Alinity i 2nd Generation Testosterone (Testo)-01

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i 2nd Generation Testosterone assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of testosterone in human serum and plasma on the Alinity i analyzer.

The Alinity i 2nd Generation Testosterone assay is to be used for the measurement of testosterone 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 Alinity i 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*, *4*, *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

This assay is a delayed one-step immunoassay for the quantitative determination of testosterone in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-testosterone (sheep, monoclonal) coated paramagnetic microparticles, and assay specific diluent are combined and incubated. The testosterone present in the sample binds to the anti-testosterone coated microparticles. Testosterone acridinium-labeled conjugate is added to create a reaction mixture. The reaction mixture is incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

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 system 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 Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i 2nd Generation Testosterone Reagent Kit 07P68

NOTE: This product is composed of 4 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	07P6821	07P6831
Tests per cartridge set	100	400
Number of cartridge sets per kit	2	2
Tests per kit	200	800
MICROPARTICLES	6.6 mL	21.9 mL
CONJUGATE	6.9 mL	25.0 mL
ASSAY SPECIFIC DILUENT	25.0 mL	33.8 mL
SPECIMEN DILUENT	12.6 mL	46.9 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 Phosphate and glycine in citrate buffer. Preservative: ProClin 300.

SPECIMEN DILUENT PBS buffer. Preservative: ProClin 300.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

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], [8], [9], [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	Contain 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	
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/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity**

ci-series Operations Manual, Section 8.

Reagent Handling

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
    - **Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.**
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- **Prior to loading on the analyzer for the first time, gently invert cartridges 20 times.**
- **Reagent cartridges cannot be inverted after the septum has been pierced by the analyzer.**
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use. Prior to loading on the analyzer for the first time, gently invert cartridges 20 times.
Onboard	System Temperature	30 days	

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Reagent cartridges cannot be inverted after the septum has been pierced by the analyzer. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents**, refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the **Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i 2nd Generation Testosterone assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) / (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
ng/dL	28.84	nmol/L
	100	ng/mL

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Dipotassium EDTA

Specimen Conditions

Do not use:

- heat-inactivated specimens
- pooled specimens
- grossly hemolyzed specimens
- specimens with 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.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at ≥ 1000 RCF (Relative Centrifugal Force) for 10 minutes before testing.
- 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.
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Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature	8 hours	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.
	2 to 8°C	7 days	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 to 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.

PROCEDURE

Materials Provided

07P68 Alinity i 2nd Generation Testosterone Reagent Kit

Materials Required but not Provided

- Alinity i 2nd Generation Testosterone assay file
- 07P6801 Alinity i 2nd Generation Testosterone Calibrators
- 07P6810 Alinity i 2nd Generation Testosterone Controls or other commercially available controls
- Alinity Pre-Trigger Solution
- Alinity Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, **refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.**
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

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 **1009.40 ng/dL** (35.00 nmol/L) and less than or equal to 1500.00 ng/dL (52.01 nmol/L), the instrument automatically orders a retest using the **1:4 dilution** protocol. If sample results are less than 12.98 ng/dL (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 28.84 ng/dL (1.00 nmol/L).

- Priority
  - Sample volume for first test: 100 µL
  - Sample volume for each additional test from same sample cup: 50 µL
- ≤ 3 hours on the reagent and sample manager:
  - Sample volume for first test: 150 µL
  - Sample volume for each additional test from same sample cup: 50 µL
- > 3 hours on the reagent and sample manager:
  - Replace with a fresh aliquot of sample.

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 1009.40 ng/dL (35.00 nmol/L) and less than or equal to 1500.00 ng/dL (52.01 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 1009.40 ng/dL (35.00 nmol/L).

- Priority
  - Sample volume for first test: 88 µL
  - Sample volume for each additional test from same sample cup: 38 µL
- ≤ 3 hours on the reagent and sample manager:
  - Sample volume for first test: 150 µL
  - Sample volume for each additional test from same sample cup: 50 µL
- > 3 hours on the reagent and sample manager:
  - Replace with a fresh aliquot of sample.

