PATHOZYME® LUTEINIZING HORMONE Ref OD357

Enzyme Immunoassay for the quantitative detection of LH in human serum.

Store at 2°C to 8°C. DO NOT FREEZE.

For in vitro use only.

INTRODUCTION

Luteinizing Hormone (LH) is produced in both males and females from the anterior pituitary gland in response to luteinizing hormone releasing hormone (LH-RH or Gn-RH) which is released by the hypothalamus. LH, also called Interstitial Cell Stimulating Hormone (ICSH) in men, is a glycoprotein of a molecular weight of 30,000 daltons. It is composed of 2 non-covalently associated dissimilar amino acid chains, alpha and beta. The alpha chain is similar to those found in Thyroid Stimulating Hormone (TSH) Follicle Stimulating Hormone (FSH), and human Chorionic Gonadotropin (hCG). The differences between these hormones lies in the amino acid composition of the beta subunits accounting for their immunological differentiation.

Basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells to produce testosterone. The variation in LH concentrations in women is subject to the ovulatory cycle of healthy menstruating women and depends on hormonal events involving the hypothalamus and pituitary glands. The decrease in progesterone and oestradiol levels from the preceding ovulation initiates each menstrual cycle. As a result of the decrease in hormone levels, the hypothalamus increases the secretion of gonadotropin releasing factors (GnRF), which in turn stimulates the pituitary to increase FSH production and secretion.

These rising FSH levels stimulate several follicles during the follicular phase and one of these will mature to contain the egg. As the follicle develops, oestradiol is secreted, slowly at first, but increases rapidly by day 12 or 13. LH is released as a result of this due to direct stimulation of oestradiol on the pituitary which in turn increases levels of GnRF and FSH. These events mark the preovulatory phase. Ovulation occurs approximately 12 to 18 hours after the LH reaches maximum levels.

After the egg is released, the corpus luteum is formed which secretes progesterone and oestrogen, which are feed back regulators of LH.

The Luteal phase follows the ovulatory phase which is characterised by high progesterone levels, a second oestradiol increase and low LH and FSH levels. Low levels of LH and FSH are a result of negative feedback effects of oestradiol and progesterone.

After conception, the developing embryo produces hCG, which causes the corpus luteum to continue producing progesterone and oestradiol. The corpus luteum regresses if the pregnancy does not occur and the corresponding drops in progesterone and oestradiol levels results in menstruation. The hypothalamus initiates the menstrual cycle again as a result of low hormone levels.

Patients suffering from Hypogonadism show increased concentrations of LH in their serum. A decrease in steroid hormone production in females is a result of immature ovaries, primary ovarian failure, polycystic ovary disease or menopause and in these cases LH secretion is not regulated.

A similar loss of hormone regulation occurs in males with abnormal testes development or anorchia. In cases of Klinefelter Syndrome and primary testicular failure, high concentrations of LH may be found. However, LH levels will not necessarily be elevated if the secretion of androgens continues. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism and severe starvation.

A lack of secretion by the anterior pituitary may cause lower levels of LH. These low levels can result in infertility in both males and females. Low levels may also be due to decreased secretion of GnRH by the hypothalamus or an inability of the pituitary to respond to these factors.

Therefore, low levels of LH may indicate dysfunction of the pituitary or hypothalamus, although the actual source of the dysfunction must be confirmed by other tests.

In conjunction with FSH assays, LH assays are routinely performed in the differential diagnosis of hypothalamic, pituitary and gonadal dysfunction. Such hormone levels are also used to determine menopause, pinpoint ovulation and monitor endocrine therapy.

The following preparations were tested as negative: HCG (WHO 2nd International Standard 61/2) at less than 1,000 mIU/ml, FSH (WHO 2nd International Reference Preparation HMG) at less than 125 mIU/ml and TSH (WHO 2nd IRP 80/558) at less than 62.5µIU/ml.

INTENDED USE

PATHOZYME LH is an Enzyme Immunoassay (EIA) for the quantitative determination of Luteinizing Hormone (LH) in human serum.

For professional use only.

PRINCIPLE OF THE TEST

Specific anti-LH antibodies are prepared, purified and coated onto microtitration wells. Test sera are applied. Then Monoclonal anti-LH labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If human LH is present in the sample, it will combine with the antibody on the well and the enzyme Conjugate, resulting in LH molecules being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed with distilled water to remove unbound labelled antibodies.

On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of LH. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of LH is directly proportional to the colour intensity of the test sample.

