Luteinizing Hormone (LH) is produced in both males and females from the anterior pituitary gland in response to luteinizing hormone releasing hormone (LHRH or GnRH) 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 comprised of 2 non-covalently associated disulphide 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 Gonadotrophin (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 oestraediol levels from the postovulatory rise in LH result in increased LH and prolactin levels. 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 and prolactin 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, oestraediol is secreted, slowly at first, inhibiting the anterior pituitary and further stimulating LH production. LH, in turn stimulates the ovulation of the mature follicle resulting in the release of the oocyte. The release of the oocyte initiates the menstrual cycle again as a result of LH stimulation from the corpus luteum.

After the egg is released, the corpus luteum is formed which secretes progesterone and oestraediol, which are feed back regulators of LH. The Luteal phase follows the ovulatory phase which is characterised by high LH, progesterone levels, a second oestraediol increase and low LH and FSH levels. Low levels of LH and FSH are a result of negative feedback effects of oestraediol and progesterone.

After conception, the developing embryo produces hCG, which causes the corpus luteum to continue producing progesterone and oestraediol. The corpus luteum regresses if the pregnancy fails to continue. LH does not occur during renal failure, cirrhosis, 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 and also due to decreased secretion of GnRF 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. 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 hyperprolactinemia 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 97/1) at less than 1,000 mIU/ml, FSH (WHO 2nd International Reference Preparation HMG) at less than 125 mIU/ml and TSH (WHO 2nd IOIP 80/558 ) at less than 82.5 mIU/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 microtiter wells. Test sera are applied. Then Monoclonal anti-LH labelled with Norovirgin Peroxidase enzyme (Corpiugate) is added. If human LH is present in the sample, it will combine with the antibody on the well and the enzyme Corpiugate, 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 a Substrate (TMB), a colour will develop only in those wells in which LH is present, indicating the presence of LH. The enzyme reaction is stopped by the addition of Sulphuric 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 IOIP 69/40

Microtitre Plate

12 x 8 wells x 1

Reference standard: Human serum free of LH

Lyophilised

1

Cat A 1

5mlU/ml

Lyophilised

1

Cat B 5mlU/ml

Lyophilised

1

Cat C 1mlU/ml

Lyophilised

1

Cat D 50mlU/ml

Lyophilised

1

LH Standard 500mlU/ml

Lyophilised

1

Cat E 100mlU/ml

Lyophilised

1


Lyophilised

1

Cat F 200mlU/ml

Lyophilised

1


Lyophilised

1

Cal G 1ml

Lyophilised

1

Cal H 1ml

Lyophilised

1

Cal I 1ml

Lyophilised

1

Cal J 1ml

Lyophilised

1

Cal K 1ml

Lyophilised

1

Stop Solution: Hydrochloric Acid diluted in purified water.

Ready to use. (Colourless)

Instruction leaflet and EIA Data Recording Sheet

1 x 1

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes, 10ul, 200ul and 1000ul

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 patho tested and confirmed negative for HAV, HEP and HIV 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 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. No precautionary measures 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 Dinitro Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with 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 adversely 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 Acetate 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 4°C to 8°C for 30 days. For long term storage freeze and thaw at -20°C. Thawed standards must be mixed gently 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. Diagnoses 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 assay.
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 50l of Standards and test serum into the assigned wells.
5. Dispense 100l of Anti-LH Conjugate Reactant into each well.
6. Thoroughly mix for 30 seconds. It is very important to have complete mixing at this stage.
7. Incubate 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 an adequate disinfectant is contained in the Biohazard container.
9. Hand Washing. Fill the wells with a minimum of 300l of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure an adequate disinfectant is contained in the Biohazard container.
10. Rinse the strips 7 times with distilled water.
11. Machine Washing: Ensure that 300l 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 100l 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 100l 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.

Calculated to major competitors and in house standards.

The co-efficients 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.

EVALUATION DATA

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>113</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>1.00</td>
</tr>
<tr>
<td>Slope</td>
<td>1.11</td>
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<tr>
<td>Intercept</td>
<td>0.49</td>
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<tr>
<td>Nichols Allegro Mean</td>
<td>6.6 mIU/ml</td>
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These kits were shown to give good correlation.

REFERENCES


QUICK REFERENCE TEST PROCEDURE

1. Dispense 50l of standards or Standards and 100l 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 100l 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 100l 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.

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.

Add 100l of Standards and test serum into the assigned wells. Add 100l of Substrate Solution into each well and mix gently for 5 seconds. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C). Stop the reaction by adding 100l Stop Solution to each well. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour. 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, 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 inoperable. 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 mean absorbance value (A0) for each set of Standards and test sera. Construct a standard curve by plotting the mean absorbance from each standard against its concentration in mIU/ml on graph paper. Use the mean absorbance values for each test sera to determine the corresponding concentration of LH in mIU/ml from the standard curve. If levels of controls or unknown 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 range based on patient populations. The results provided below are based on randomly selected un-patient clinical laboratory samples.

The minimum detectable concentration of human Luteinising 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.

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