Neat (Undiluted) Assay Protocol

The neat protocol tests samples undiluted. This protocol is utilized for patient samples with results between **0.00 and 28.84 ng/dL** (0.00 and 1.00 nmol/L), and the low control. Samples may be tested using this protocol initially or if the result produced is less than 12.98 ng/dL (0.45 nmol/L).

- Priority
  - Sample volume for first test: 200 µL
  - Sample volume for each additional test from same sample cup: 150 µL
- ≤ 3 hours on the reagent and sample manager:
  - Sample volume for first test: 200 µL
  - Sample volume for each additional test from same sample cup: 150 µL
- > 3 hours on the reagent and sample manager:
  - Replace with a fresh aliquot of sample.

Refer to the Alinity i 2nd Generation Testosterone calibrator package insert and/or Alinity i 2nd Generation Testosterone control package insert for preparation and usage.

For general operating procedures**, refer to the Alinity ci-series Operations Manual, Section 5**.

For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with a testosterone value exceeding 1009.40 ng/dL (35 nmol/L) are flagged with the code "> 1009.40 ng/dL" ("> 35 nmol/L") and may be diluted with 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 by multiplying the result by the dilution factor.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Each assay control must be tested to evaluate the assay calibration.

Once a 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.
- Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.
    - If statistically-based quality control limits are not available, then the calibration should not exceed a 30-day limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

At least 2 quality control will be run every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of **20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[11]

- If more frequent control monitoring is required, follow the laboratory quality control procedures.
- If quality control results do not meet the acceptance criteria laboratory procedure, sample results may be suspect. Follow the laboratory quality control procedures to troubleshoot. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[12]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

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

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

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 Alinity ci-series Operations Manual, Section 5**.

Measuring Interval

Measuring interval is defined as the range of values in ng/dL (nmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias across all available assay file dilutions.

The measuring interval of the Alinity i 2nd Generation Testosterone assay is 4.33 to 1500.00 ng/dL (0.15 to 52.01 nmol/L).

LIMITATIONS OF THE PROCEDURE

- 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 Alinity i 2nd Generation Testosterone that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[13, 14]
- A strong interaction with D-(-)Norgestrel (1000 ng/mL), 19-nortestosterone (Nandrolone), Ethisterone, 11b-Hydroxytestosterone, and 11-Ketotestosterone was found. Do not use samples from patients receiving these compounds.
- 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.[15]
- Samples positive for Rheumatoid Factor (RF), that were spiked to testosterone concentrations of 201.9 ng/dL and 700.8 ng/dL, had a mean percent recovery of 103.0% (range: 78.2 - 129.6%, n = 25) and 94.0% (range: 80.6 - 101.1%, n = 25), respectively.
- Samples positive for Heterophilic Antibodies, that were spiked to testosterone concentrations of 201.9 ng/dL and 700.8 ng/dL, had a mean percent recovery of 103.0% (range: 74.5 - 109.2%, n = 23) and 94.0% (range: 80.6 - 104.0%, n = 25), respectively.

EXPECTED VALUES

The provided manufacturer refrence ranges will be adopted. Effort will be made to verify in house

The ARCHITECT 2nd Generation Testosterone expected values study was conducted in 2012. Testing was performed on a minimum of 120 samples from apparently healthy individuals in the following categories: normal males 21-49 years of age, and normal females 21-49 years of age. Additional samples were tested from apparently healthy males ≥ 50 years of age and apparently healthy postmenopausal females ≥ 50 years of age.

The following inclusion/exclusion criteria apply to normal males and females 21-49 years of age: Samples within normal ranges for thyroid stimulating hormone (TSH), free thyroxine (Free T4), prolactin and creatinine were included in the study. Males with intact reproductive system and no known diagnosis of diabetes (type I or type II), disorder of pituitary gland or associated hormones, thyroid or adrenal disorder, with no history of chronic illness or systemic disease were included in the study. Normal ovulating females not being treated for infertility, with no known diagnosis of diabetes, thyroid or adrenal disorder, with no history of chronic illness or systemic disease and not using birth control medication were included in the study.

The data are summarized in the following table.

