KAISER PERMANENTE®
COLORADO LABORATORY

hLH TEST FOR BECKMAN / COULTER ACCESS II

PRINCIPLE

Human Luteinizing Hormone (hLH, Lutropin) is made up of two non-identical, non-covalently associated glycoprotein subunits, denoted alpha and beta. It has been reported that the 28,500 dalton molecular weight hLH contains two N-linked carbohydrate chains on the alpha subunit and one asparagine-linked oligosaccharide on the beta subunit. The alpha subunit is similar in structure for the glycoproteins hLH, hCG, hFSH, and hTSH. It is the differences in the beta subunit of these glycoproteins which contributes to immunological and physiological specificity.1,2,3

In the female, hLH stimulates the final maturation of the follicle, follicular rupture, and ovulation.3 Human LH is secreted by the gonadotropic cells of the anterior lobe of the pituitary gland in response to gonadotropin releasing hormone (GnRH) from the medial basal hypothalamus. Both hLH and hFSH are secreted in a pulsatile nature; however, this is less noticeable for hFSH perhaps due to the longer half life in the circulation.3 In a normal menstrual cycle negative feedback by estradiol suppresses hLH secretion in the follicular phase. As the follicle develops (in response to hFSH) estradiol production increases which triggers an increase in GnRH and an increased sensitivity of the pituitary to GnRH. A GnRH surge results in the preovulatory (mid-cycle) surge of hLH and ovulation. Following this surge, hLH is suppressed during the luteal phase due to negative feedback from progesterone and estradiol.

Variation in cycle lengths are observed in normally menstruating females due to variations in the length of the follicular phase. In the menopausal female, hLH levels are elevated in response to decreased production of ovarian estrogens and progestogens, which eliminates the negative feedback mechanism on the pituitary gland. As a result, ovulation and menstrual cycles decrease and eventually cease.6

In the male, hLH is often referred to as interstitial cell-stimulating hormone and influences the production of testosterone by the Leydig cells of the testes.7

Concentrations of hLH and hFSH are commonly determined in investigations of menstrual cycle, fertility, and pubertal developmental abnormalities, such as premature ovarian failure, menopause, ovulatory disorders and pituitary failure.8 The ratio of hLH/hFSH has been used to assist in the diagnosis of polycystic ovary disease. Low concentrations of hLH and hFSH may indicate pituitary failure while elevated concentrations of hLH and hFSH along with decreased concentrations of gonadal steroids may indicate gonadal failure (menopause, ovariectomy, premature ovarian syndrome, Turners Syndrome).9 Low concentrations of gonadotropin are usually observed in females taking oral steroid based contraceptives.10 In the male, elevated hLH and hFSH with low concentrations of gonadal steroids may indicate testicular failure or anorchia. In Klinefelter's syndrome hLH may be elevated due to Sertoli cell failure.11

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Franklin Laboratory.

SPECIMEN REQUIREMENTS

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
3. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
4. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
5. Thaw samples only once.
6. Use the following guidelines when preparing specimens, unless instructed otherwise in the product insert:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

7. Frozen specimens can be stored up to six months before testing

Order Codes

Cerner Code	Cerner Description	Health Connect Code	Health Connect Description
LH	LUTEINIZING HORMONE	83002B	LH

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EQUIPMENT AND MATERIALS

1. Beckman Coulter Access II Analyzer(Franklin Facility ID# 982972/SN #504760)
2. Beckman Coulter Access reaction Vessels Ref#81901
3. Beckman Coulter Access Waste Bags Ref#81904
4. Transfer Pipettes Item # 9990 5580
5. Fiber-free polyester swabs
6. 2.0 mL sample cups(3 for maintenance)

REAGENTS

Access hLH Reagent Pack
Cat. No. 33510: 100 determinations, 2 packs, 50 tests/pack
• Provided ready to use.
• Store upright and refrigerate at 2 to 10°C.
• Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
• Stable until the expiration date stated on the label when stored at 2 to 10°C.
• Stable at 2 to 10°C for 28 days after initial use.
• Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
• If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
• All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic Particles coated with goat anti mouse IgG: mouse monoclonal anti-hLH complexes suspended in TRIS buffered saline with bovine serum albumin (BSA), surfactant, <0.1% sodium azide, and 0.1% ProClin**300.
R1b:	TRIS-buffered saline with BSA, protein(mouse,goat),surfactant, <0.1% sodium azide, and 0.1% ProClin 300.
R1c:	Goat anti-hLH-alkaline phosphatase conjugate in TRIS saline buffer with BSA, protein(goat), surfactant, <0.1% sodium azide, and 0.1% ProClin 300.

