generation Testosterone assay from testing a minimum of 200 samples divided by males 7-18 years of age (n=120) and females 7-18 years of age (n=116). The individuals included in the study had no clinical or endocrinological signs of premature or delayed puberty or virilization. The data are summarized in the following tables.

		Males nmol/L (ng/dL)				
Stage	n	Median	Min.	Max.	5th percentile	95th percentile
Tanner Stage I	22	0.28 (7.93)	0.08 (2.31)	1.05 (30.28)	0.09 (2.52)	1.02 (29.29)
Tanner Stage II	23	0.87 (25.09)	0.13 (3.75)	9.78 (282.06)	0.13 (3.86)	9.66 (278.59)
Tanner Stage III	25	0.86 (24.80)	0.30 (8.65)	23.64 (681.78)	0.31 (8.91)	22.73 (655.39)
Tanner Stage IV	25	7.62 (219.76)	0.62 (17.88)	27.24 (785.60)	0.69 (19.96)	26.16 (754.45)
Tanner Stage V	25	18.44 (531.81)	0.46 (13.27)	31.42 (906.15)	0.58 (16.64)	31.28 (902.09)

		Females nmol/L (ng/dL)				
Stage	n	Median	Min.	Max.	5th percentile	95th percentile
Tanner Stage I	25	0.28 (8.08)	0.02 (0.58)	1.15 (33.17)	0.04 (1.18)	1.12 (32.30)
Tanner Stage II	24	0.42 (12.11)	0.15 (4.33)	0.80 (23.07)	0.18 (5.05)	0.77 (22.21)
Tanner Stage III	24	0.89 (25.67)	0.24 (6.92)	1.49 (42.97)	0.26 (7.43)	1.43 (41.24)
Tanner Stage IV	19	0.94 (27.11)	0.53 (15.29)	1.86 (53.64)	0.53 (15.29)	1.86 (53.64)
Tanner Stage V	24	1.31 (37.78)	0.52 (15.00)	3.55 (102.38)	0.59 (17.02)	3.43 (98.78)

A third study was conducted testing a minimum of 120 samples from individuals in the following categories: normal males 21-49 years of age, normal males ≥ 50 years of age, premenopausal normal females 21-49 years of age, and postmenopausal normal females ≥ 50 years of age not on hormone replacement therapy. The Free Testosterone Index (% FTI) or Free Androgen Index (% FAI) correlates with the value of free testosterone. Therefore, in addition to ARCHITECT 2nd Generation Testosterone (List Number 2P13), all samples were tested with ARCHITECT Sex Hormone Binding Globulin (ARCHITECT SHBG, List Number 8K26). The % FTI or % FAI was calculated on a molar/molar basis. These samples gave values for the different groups summarized in the following table*.

SHBG and Total Testosterone

Category	n	SHBG nmol/L Median	SHBG nmol/L 2.5th percentile	SHBG nmol/L 97.5th percentile	Testosterone nmol/L (ng/dL) Median	Testosterone nmol/L (ng/dL) 2.5th percentile	Testosterone nmol/L (ng/dL) 97.5th percentile
Males (21-49 years of age)	163	31.1	16.2	68.5	15.33 (442.07)	8.76 (252.73)	27.85 (803.24)
Males (≥50 years of age)	144	35.3	13.7	69.9	14.42 (415.85)	8.58 (247.50)	23.37 (674.13)
Females (Premenopausal, 21-49 years of age)	174	48.6	14.7	122.5	1.05 (30.43)	0.52 (14.92)	1.72 (49.56)
Females (Postmenopausal, ≥50 years of age)	175	49.9	16.7	124.4	0.76 (21.83)	0.46 (13.34)	1.18 (33.90)

Free Testosterone Index or Free Androgen Index

Category	n	FTI or FAI (%) [a] Median	FTI or FAI (%) [a] 2.5th percentile	FTI or FAI (%) [a] 97.5th percentile
Males (21-49 years of age)	163	46.6	24.5	113.3
Males (≥50 years of age)	144	40.7	19.3	118.4
Females (Premenopausal, 21-49 years of age)	174	2.0	0.7	8.7
Females (Postmenopausal, ≥50 years of age)	175	1.5	0.5	4.7

[a] $\text{FTI or FAI (\%)} = \frac{\text{ARCHITECT 2nd Generation Testosterone Value (nmol/L)}}{\text{ARCHITECT SHBG (nmol/L)}} \times 100$

* Representative performance data are shown. Results obtained at individual laboratories may vary.