Testosterone ng/dL							
Category **Apparently Healthy**	**n**	**Age Range (years)**	**Median**	**Min.**	**Max.**	**5th percentile**	**95th percentile**
Males (21-49 years of age)	129	21-49	494.03	47.01	980.56	240.24	870.68
Males (≥ 50 years of age)	71	50-77	442.41	127.18	1020.36	220.91	715.81
Females (21-49 years of age)	129	21-49	24.80	7.21	79.31	13.84	53.35
Females (≥ 50 years of age)	52	50-82	23.50	8.65	36.92	12.40	35.76

CALIPER Pediatrics:

Male Reference Intervals

Age	Lower Limit	Upper Limit	Sample Size	Lower Confidence Intervals	Higher Confidence Intervals
4 Days to < 6 Months	8.65	299	72	(0.86, 13.3)	(270, 329)
6 Months to < 9 Years	0	35.7	61	N/A	(15.0, 53.9)
9 to < 11 Years	0	23.3	45	N/A	(16.4, 30.3)
11 to < 14 Years	0	444	89	N/A	(386, 486)
14 to < 16 Years	36	632	78	(19.3, 59.7)	(592, 671)
16 to < 19 Years	148	794	94	(126, 175)	(740, 848)

Female Reference Intervals

Age	Lower Limit	Upper Limit	Sample Size	Lower Confidence Intervals	Higher Confidence Intervals
4 Days to < 9 Years	1.15	62	125	(0.86, 1.44)	(59.1, 92.8)
9 to < 13 Years	0	28.2	102	N/A	(22.5, 32.0)
13 to < 15 Years	10.4	44.4	79	(0.86, 12.1)	(40.6, 48.4)
15 to < 19 Years	14.1	49	110	(13.3, 14.7)	(46.4, 52.2)

A second study was conducted in 2014 to calculate Free Testosterone Index (FTI) (also known as Free Androgen Index (FAI)) using ARCHITECT 2nd Generation Testosterone (List Number [LN] 2P13) and ARCHITECT SHBG (LN 8K26) assay results. The %FTI or %FAI calculated as [Total Testosterone] / [SHBG] provides an index of free testosterone status.

Testing was performed on 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 following inclusion/exclusion criteria apply to the study: Samples that were within the expected values of the ARCHITECT 2nd Generation Testosterone (LN 2P13) reagent insert and the ARCHITECT SHBG (LN 8K26) reagent insert were included in the study.

The %FTI or %FAI was calculated on a molar/molar basis. The data from this study are summarized in the following tables.

ARCHITECT SHBG and ARCHITECT 2nd Generation Testosterone Assay Results*

Category	n	SHBG nmol/L			Testosterone nmol/L (ng/dL)		
		Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	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 Expected Values

Category	N	FTI or FAI (%) [a]		
		Median	2.5th percentile	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

[*] The table provides the SHBG and testosterone assay results. These should not be used as expected values for SHBG and testosterone.

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

The expected values for %FTI or %FAI provided in the table above were generated using ARCHITECT 2nd Generation Testosterone and ARCHITECT SHBG assay results. Expected values for %FTI or %FAI should be determined with the assays used to generate the SHBG and testosterone assay results. %FTI or %FAI results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc. and should not solely be used to make medical decisions.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.*16* Testing was conducted using 1 lot of the Alinity i 2nd Generation Testosterone Reagent Kit, 1 lot of the Alinity i 2nd Generation Testosterone Calibrators, and 1 lot of the Alinity i 2nd Generation Testosterone Controls and 1 instrument. Three controls and 1 human serum panel were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

			Within-Run (Repeatability)		**Within-Laboratory (Total)[a]**	
Sample	**n**	**Mean (ng/dL)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	120	9.09	0.321	3.5	0.732	8.1
Control Level 2	120	73.00	1.796	2.5	2.760	3.8
Control Level 3	120	243.00	5.468	2.3	6.433	2.6
Panel	120	66.35	1.886	2.8	2.073	3.1

Sample	n	Mean (nmol/L)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory (Total)[a] SD	Within-Laboratory (Total)[a] %CV
Control Level 1	120	0.32	0.011	3.5	0.025	8.1
Control Level 2	120	2.53	0.062	2.5	0.096	3.8
Control Level 3	120	8.43	0.190	2.3	0.223	2.6
Panel	120	2.30	0.065	2.8	0.072	3.1

