| **Luteinizing Hormone (LH)** |
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| **Purpose** | This procedure provides instructions for performing LUTEINIZING HORMONE (LH) on ABBOTT INSTRUMENTATION. The Alinity i LH assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of human luteinizing hormone (LH) in human serum and plasma on the Alinity i analyzer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating Alinity i at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of luteinizing hormone (LH) in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and anti-β LH coated paramagnetic microparticles are combined and incubated. The LH present in the sample binds to the anti-β LH coated microparticles. The mixture is washed. Anti-α LH acridinium-labeled conjugate is added to create a reaction mixture and 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 a direct relationship between the amount of LH in the sample and the RLUs detected by the system optics. For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | Human luteinizing hormone (LH, lutropin) is a glycoprotein hormone with two dissimilar subunits (α and β). The α-subunit is essentially identical to the α-subunits of follicle stimulating hormone (FSH, follitropin), thyroid stimulating hormone (TSH, thyrotropin), and human chorionic gonadotropin (hCG). The β-subunit is considerably different from that of FSH and TSH.However, the β-subunits of LH and hCG are very similar.LH, together with FSH, is secreted by the gonadotroph cells in the pituitary in response to the secretion of the gonadotropin releasing hormone (LHRH, GnRH) from the medial basal hypothalamus. Ovarian steroids, principally estrogens, modulate the secretion of LH and FSH which in turn regulate the menstrual cycle in females. When the follicle and the ovum contained within it, reach maturity, a surge of LH causes the follicle to rupture releasing the ovum. The follicular remnant is transformed into a corpus luteum, which secretes progesterone and estradiol. During the follicular and luteal phases, LH concentrations are much lower than the levels observed at the time of the LH surge. During the follicular and luteal phases, the estrogens exert a negative feedback on the release of LH. Shortly before the mid-cycle surge in LH, ovarian steroids, specifically estradiol, exert a positive feedback on the release of LH. Determination of the concentration of LH is essential for the prediction of ovulation, in the evaluation of infertility, and the diagnosis of pituitary and gonadal disorders. Increasing concentrations of LH precede ovulation and in cases in which the period of optimal fertility needs to be defined for the timing of intercourse or artificial insemination, daily concentrations of LH are important for the prediction of ovulation. More frequent sampling is required if the precise time of follicular rupture is needed for egg aspiration for *in vitro* fertilization. At menopause, or following ovariectomy in women, concentrations of estrogens decline to low levels. The lowered concentrations of estrogens result in a loss of the negative feedback on gonadotropin release. The consequence is an increase in the concentrations of LH and FSH.The primary role of LH in the male is to stimulate the production of testosterone by the Leydig cells. LH, through the production of testosterone together with FSH, regulates spermatogenesis in the Sertoli cells of the seminiferous tubules of the testes. Testosterone exerts a negative feedback on the release of LH. In sexually mature adults, gonadotropin deficiency is usually an early indication of the development of panhypopituitarism. Low concentrations of LH, FSH, and steroids are observed with this disorder. In contrast, gonadotropin secreting tumors of the hypothalamus and pituitary result in elevated concentrations of LH and FSH.Gonadal failure, a cause of infertility, is indicated by elevated concentrations of LH and FSH accompanied by low concentrations of gonadal steroids. In the female, elevated concentrations of LH can indicate primary amenorrhea, menopause, premature ovarian failure, polycystic ovarian syndrome, hypergonadotropic hypogonadism, or ovulation. In the male, elevated concentrations of LH can result from primary testicular failure, seminiferous tubule dysgenesis (Klinefelter’s syndrome), Sertoli cell failure, anorchia, or hypergonadotropic hypogonadism. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup:** Hold samples until instrument is back in service. If urgent or directed by provider, send to Mayo Medical Laboratories (FSH) |
| **Sunquest Test Codes** | **LH** LH**FSLH** FSH and LH |
| **Specimen** | Sample: Serum or Plasma (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, EDTA**Minimum sample volume:** 0.75 mL blood, 0.25 mL serum/plasmaMaximum number of replicates sampled from the same sample cup: 10Priority loaded: Sample volume for first test: 75 μL Sample volume for each additional test from same sample cup: 25 μLLoaded routinely: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 25 μL**Stability when separated from cells/gel:** **20 to 25°C** Not specified. Remove to storage at least once per shift.**2 to 8°C** 7 days**-20°C** 14 days**Rejection criteria:** * Unlabeled tube
* Sample type other than serum or acceptable plasma
* Heat-inactivated specimens
* Pooled specimens
* Grossly hemolyzed specimens: append –HP if samples are grossly hemolyzed. See interferences and reporting instructions.
* Specimens with obvious microbial contamination