This test has been calibrated against WHO, 1st IRP 68/40

CONTENTS

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96

Microtitre Plate	**12 x 8 wells x 1**
Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.	
Cal A 0mIU/ml	**1**
Reference Standard: Human serum free of LH. Lyophilised.	
Cal B 5mIU/ml	**1**
Reference Standard: LH diluted in human serum. Lyophilised.	
Cal C 15mIU/ml	**1**
Reference Standard: LH diluted in human serum. Lyophilised.	
Cal D 50mIU/ml	**1**
Reference Standard: LH diluted in human serum. Lyophilised.	
Cal E 100mIU/ml	**1**
Reference Standard: LH diluted in human serum. Lyophilised.	
Cal F 200mIU/ml	**1**
Reference Standard: LH diluted in human serum. Lyophilised.	
Conj	**11 ml**
Anti-LH HRP Conjugate: Anti-LH conjugated to Horseradish Peroxidase. Ready to use (Pink)	
Subs TMB	**11ml**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	
Soln Stop HCl 1M	**11ml**
Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)	
Instruction leaflet and EIA Data Recording Sheet	**1 + 1**

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl and 1000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME LH contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All Reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

PATHOZYME LH Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME LH Stop Solution is Dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME LH reagents contain 1% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

Proclin™ 300 is a trade mark of ROHM & HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS(Except Standards for storage) as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversly affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

Add 1ml of distilled water to each standard vial in order to reconstitute the lyophilised standards. Allow to stand for at least 20 minutes then mix gently. Rehydrated standards can be stored at 2 °C to 8 °C for 30 days. For long term storage freeze and aliquot at -20 °C. Freeze thaw only once. Thawed standards must be mixed prior to testing.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.
Pregnancy results in elevated levels of hCG therefore the diagnostic use of **PATHOZYME LH** is not recommended during pregnancy or immediately post pregnancy.

ASSAY PROCEDURE

1. Bring all the kit components and test serum to room temperature (20°C to 25°C) prior to the start of the assy.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 50µl of Standards and test serum into the assigned wells.
5. Dispense 100µl of Anti-LH Conjugate Reagent into each well.
6. Thoroughly mix for 30 seconds. It is very important to have complete mixing at this stage.
7. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).
8. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
9. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper.
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense 100µl Substrate Solution into each well and mix gently for 5 seconds.
13. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
14. Stop the reaction by adding 100µl Stop Solution to each well.
15. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
16. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

CALCULATION OF RESULTS

Calculate the meen absorbance value (A_{450}) for each set of Standards and test sera. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in mlU/ml on graph paper. Use the mean absorbance values for each test sera to determine the corresponding concentration of LH in mlU/ml from the standard curve.
If levels of controls or users known samples do not give expected results, test results must be considered invalid.
If using a software package choose a quadratic regression curve fit

EXPECTED VALUES AND SENSITIVITY

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators proportional to their concentration. The OD of Calibrator A should be less than 0.75 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid.

Each laboratory must establish its own normal ranges based on patient populations. The results provided below are based on randomly selected out-patient clinical laboratory samples.

	No. of		LH(mIU/ml)	
	Age	**Patients**	**Mean**	**Range**
Male	<10	25	1.3	<2.5
Male	15-60	56	4.8	1.0 to 15.0
Female	<10	25	1.1	<2.0
Female	20-35	60	15.0	1.0 to 90.0
Female	46-60	40	38.0	8.0 to120.0

The minimum detectable concentration of human Luteinizing Hormone by **PATHOZYME LH** is estimated to be 1mIU/ml.

Concentrations of 4,000 mIU/ml have been observed using **PATHOZYME LH** with no prozone (Hook) effect.

EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of **PATHOZYME LH** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme LH kit and the Nichols Allegro LH IRMA Kit for samples with levels between 0.2 and 55.8 mIU/ml the following data was generated.

Number of Samples	113
Correlation Co-efficient	0.958
Slope	1.115
Intercept	0.49
Omega Mean	10.1 mIU/ml
Nichols Allegro Mean	8.6 mIU/ml

These kits were shown to give good correlation.

REFERENCES

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4. **Shome, B., and Parlow, A.F.** *J. Clin. Endocrinol. Metab.* 39:203-205; 1974.
5. **Uotila, M., Ruoslahti, E., and Engval, E.** *J. Immuno. Methods.* 42:11-15; 1981.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of samples or Standards and 100µl of Anti-LH Conjugate into each well and mix thoroughly for 30 seconds.
2. Incubate for 45 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash 5 times with distilled water.
4. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.
5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
6. Add 100µl Stop Solution to each well and gently shake for 30 seconds.
7. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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