[a]Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*17* Testing was conducted using 3 lots of the Alinity i 2nd Generation Testosterone Reagent Kit on each of 2 instruments over a minimum of 3 days. The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below. These representative data support the lower limit of the measuring interval.

	ng/dL	nmol/L
LoB[a]	0.58	0.02
LoD[b]	1.22	0.04
LoQ[c,d]	**4.33**	0.15

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ is defined as the lowest concentration at which a maximum allowable precision of 20% CV was met.

[d] This value represents the observed LoQ on the ARCHITECT System. The LoQ observed on the Alinity i analyzer supports this LoQ.

Linearity

A study was performed based on guidance from CLSI EP06-A.*18*

This assay is linear across the measuring interval of **4.33 to 1500.00 ng/dL** (0.15 to 52.01 nmol/L).

Interference

These studies were performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

Interference was demonstrated by a study based on guidance from CLSI EP7-A2.[19]

Potentially Interfering Endogenous Substance	Interferent Concentration	% Interference[a]	
		Testosterone Concentration	
		201.9 ng/dL	700.8 ng/dL
Bilirubin (unconjugated)	20 mg/dL	-0.2	1.9
Bilirubin (conjugated)	20 mg/dL	-2.0	4.4
Hemoglobin	500 mg/dL	2.5	2.2
Total Protein	12 g/dL	-4.6	-7.0
Triglycerides	2000 mg/dL	-6.5	-1.1
Biotin	30 ng/mL	-2.1	-0.8
SHBG	200 nmol/L	-5.3	-9.1

[a] % Interference = $\frac{\text{Concentration Difference}}{\text{Reference Sample Mean (or Median) Concentration}} \times 100$

Potentially Interfering Drugs and Other Compounds

NOTE: Test compound concentration is in ng/mL unless noted otherwise.

A study was performed based on guidance from the CLSI document EP7-A2.[19] Potentially interfering drugs and other compounds were evaluated to determine whether testosterone concentrations were affected when using the ARCHITECT 2nd Generation Testosterone assay.

Test Compound (Drugs)	Test Compound Conc.[a] ng/mL	Testosterone Concentration			
		201.9 ng/dL		700.8 ng/dL	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
Danazol	1000	724.19	0.7	606.24	0.6

Test Compound (Drugs)	Test Compound Conc.[a] ng/mL	Testosterone Concentration			
		201.9 ng/dL		700.8 ng/dL	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
Dexamethasone	2000	1.12	0.0	-0.58	0.0
Ethisterone	1000	**	NA	**	NA
Mestranol (17α-Ethynylestradiol 3 methyl ether)	1000	-3.75	0.0	2.31	0.0
D-(-) Norgestrel	20	-16.83	-0.8	-12.69	-0.6
	1000	1072.19	1.1	783.66	0.8
19-nortestosterone (Nandrolone)	30 nmol/L	**	NA	**	NA
Prednisolone	1000	2.64	0.0	5.44	0.0
Prednisone	1000	1.92	0.0	-9.59	0.0
Spironolactone	500	0.43	0.0	-4.21	0.0
Testosterone Propionate	100	2843.19	28.4	883.00	8.8

Test Compound (Other Compounds)	Test Compound Conc.[a] ng/mL	Testosterone Concentration			
		201.9 ng/dL		700.8 ng/dL	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
5α-Androstane-3β,17β-diol	1000	477.51	0.5	348.41	0.3
Androstenediol	1000	-3.11	0.0	-7.94	0.0
Androstenedione	100	177.29	1.8	121.47	1.2
Cortisol	1000	0.85	0.0	15.36	0.0
Cortisone	2000	1.06	0.0	10.11	0.0
DHEA	1000	-13.07	0.0	-9.34	0.0
DHEAS	50 000	96.65	0.0	99.94	0.0