It is recommended that each laboratory establish its own reference range that is appropriate for the laboratory's patient population (i.e., a normal range that reflects the type of specimen and demographic variables such as age and sex, as applicable).

SPECIFIC PERFORMANCE CHARACTERISTICS

Data in the SPECIFIC PERFORMANCE CHARACTERISTICS section were generated using the ARCHITECT i 2000SR System. Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT 2nd Generation Testosterone assay is designed to have a within-laboratory (total) imprecision of ≤ 10% CV for samples with testosterone concentrations ≥ 0.5 nmol/L to 35 nmol/L.

Within-Laboratory Precision

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[14] Testing was conducted using two instruments, two ARCHITECT 2nd Generation Testosterone Reagent Kit lots, and one lot each of ARCHITECT 2nd Generation Testosterone Calibrators and Controls. Three levels of controls and 1 human serum panel were assayed with a minimum of 2 replicates at 2 separate times per day for 20 different days. The data are summarized in the following table.

Sample	Instrument	Reagent Lot	n	Mean nmol/L (ng/dL)	Within Run SD	Within Run %CV	Within - Laboratory (Total) SD	Within - Laboratory (Total) %CV
Control Level 1	1	1	80	0.34 (9.88)	0.016 (0.456)	4.6	0.017 (0.477)	4.8
		2	80	0.32 (9.24)	0.012 (0.358)	3.9	0.016 (0.472)	5.1
	2	1	80	0.33 (9.56)	0.014 (0.390)	4.1	0.015 (0.439)	4.6
		2	80	0.31 (9.02)	0.016 (0.459)	5.1	0.016 (0.468)	5.2
Control Level 2	1	1	80	2.64 (76.07)	0.081 (2.339)	3.1	0.095 (2.734)	3.6
		2	80	2.50 (72.07)	0.079 (2.277)	3.2	0.082 (2.361)	3.3
	2	1	80	2.57 (74.24)	0.086 (2.492)	3.4	0.092 (2.661)	3.6
		2	80	2.53 (72.86)	0.061 (1.769)	2.4	0.093 (2.684)	3.7
Control Level 3	1	1	80	7.92 (228.38)	0.167 (4.811)	2.1	0.208 (5.993)	2.6
		2	80	7.86 (226.67)	0.161 (4.643)	2.0	0.208 (6.008)	2.7
	2	1	80	7.88 (227.22)	0.199 (5.732)	2.5	0.260 (7.496)	3.3
		2	80	7.97 (229.95)	0.191 (5.504)	2.4	0.222 (6.391)	2.8
Panel	1	1	80	2.16 (62.35)	0.079 (2.287)	3.7	0.082 (2.379)	3.8
		2	80	2.11 (60.72)	0.045 (1.285)	2.1	0.062 (1.796)	3.0
	2	1	80	2.13 (61.30)	0.065 (1.888)	3.1	0.076 (2.184)	3.6
		2	80	2.13 (61.53)	0.054 (1.558)	2.5	0.070 (2.028)	3.3

Linearity

A study was performed based on guidance from the NCCLS document EP6-A.[15] Using an absolute deviation from linearity of 0.125 nmol/L for samples with concentrations of ≤ 0.5 nmol/L, and 10% for samples with concentrations > 0.5 nmol/L, a linear range of 0.13 – 64.57 nmol/L (3.82 – 1862.27 ng/dL) was demonstrated for the ARCHITECT 2nd Generation Testosterone assay.

Sensitivity

Limit of Quantitation

The ARCHITECT 2nd Generation Testosterone assay is designed to have a Limit of Quantitation (LoQ) of ≤ 0.15 nmol/L, where the LoQ is defined as the lowest analyte concentration that yields an estimated total error less than the total allowable error (TEa), where, the TEa is:

- TEa = 0.125 nmol/L for samples with concentrations ≤ 0.5 nmol/L
- TEa = 25% for samples with concentrations > 0.5 nmol/L

The LoQ was determined based on guidance from NCCLS document EP17-A.[16] The observed LoQ for the ARCHITECT 2nd Generation Testosterone assay was 0.08 nmol/L (2.30 ng/dL).

Limit of Blank and Limit of Detection

In the same study, the Limit of Blank (LoB) and Limit of Detection (LoD) were determined. The LoB was 0.03 nmol/L (0.87 ng/dL) and the LoD was 0.05 nmol/L (1.44 ng/dL).

