Snibe Diagnostic

CE REF 130202002M: 100 tests
130602002M: 50 tests

MAGLUMI® LH (CLIA)

INTENDED USE
The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Luteinizing hormone (LH) in human serum with the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST
Luteinizing hormone (LH, also known as lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland. LH is a heterodimeric glycoprotein. Each monomeric unit is a glycoprotein molecule; one alpha and one beta subunit make the full, functional protein. Its structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The protein dimer contains 2 glycopeptides subunits, labeled alpha and beta subunits that are non-covalently associated[1].
In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum[2]. LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells. With the rise in estrogens, LH receptors are also expressed on the maturing follicle, which causes it to produce more estradiol. Eventually, when the follicle has fully matured, a spike in 17α-hydroxyprogesterone production by the follicle inhibits the production of estrogens, leading to a decrease in estrogen-mediated negative feedback of GnRH in the hypothalamus, which then stimulates the release of LH from the anterior pituitary[3-4].
In males, where LH had also been called interstitial cell–stimulating hormone (ICSH), it stimulates Leydig cell production of testosterone. It acts synergistically with FSH[2,5]. LH acts upon the Leydig cells of the testis and is regulated by gonadotropin-releasing hormone (GnRH). The Leydig cells produce testosterone (T) under the control of LH, which regulates the expression of the enzyme 17β-hydroxysteroid dehydrogenase that is used to convert androstenedione, the hormone produced by the testes, to testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis[6-7].
In children with precocious puberty of pituitary or central origin, LH and FSH levels may be in the reproductive range instead of the low levels typical for their age. During the reproductive years, relatively elevated LH is frequently seen in patients with polycystic ovary syndrome; however, it would be unusual for them to have LH levels outside of the normal reproductive range. Persistently high LH levels are indicative of situations where the normal restricting feedback from the gonad is absent, leading to a pituitary production of both LH and FSH. While this is typical in menopause, it is abnormal in the reproductive years. Diminished secretion of LH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In females, amenorrhea is commonly observed.

PRINCIPLE OF THE TEST
The LH assay is a sandwich chemiluminescence immunoassay.
The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-LH monoclonal antibody, ABEI labeled with another monoclonal antibody are mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of LH present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS
Material Provided

Components	Contents	100 tests (REF: 130202002M)	50 tests (REF: 130602002M)
Magnetic Microbeads	Magnetic microbeads coated with anti-LH monoclonal antibody, Containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing bovine serum and LH antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing bovine serum and LH antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
ABEI Label	Anti-LH monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
Internal Quality Control	Containing bovine serum and LH antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided
MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION
To perform an accurate calibration, we have provided the test calibrators standardized against the WHO 2nd International Standard 80/552.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification

(RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:
- After each change of lots (Reagent or Starter 1+2).
- Every 4 weeks and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If control results lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***LH (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:
- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed one time. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 24 hours at 2-8°C. Freeze samples at or below -20°C if the sample is not assayed within 24 hours.
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of LH is 40 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

High-Dose Hook

For the LH assay, no high dose hook effect was observed when samples containing LH up to 3,000 mIU/mL.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

Calculation of Results

The analyzer automatically calculates the LH concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of mIU/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected ranges for the LH assay were obtained by testing 400 apparently healthy individuals (111 males and 289 females) in China, and gave the following expected value:
Males: 1.1-25 mIU/mL (2.5^{th}-97.5^{th} percentiles).
Femals:

Phase	N	2.5^{th}-97.5^{th} percentiles (mIU/mL)
Follicular phase	65	1.2-12.5
Ovulatory phase	33	12-82
Luteal phase	66	0.4-19
Postmenopause	125	14-48

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the LH assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 2 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(mIU/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(mIU/mL)	%CV	SD(mIU/mL)	%CV	SD(mIU/mL)	%CV
Serum Pool 1	1.155	0.063	5.46	0.057	4.94	0.085	7.36
Serum Pool 2	12.499	0.580	4.64	0.550	4.40	0.799	6.39
Serum Pool 3	84.086	2.493	2.97	1.770	2.10	3.057	3.64
Control 1	24.884	1.088	4.37	1.027	4.13	1.496	6.01
Control 2	48.541	1.941	4.00	1.261	2.60	2.315	4.77

Limit of Blank (LoB)

The LoB for the LH assay is 0.1 mIU/mL.

Limit of Detection (LoD)

The LoD for the LH assay is 0.2 mIU/mL.

Measuring Range

0.1-250 mIU/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.1 mIU/mL. Values above the measuring range are reported as >250 mIU/mL.

Linearity

The assay is linear between 0.2 mIU/mL and 250 mIU/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a serum sample containing LH 260 mIU/mL with a serum sample depleted of LH (0.0 mIU/mL).The mean sample recovery ranged from 90% to 110%.

Method Comparison

A total of 100 samples in the range of 1.12 to 244.79 mIU/mL were tested by the LH assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=0.965x+1.050$. $r^2=0.988$.

Analytical Specificity

The specificity of the assay was obtained by adding FSH (150 mIU/mL), HCG (200000 mIU/mL), and TSH (100 μIU/mL) to serum samples at the

indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 66 mg/dL
- Hemoglobin 1000 mg/dL
- Triglyceride 1900 mg/dL

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code