Test Compound (Other Compounds)	Test Compound Conc.[a] ng/mL	Testosterone Concentration			
		201.9 ng/dL		700.8 ng/dL	
		Conc. Diff.[b]	% Cross-Reactivity[c]	Conc. Diff.[b]	% Cross-Reactivity[c]
Dihydrotestosterone	500	352.85	0.7	223.05	0.4
Epitestosterone	100 nmol/L	2.71	0.1	12.32	0.4
Estradiol (17β-Estradiol)	1000	8.67	0.0	-5.50	0.0
Estrone	1000	-8.90	0.0	-9.09	0.0
Ethynodiol diacetate	50	-5.44	-0.1	-2.19	0.0
17α-Ethynylestradiol	1000	-11.79	0.0	3.23	0.0
11β-Hydroxytestosterone	100	**	NA	**	NA
11-Ketotestosterone	1000	**	NA	**	NA
Progesterone	1000	-1.75	0.0	18.65	0.0

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

[b] Conc. Diff. = Concentration Difference (ng/dL)

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

[**] These compounds tested above the measuring interval and the % Crossreactivity could not be calculated. See LIMITATIONS OF THE PROCEDURE.

Tube Type Matrix Comparison

This study was performed on the ARCHITECT i System.

A study was performed to evaluate the types of blood collection tubes that can be used with the ARCHITECT 2nd Generation Testosterone assay. The tube types were evaluated using the Passing-Bablok regression method to compare each evaluation tube type (n =54) to the control tube type (serum plastic). The data are summarized in the following table.

Evaluation Tube Type	Control Tube (serum plastic) Range (ng/dL)	Evaluation Tube Range (ng/dL)	r[a]	Intercept (ng/dL)	Slope
Serum, glass	14.61-1430.75	14.42-1469.01	1.000	-0.38	1.01
Serum separator, plastic		14.23-1477.86	0.999	-0.42	0.99
Serum separator II Advance		14.32-1419.41	0.999	-0.07	0.98
Dipotassium EDTA		13.41-1526.41	0.997	-0.96	1.02

[a] r = Correlation Coefficient

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[20]

		Unit	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i 2nd Generation Testosterone vs ARCHITECT 2nd Generation Testosterone	Serum	ng/dL (nmol/L)	316	1.00	0.78 (0.02)	1.10	5.63-1558.23 (0.20-54.03)

This study was performed on the ARCHITECT i System.

A method comparison study was performed based on guidance from the CLSI document EP9-A2-IR[21] using the Passing- Bablok regression method to compare the ARCHITECT 2nd Generation Testosterone assay to the LCMS testosterone method. The data are summarized in the following tables.

ARCHITECT 2nd Generation Testosterone vs. LCMS (n = 138)

Concentration Range ng/dL		Correlation Coefficient	Intercept	95%		95%
ARCHITECT	LCMS	(r)	ng/dL	CI[a]	Slope	CI[a]
13.74-1429.61	6.0-	0.994	-3.70	(-5.00, -	1.00	(0.98,

Concentration Range ng/dL		Correlation Coefficient	Intercept	95%		95%
ARCHITECT	**LCMS**	**(r)**	**ng/dL**	**CI[a]**	**Slope**	**CI[a]**
	1330.0			1.66)		1.03)

[a] CI = Confidence Interval

ARCHITECT 2nd Generation Testosterone vs. LCMS Female Specimens (n = 73)

Concentration Range ng/dL		Correlation Coefficient	Intercept	95%		95%
ARCHITECT	**LCMS**	**(r)**	**ng/dL**	**CI[a]**	**Slope**	**CI[a]**
13.74-349.97	6.0-346.5	0.985	2.77	(0.53, 4.45)	0.82	(0.77, 0.88)

[a] CI = Confidence Interval

ARCHITECT 2nd Generation Testosterone vs. LCMS Male Specimens (n = 65)

Concentration Range ng/dL		Correlation Coefficient	Intercept	95%		95%
ARCHITECT	**LCMS**	**(r)**	**ng/dL**	**CI[a]**	**Slope**	**CI[a]**
86.93-1429.61	115.0-1330.0	0.990	-48.63	(-62.25, -32.25)	1.10	(1.07, 1.13)

[a] CI = Confidence Interval

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