Interference

Potentially Interfering Endogenous Substances

Potential interference in the ARCHITECT 2nd Generation Testosterone assay from hemoglobin, bilirubin, triglycerides, protein and biotin was evaluated to be ≤ 10%. Interference was demonstrated by a

study based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) protocol EP7-A2.[17] The data are summarized in the following table.

Potentially Interfering Endogenous Substance	Minimum Interferent Concentration	% Interference	
		Testosterone Concentration	
		7 nmol/L	24.3 nmol/L
Bilirubin (unconjugated)	15 mg/dL	3.3	4.2
Bilirubin (conjugated)	15 mg/dL	1.5	4.1
Hemoglobin	100 mg/dL	-1.4	-0.5
Total Protein	12 g/dL	-4.6	-7.0
Triglycerides	1,000 mg/dL	-6.4	-4.4
Biotin	30 ng/mL	-2.1	-0.8

Potentially Interfering Drugs and Other Compounds

A study was performed based on guidance from the CLSI document EP7-A2.[17] Potentially interfering drugs and other compounds were evaluated to determine whether testosterone concentrations were affected when using the ARCHITECT 2nd Generation Testosterone assay. The data are summarized in the following table.

NOTE: Test compound concentration is in nmol/L unless noted otherwise.

Test Compound (Drugs)	Test Compound Conc.[a] nmol/L	Testosterone Concentration			
		2.4 nmol/L		10 nmol/L	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
Buserelin	300 ng/mL	-0.03	0.0	0.04	0.0
Clomiphene Citrate	10,000	-0.08	0.0	0.01	0.0
Cyproterone acetate	2,000	-0.07	0.0	-0.16	0.0
Danazol	1,000	2.48	0.2	0.04	0.0
11-deoxy-17- hydroxycorticosterone	1,000	-0.01	0.0	0.12	0.0
Desoxycorticosterone acetate	5,000	0.06	0.0	0.04	0.0
Dexamethasone	5,000	-0.01	0.0	0.04	0.0
Diethylstilbestrol (DES)	2 µg/mL	0.01	0.0	-0.06	0.0
17β-estradiol-17-propionate	10,000	0.03	0.0	-0.24	0.0
17β-estradiol-17-valerate	10,000	0.05	0.0	0.00	0.0
Ethisterone	20	0.01	0.0	-0.37	-1.8
Flunisolide	1,000	-0.01	0.0	0.01	0.0
Fluoxymesterone	500	0.00	0.0	-0.34	-0.1
Flutamide	250 ng/mL	0.03	0.0	-0.01	0.0
Goserelin acetate	10 ng/mL	-0.05	-0.6	-0.05	-0.6
Hydroxyflutamide	5 µg/mL	0.01	0.0	0.02	0.0
Ketoconazole	20 µg/mL	0.02	0.0	0.06	0.0
Leuprolide acetate	150 ng/mL	0.03	0.0	0.04	0.0
Livial (Tibolone)	1,000	0.06	0.0	-0.21	0.0
Lynestrenol	1,000	0.25	0.0	-0.58	-0.1
Medroxyprogesterone	5,000	-0.06	0.0	-0.28	0.0
Mestranol (17α-ethynylestradiol 3 methyl ether)	1,000 ng/mL	0.02	0.0	0.24	0.0
Nilutamide	25 µg/mL	0.03	0.0	-0.27	0.0
Norethindrone	10	0.07	0.7	0.11	1.1
Norethindrone acetate	500	0.05	0.0	-0.22	0.0
Norgestrel	20 ng/mL	0.19	0.3	-0.61	-1.0
19-nortestosterone (Nandrolone)	30	106.11	353.7	98.65	328.8
Oxymethalone	100	0.04	0.0	0.16	0.2
Prednisone	2000	0.03	0.0	-0.02	0.0
Stanazolol	400	0.17	0.0	-0.35	-0.1
Tamoxifen	1,000	0.01	0.0	-0.07	0.0
Testosterone Acetate	250	4.80	1.9	2.33	0.9
Testosterone Enanthate	250	0.43	0.2	0.88	0.4
Triamcinolone	100	0.00	0.0	0.05	0.0
Spironolactone	500 ng/mL	-0.02	0.0	-0.10	0.0

