| **FSH** |
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| **Purpose** | This procedure provides instructions for performing FOLLICLE STIMULATING HORMONE (FSH) on ABBOTT INSTRUMENTATION. The Alinity i FSH assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of follicle stimulating hormone (FSH) in human serum and plasma on the Alinity i analyzer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Alinity ci at Children’s Minnesota Laboratory, Minneapolis. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of FSH in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and anti-β FSH coated paramagnetic microparticles are combined and incubated. The FSH present in the sample binds to the anti-β FSH coated microparticles. The mixture is washed. Anti-α FSH 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 FSH 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.  |
| **Clinical Significance** | Human Follicle Stimulating Hormone (FSH, follitropin) is a glycoprotein of approximately 30 000 daltons which, like luteinizing hormone (LH, lutropin), human chorionic gonadotropin (hCG) and thyroid stimulating hormone (TSH, thyrotropin), consists of two noncovalently associated subunits designated α and β. The α subunit of FSH contains 92 amino acids and is very similar to the α subunits of LH, hCG, and TSH. The β subunit of FSH is unique and confers its immunological and functional specificity.FSH and LH control growth and reproductive activities of the gonadal tissues. FSH promotes follicular development in the ovary and gametogenesis in the testis. The gonadotroph cells of the anterior pituitary secrete both FSH and LH in response to gonadotropin releasing hormone (LHRH or GnRH) from the medial basal hypothalamus. Both FSH and LH are secreted in a pulsatile manner, with rapid fluctuations over the normal range. The pulsatility of FSH is less pronounced than that of LH. Release of both FSH and LH from the pituitary is under negative feedback control by the gonads.FSH in mature females acts to stimulate development of the ovarian follicles. Circulating FSH levels vary throughout the menstrual cycle in response to estradiol and progesterone. A small, but significant increase in circulating FSH accompanies the mid-cycle LH surge. However, the physiological significance of this increase is unknown. Circulating levels of FSH decline in the luteal phase in response to estradiol and progesterone production by the developing corpus luteum.At menopause, ovarian function is diminished with concomitant decrease in estradiol secretion. FSH and LH then increase significantly in response to diminished feedback inhibition of gonadotropin release. In males, FSH, LH, and testosterone regulate spermatogenesis by the Sertoli cells in the seminiferous tubules of the testes. FSH is less sensitive to feedback inhibition by testosterone than is LH and is thought to be regulated independently by the inhibitory peptide inhibin produced by the Sertoli cells. Because of the negative feedback mechanisms regulating gonadotropin release, elevated concentrations of LH and FSH are indicative of gonadal failure when accompanied by low concentrations of the gonadal steroids. In males, these observations suggest primary testicular failure or anorchia. FSH may also be elevated in Klinefelter’s syndrome (seminiferous tubule dysgenesis) or as a consequence of Sertoli cell failure. In females, situations in which FSH is elevated and gonadal steroids are depressed include menopause, premature ovarian failure, and ovariectomy, while with polycystic ovarian syndrome the LH/FSH ratio may be increased. Abnormal FSH concentrations may also indicate dysfunction of the hypothalamic-pituitary axis. In sexually mature adults, FSH deficiency, together with low concentrations of LH and sex steroids, may indicate panhypopituitarism. This can result either from a decrease in the release of GnRH or from a lack of response of the pituitary to GnRH. Determination of serum FSH, following administration of GnRH, may allow differentiation of these two conditions. The use of oral contraceptives usually results in reduction of gonadotropin. |
| **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** | **FSH** FSH**FSLH** FSH and LH |
| **Specimen** | Sample: Serum or Plasma (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, EDTA**Minimum sample volume:** 0.75 mL blood, 0.25 mL serum/plasma*Maximum number of replicates sampled from the same sample cup: 10*Priority Loaded: Sample volume for first test: 75 μLSample volume for each additional test from same sample cup: 25 μLLoaded Routinely:Sample volume for first test: 150 μLSample 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:** 12 months **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** 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.**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 FSH Reagent Kit | 07P4920 | **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 printed expiration date**On-board**: 30 days**Opened and stored in fridge:** Until printed expiration date or until onboard stability time is reached. Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance. |
| Alinity i FSH Calibrators | 07P4901 | **Store at:** 2 to 8°C**Unopened:** Until printed expiration date**Opened expiration:** Until expiration date. 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: *CAL1 and CAL2* Contains sodium azide. Contact with acids liberates very toxic gas.**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.The purified human FSH used in calibrator 2 was derived from donor units tested and found to be nonreactive for HIV-1, HIV-2, hepatitis B, and hepatitis C.For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.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: | 0.1 to 150.0 mIU/mL |
| Reference Material: | Alinity i FSH Calibrator kit |
| Suggested Calibration Levels: | CAL 1: 0 mCAL 2: 100 |
| Calibration Scheme: | 2 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 twice annually using the AUDIT K833M-5 by running all applicable levels in triplicate. Assay results are submitted to Audit Microcontrols and/or entered in EP Evaluator for compilation. Results are reviewed and approved by the Technical Specialist. Questionable results are investigated and corrective actions documented. |

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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:* Hemoglobin: up to ≤ 500 mg/dL
* Bilirubin up to: ≤ 20 mg/dL
* Triglycerides up to: ≤ 3000 mg/dL
* Protein up to: ≤ 12 g/dL
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| **Reference Intervals** | **Female:** < 1 Year: 0.38-10.4 1 to < 9 Years: 0.42-5.45 9 to < 11 Years: 0.44-4.22 11 to < 19 Years: 0.26-7.77 **Included on all Female test results:** Normally menstruating females: Follicular Phase: 3.03 - 8.08 Mid-Cycle Peak: 2.55 - 16.69Luteal Phase: 1.38 - 5.47 Post-menopausal females: 26.72 - 133.41 **Male**: < 1 Year: 0.09-2.411 to < 5 Years 0-0.91 5 to < 10 Years: 0-1.62 10 to < 13 Years: 0.35-3.91 13 to < 19 Years 0.78-5.1 Adult: 0.95 - 11.95 |
| **Critical Values** | None specified |
| **Limitations** | For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.If the FSH results are inconsistent with clinical evidence, additional testing is suggested 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 FSH 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: | 1:5 |
| 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 150.0 mIU/mL without error messages are released
* Results below 0.1 without error messages are reported as < 0.1 mIU/mL.
* Results > 150.0 should be diluted using the onboard automated 1:5 dilution. Release results without error messages following this dilution.
* Results > 900.0 following automated dilution are reported as > 900.0 mIU/mL.

**Included on all Female test results:** Normally menstruating females: Follicular Phase: 3.03 - 8.08 Mid-Cycle Peak: 2.55 - 16.69Luteal Phase: 1.38 - 5.47 Post-menopausal females: 26.72 - 133.41  |
| **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 FSH Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised April 2018
2. Abbott Alinity i FSH 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 11, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected instrument name, number, added more information: AMR, QC material, reference intervals, references, dilution, etc for new procedure. |