Warnings and Precautions
• For in vitro diagnostic use.
• Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
• Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.12
• Xi. Irritant: 0.1% ProClin 300.
• The Material Safety Data Sheet (MSDS) is available upon request.

CALIBRATION- See Additional Access Calibration Procedure

QUALITY CONTROL- See Additional Access QC procedure.

PROCEDURE

- **Loading patient samples – Step by step instructions**

1. Place SST tube in appropriate Rack
2. Load Rack and press run.
3. For Manual Entry:
    - Go to “sample manager” and barcode the rack.
    - Select “test request”
    - Use the barcode scanner and read the barcode on the plasma cup.
    - Select the test “LH” and press ENTER.
    - Turn on batch mode to program the rest of the samples on the same rack for the same test.
    - Use the barcode scanner and read the barcode of all the samples you have on the rack.
4. Load the rack on the instrument – remember to tell, wait, do, done – then press RUN.
5. Check QC on log to make sure it is OK before reporting patient results.
6. The completed report will automatically print
7. Enter results in CERNER – see below
8. Cap and save samples in the back refrigerator and discard after 24 hours.

- **Procedural Comments**

A. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

B.. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

C. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes. Use one hundred fifty-five (155) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

D. The system default unit of measure for sample results is mIU/mL. To change sample reporting units to the International System of Units (SI units), IU/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply mIU/mL by multiplication factor 1.

REPORTING RESULTS

1. Patient results are entered in the “ARE” application of CERNER – accession or instrument queue mode.
2. Enter the accession # (by typing or scanning the barcode) in the accession # field.
3. PERFORM then VERIFY.
4. Patient test results are determined automatically by the system software using a smoothing spline math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

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- **Imprecision**

  This assay exhibits total imprecision of less than 10% across the assay range. One study, using commercially available human serum based control material generating a total of two assays,two replicates per assay, over 10 days provides the following data, analyzed via analysis of variance (ANOVA).[17,18]

REFERENCE RANGES

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. hLH levels were measured in human serum samples from 50 adult males, 50 postmenopausal females, and 26 normal cycling females. The cycles were synchronized to the mid-cycle hLH peak. The range of hLH levels generated at Beckman Coulter, Inc., are summarized below:

	Males		**Females hLH**	(mIU/mL)	
	hLH(mIU/ml)	Mid-Follicular	Mid-Cycle Peak	Mid-Luteal Phase	Postmenopausal
Number	50	29	26	27	50
Mean	3.75	5.88	52.84	4.84	30.55
Range	1.24-8.62	2.12-10.89	19.18-103.03	1.20-12.86	10.87-58.64

Laboratory	**Analytical Measurement Range**	**Reportable Range**
Franklin (hLH)	0.2 - 250 mIU/mL	0.2 – 250 mIU/mL

LIMITATIONS OF THE PROCEDURE

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.2-250 mIU/mL [IU/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.2 mIU/mL [IU/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 213 mIU/mL (IU/L).
   - If a sample contains more than the stated value of the highest Access hLH Calibrator (S5), report the result as greater than that value (i.e., > 250 mIU/mL [IU/L]). Alternatively, dilute one volume of sample with one volume of Access hLH Calibrator S0 (zero) or Access Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
     The DxI system onboard dilution feature automates the dilution process, using one volume of sample with one volume of Access Sample Diluent A, allowing samples to be quantitated up to approximately 500 mIU/mL (IU/L). The system reports the results adjusted for the dilution.

2. For assays employing antibodies, the possibility exists for interference by heterophile

antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[15,16]
Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

3. The Access hLH results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

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