| **Testosterone** |
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| **Purpose** | This procedure provides instructions for performing TESTOSTERONE on ABBOTT INSTRUMENTATION. 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.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating Alinity ci and Architect i1000 at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | 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. |
| **Clinical Significance** | 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 109 Lmol-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. 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.The concentration of testosterone in an individual fluctuates over 24 hours. 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. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACI)****Backup:** Mayo Medical Laboratories, Mass Spec, Sunquest test code TTST- required RED NO GEL. Also use this test for children under 1 year of age. |
| **Sunquest Test Codes** | **TESTO** |
| **Specimen** | Sample: Serum or EDTA plasma (RED NO GEL to be eligible for MML testing)**Preferred:** Serum (with or without gel barrier) **Alternative:** EDTA plasma**NOTE**: Samples for Mayo Medical Laboratories TTST require Red/Serum drawn without gel, preferred 3 mL draw, 0.9 mL sample volume. Children under the age of 1 year should be sent to MML, and the minimum specimen volume is 0.215 mL. **Minimum sample volume:** 0.75 mL blood, 0.25 mL serum/plasma: Minimum sample volume does not allow for repeats or sendout.**ALWAYS LOAD SAMPLES IN A PRIORITY LANE.****DEFAULT 1:3 ASSAY DILUTION PROTOCOL**:Priority Loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 50 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 50 μL**1:4 Assay Dilution Protocol:**Priority Loaded: Sample volume for first test: 88 μL Sample volume for each additional test from same sample cup: 38 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 50 μL**Neat (Undiluted) Assay Protocol:**Priority Loaded: Sample volume for first test: 200 μL Sample volume for each additional test from same sample cup: 150 μLRoutinely loaded: Sample volume for first test: 200 μL Sample volume for each additional test from same sample cup: 150 μL**Stability when separated from cells/gel:** **20 to 25°C** 8 hours**2 to 8°C** 7 days**-20°C** 7 days- AVOID MORE THAN ONE FREEZE/THAW CYCLE**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** **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.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. |
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i 2nd Generation Testosterone Reagent Kit | 07P6821 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**On-board**: 30 daysStore 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. Do not invert after the cartridge has been loaded on the system.** |
| Alinity i 2nd Generation Testosterone Calibrator Kit | 07P6801 | **Store at:** -10°C or colder**Unopened**: Until manufacturer’s expiration date• Calibrators may be thawed at room temperature for 90 to 120 minutes or overnight at 2 to 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.• Prior to each use, mix by gentle inversion (10 times).The analyzer will track in-use stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer. The analyzer will not allow use of the calibrator on the system if the in-use stability is exceeded. **Opened expiration:** 2 to 8°C for up to 3 months, not to exceed the expiration date printed on the bottle. Store tightly capped. Return to refrigerated storage after use. Do not refreeze the calibrators after thaw.  |

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| **Risk and Safety** | **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.**The following warnings and precautions apply to:** *Microparticles* **WARNING** Contains methylisothiazolones and sodium azide. May cause an allergic skin reaction. Contact with acids liberates very toxic gas.**The following warnings and precautions apply to:** *conjugate and specimen diluent* **WARNING** Contain methylisothiazolones. May cause an allergic skin reaction. **The following warnings and precautions apply to:** *assay specific diluent* **WARNING** Contains hydrochloric acid and methylisothiazolones. May cause an allergic skin reaction. May be corrosive to metals. No special disposal indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 4.33 – 1500.00 ng/dL |
| Reference Material: | Abbott Alinity i Calibrator Set |
| Suggested Calibration Levels: | CAL A = 0.00 ng/dLCAL B = 2.88 ng/dLCAL C = 5.77 ng/dLCAL D = 46.14 ng/dLCAL E = 360.50 ng/dLCAL F = 865.20 ng/dL |
| Calibration Scheme: | 6 Levels |
| Calibration Frequency: | For each new lot of reagent, after major maintenance or service, if indicated by quality control results, as indicated in laboratory quality control procedures |
| AMR | AMR is verified with every calibration and at least once every 6 months. |