**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** Reagents are shipped on wet ice or frozen gel packs. 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. 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. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i LH Reagent Kit | 07P9120 | **Store at:** 2 to 8°C 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.**Unopened:** Until expiration Date**On-board**: 30 daysReagents 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. |
| Alinity i LH Calibrators | 07P9101 | **Store at:** 2 to 8°C**Unopened:** Until printed expiration date**Opened expiration:** 4 months from the date opened. Do not exceed the lot expiration date printed on the bottle. Store tightly capped with new replacement cap. Return to refrigerated storage after use. |

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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 and Conjugate***WARNING** Contains methylisothiazolones.May cause an allergic skin reaction.**The following warnings and precautions apply to:** *Cal A- Cal F* **CAUTION:** This product contains human-sourced and/ or potentially infectious components. Refer to the CONTENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that this product and human specimens be 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. **WARNING** Contains methylisothiazolones.May cause an allergic skin reaction.No special disposal of reagents 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: | 0.1 – 250.0 mIU/mL |
| Reference Material: | Alinity i LH Calibrator kit |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 1.0CAL C: 3.5CAL D: 15.0CAL E: 50.0CAL F: 250.0 |
| Calibration Scheme: | 6 Levels |
| Calibration Frequency: | With every new lot number, after maintenance or service of major instrument parts, as indicated by quality controls, and as directed by field service representatives. |
| AMR | AMR is verified with every calibration, at least every 6 months. |

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| **Quality Control** | **QC Material:** BioRad Liquichek Immunoassay Plus Levels 1,2 and 3 **Frequency:** Three levels each day of use. **Stability:** 5 Days at 2-8°C (due to the inclusion and use of Estradiol in this control) **Preparation**: Let vials thaw for 30 minutes at room temperature and gently swirl to ensure homogeneity. Do not allow to stand at room temperature longer than 20 minutes after completely thawed. **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** | Interference is less than 10% at the following levels for endogenous potentially interfering substances at LH concentrations of 10-20 and 50-70 mIU/mLBilirubin ≥ 20 mg/dL Protein ≥ 12 g/dL Triglycerides ≥ 3000 mg/dL Hemoglobin ≥ 500 mg/dL Visibly grossly hemolyzed samples should have –HP (Hemolysis present) appended. |
| **Reference Intervals** | **Female:**< 3 Months: 0 - 2.413 Months to < 1 Year: 0 - 1.191 to < 10 Years 0 - 0.3310 to < 13 Years: 0 - 4.34 13 to < 15 Years: 0.37 - 6.5215 to < 17 Years: 0 - 13.117 to < 19 Years: 0 - 8.38**Comment included on all Female results:**Normally Menstruating Female:Follicular Phase: 1.80 - 11.78Mid-Cycle Peak: 7.59 - 89.08Luteal Phase: 0.56 - 14.00**Male:** < 3 Months: 0.19 - 3.81 3 Months to < 1 Year: 0 - 2.891 to < 10 Years: 0 - 0.3310 to < 13 Years: 0 - 4.3413 to < 15 Years: 0 - 4.1115 to < 17 Years: 0.79 - 4.7617 to < 19 Years: 0.94 - 7.1 Adult: 0.57 - 12.07 |
| **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 LH results are inconsistent with clinical evidence, additionaltesting is recommended to confirm the result.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 LH that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.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. |
| **Dilutions** |

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| Max Auto Dilution: | 1:4 |
| Maximum Manual Dilution: | None |
| 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 0.1, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 0.1 and 250.0 without error messages are released
* Results below 0.1 without error messages are reported as < 0.1 mIU/mL.
* Results > 250.0 should be diluted using the onboard automated 1:4 dilution. Release results without error messages following this dilution.
* Results > 1000.0 following automated dilution are reported as > 1000.0 mIU/mL.
* Grossly hemolyzed samples should have –HP appended to denote hemolysis present.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity i LH Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised April 2018
2. Abbott Alinity i LH Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised April 2018
3. [CALIPER Reference Interval Studies](https://caliper.research.sickkids.ca/#/login;next=search;queryParams=%7B%7D), accessed 10/27/2020.
4. BioRad Liquichek Immunoassay Plus Quality Control Package Insert, BioRad Laboratories, Irvine, CA. Revised April 2020
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | May 18, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected analyzer type, numbered procedures, added AMR, references, reference intervals, corrected QC product, etc for new analyzers. |