Test Compound (Other Compounds)	Test Compound Conc.[a] nmol/L	Testosterone Concentration			
		2.4 nmol/L		10 nmol/L	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
Aldosterone	500	-0.07	0.0	0.17	0.0
5α-androstan-3α,17β-diol	20	-0.01	-0.1	-0.10	-0.5
5α-androstan-3β,17β-diol	10	0.03	0.3	0.05	0.5
Androstanedione	1,000	0.07	0.0	-0.30	0.0
Androstenediol	40	1.34	3.3	-2.16	-5.4
Androstenedione	20	0.20	1.0	0.02	0.1
Androsterone	1,000	-0.10	0.0	0.02	0.0
Androsterone glucuronide	1,000	-0.01	0.0	-0.01	0.0
Androsterone sulphate	1,000	0.00	0.0	-0.14	0.0
Corticosterone	5,000	0.10	0.0	0.22	0.0
Cortisol	10,000	0.12	0.0	0.18	0.0
Cortisone	1,000	-0.02	0.0	0.07	0.0
Desoxycorticosterone	1,000	0.37	0.0	0.55	0.1
DHEA	50	-0.01	0.0	-0.03	-0.1
DHEAS	50,000	1.34	0.0	0.05	0.0
Dihydrotestosterone	40	0.13	0.3	-0.57	-1.4
Epiandrosterone	250	-0.02	0.0	-0.25	-0.1
Epitestosterone	100	0.05	0.0	0.01	0.0
17α-estradiol	1,000	-0.08	0.0	-0.04	0.0
17β-estradiol	200	0.06	0.0	-0.31	-0.2
17β-estradiol-3-glucuronide	500	0.22	0.0	-0.68	-0.1
17β-estradiol-3-sulphate	2,000	0.02	0.0	-0.12	0.0
Estriol	800	0.03	0.0	-0.34	0.0
Estriol 3-(β-D-glucuronide sodium salt)	500	0.02	0.0	-0.24	0.0
Estrone	500	-0.03	0.0	-0.03	0.0
Ethynodiol diacetate	50 ng/mL	0.00	0.0	0.04	0.0
17α-ethynyl estradiol	1000 ng/mL	0.0	0.0	0.0	0.0
Etiocholan-3,17-dione	500	0.00	0.0	-0.04	0.0
Etiocholan-3α,17β-diol	500	0.03	0.0	-0.34	-0.1
19-hydroxyandrostene-dione	100	0.07	0.1	-0.86	-0.9
16α-hydroxyestrone	400	0.01	0.0	-0.11	0.0
17α-hydroxypregnanolone	5,000	0.03	0.0	-0.01	0.0
17α-hydroxyprogesterone	5,000	0.05	0.0	-0.10	0.0
6β-hydroxytestosterone	5	0.42	8.4	-0.48	-9.6
11β-hydroxytestosterone	5	1.53	30.6	0.60	12.0
11-ketotestosterone	5	0.07	1.4	-0.35	-6.9
17α-methyltestosterone	10	0.62	6.2	-0.40	-4.0
Pregnanolone	2,000	-0.01	0.0	0.01	0.0
Progesterone	2,000	-0.03	0.0	0.04	0.0

[a] Test compounds were tested at or above the listed concentration.

[b] Conc. Diff. = Concentration Difference

[c] $\% \text{ Cross-Reactivity} = \frac{\text{Concentration Difference}}{\text{Test Compound Concentration}} \times 100$

Method Comparison

The ARCHITECT 2nd Generation Testosterone assay is designed to have a slope of 1.0 ± 0.2 and a correlation coefficient (r) of ≥ 0.95 for samples with testosterone concentrations ranging from 0.15 nmol/L (LoQ) to 35 nmol/L when compared to Liquid Chromatography - Tandem Mass Spectrometry (LCMS). A correlation study was performed based on guidance from the NCCLS document

EP9-A2[18] using the Passing- Bablok regression method to compare the ARCHITECT 2nd Generation Testosterone assay to the LCMS testosterone method using serum specimens (n = 198). The data are summarized in the following table.

Correlation of ARCHITECT 2nd Generation Testosterone to LCMS Testosterone

Concentration Range (nmol/L)		Correlation Coefficient (r)		Intercept		Slope	
ARCHITECT	LCMS	r	95% CL[a]	(nmol/L)	95% CI[b]	Slope	95% CI[b]
0.38-32.53	0.34-32.79	0.964	0.952	0.05	(-0.06, 0.15)	0.94	(0.92, 0.96)

[a] 95% CL= Confidence Limit (Lower, One-sided)

[b] CI = Confidence Interval

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF UK	Product of United Kingdom
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
SPECIMEN DILUENT	Specimen Diluent
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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