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| **Quality Control** | **QC Material:** Bio-Rad Liquichek Immunoassay Plus Levels 1, 2, 3**Frequency:** Three levels each day of use**Stability:** Stable until the expiration date when stored frozen between -20 and -40°C. Once thawed, opened, and stored tightly capped at 2 to 8°C, this product is stable for 5 days in Minneapolis (due to estradiol in this control). **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour.
* After thawing, the product **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | In studies completed by Abbott, interference at the following potential interfering endogenous substances were less than 10%:Bilirubin (unconjugated) 20 mg/dLBilirubin (conjugated) 20 mg/dLHemoglobin 500 mg/dLTotal Protein 12 g/dLTriglycerides 2000 mg/dLBiotin 30 ng/mLSex-Hormone Binding Globulin 200 nmol/L |
| **Reference Intervals** |

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| **Male Tanner Stages** |
| Stage I(>14 days & prepubertal) | <4.33 to 20 ng/dL |
| Stage II(Mean age 12 years) | <4.33 - 25 ng/dL |
| Stage III(Mean age 13.6 years) | <4.33 - 543 ng/dL |
| Stage IV(Mean age 15 years) | 8 - 636 ng/dL |
| Stage V(should be reached by age 18) | 99 - 760 ng/dL |

**Male Reference by age**:0 - 6 months: 8 to 300 ng/dL6 months to <9 years: <4.33 to 35 ng/dL9 - <11 years: <4.33 to 24 ng/dL11-<14 years: 8 to 445 ng/dL 14-<16 years: 36 to 632 ng/dL 16-<19 years: 147 to 795 ng/dL Adult: 240 to 871 ng/dL

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| **Female** **Tanner stages** |
| Stage I(>14 days & prepubertal) | <4.33 to 20 ng/dL |
| Stage II(Mean age 10.5 years) | <4.33 to 20 ng/dL |
| Stage III(Mean age 11.6 years) | <4.33 to 42 ng/dL |
| Stage IV(Mean age 12.3years) | 8 to 42 ng/dL |
| Stage V(Mean age 14.5 years) | <4.33 to 50 ng/dL |

**Female Reference Ranges by Age**:0-<9 years: <4.33 to 62 ng/dL 9-<13 years: <4.33 to 28 ng/dL 13-<15 years: 10 to 44 ng/dL 15-<19 years: 14 to 49 ng/dL 19 years+: <4.33 to 50 ng/dL  |
| **Critical Values** | None specified |
| **Limitations** | 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.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.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.See the package insert for more potentially interfering compounds  |
| **Dilutions** |

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| **The Alinity i analyzer runs 1:3 dilution on all patients. If the result is less than 12.98 with initial dilution, the instrument will run the specimen neat. If the sample is above 1009.40 ng/dL, the 1:4 automated dilution can be used.** |
| Max Auto Dilution: | 1:4 |
| Maximum Manual Dilution: | Not specified |
| Diluent: | Onboard Diluent |
| Automated Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 4.33, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 4.33 – 600.00 ng/dL without error messages are released automatically
* If there is not enough sample to repeat or there is an error on a lower dilution, report as applicable (<12.98 or >1009.50) and append the code “-UNQ” (Unable to Quantitate Further) to the result
* Result below 4.33 ng/dL report as < 4.33 ng/dL
* If result is <12.98, the instrument performs testing neat.
* Results above 1500.00 ng/dL report as >1500.00 ng/dL.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to freezer storage. Samples are retained 14 days in specimen storage freezer, but may not be appropriate for repeat following 7 days frozen. |
| **References** | 1. Abbott Alinity i Testosterone Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 20182. Abbott Alinity i Testosterone Calibrator Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 20183. Bio-Rad Liquichek Immunoassay Plus Package Insert, Bio-Rad Laboratories, Irvine CA USA. |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | May 29, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected mistakes, added Alinity i AMR, dilutions, references, reference intervals, etc. |