PATHOZYME® HUMAN CHORIONIC GONADOTROPHIN

Ref OD347

Enzyme-Immunoassay (EIA) for the quantitative determination of hCG in human serum

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro use only.

INTRODUCTION

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by the placenta during pregnancy. The hCG molecule consists of two combined, dis-similar subunits designated alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 daltons, confers biological and immunological specificity to the entire hCG molecule by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH).
The appearance of hCG in urine or serum soon after conception and its rapid rise in concentration makes it an ideal indicator for the detection and confirmation of pregnancy. However, elevated hCG levels are also frequently associated with trophoblastic and non-trophoblastic neoplasmas; these conditions should be considered before a diagnosis of pregnancy can be made.
Immunoassays utilising antibodies specific to the beta subunit of hCG provide a sensitive and specific technique allowing early detection of pregnancy around the time of the first missed menstrual period. In women with a multiple pregnancy (twins, triplets, etc.) levels of the hCG have been reported to be higher than those expected during a normal single pregnancy. This is probably the result of the increased placental mass necessary to sustain multiple foetuses. Also, as one might expect, cases of placental insufficiency show levels of hCG lower than those expected during normal pregnancy. Decreased values have also been associated with threatened abortion and ectopic pregnancy.

The following preparations were tested as negative: TSH (WHO International Reference Preparation 80/558) at less than 250 μ IU/ml, FSH (WHO International Reference Preparation - HMG) at less than 500 mIU/ml, Prolactin (WHO International Reference Preparation 84/500) at less than 500 ng/ml and HCG (WHO International Reference Preparation 65/217) at less than 100 ng/ml.
A cross reaction of 1.6% was detected with LH (WHO International Reference Preparation 68/40) at a level of 500 mIU/ml.

INTENDED USE

PATHOZYME hCG is an Enzyme Immunoassay (EIA) for the quantitative determination of Human Chorionic Gonadotropin (hCG) in human serum.
For professional use only.

PRINCIPLE OF THE TEST

Specific monoclonal anti-hCG antibodies are coated on to microtitration wells. Test sera are applied and incubated with Zero Buffer. If human hCG is present in the sample, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen and then hCG antibody, labelled with Horseradish Peroxide enzyme (Conjugate) is added. This results in the hCG molecules being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed 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 hCG. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of hCG is directly proportional to the colour intensity of the test sample.
This test has been calibrated to the WHO 1st IRP / 3rd IS 75/537.

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Microtitre Plate — **12 x 8 wells x 1**
Breakable wells coated with specific antibody contained in a resealable foil bag with a desiccant.

Cal A 0 mIU /ml — **1 ml**
Reference Standard: Human serum free of hCG.
Ready to use. (Colourless)

Cal B 5 mIU /ml — **1 ml**
Reference Standard: hCG diluted in human serum.
Ready to use. (Colourless)

Cal C 20 mIU /ml — **1 ml**
Reference Standard: hCG diluted in human serum.
Ready to use. (Colourless)

Cal D 50 mIU/ml — **1 ml**
Reference Standard: hCG diluted in human serum.
Ready to use. (Colourless)

Cal E 150mIU/ml — **1 ml**
Reference Standard: hCG diluted in human serum.
Ready to use. (Colourless)

Cal F 300mIU/ml — **1 ml**
Reference Standard: hCG diluted in human serum.
Ready to use. (Colourless)

Washbuf X20 — **50 ml**
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)

BUF AS — **11 ml**
Zero Buffer. Phosphate based buffer containing stabilising proteins. Ready to use. (Green)

Conj — **16.5 ml**
Anti- hCG HRP Conjugate: Anti- hCG conjugated to HRP. Ready to use (Red)

Subs TMB — **11ml**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)

Soln Stop HCl 1 M — **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, 1000μl and 5000μl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

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

PATHOZYME hCG 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 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.

Wash Buffer:
Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with 19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml of distilled water. Prepare fresh diuluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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.

ASSAY PROCEDURE

1. Bring all the kit components and the 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, Control and Test Serum into the assigned wells and gently mix for 30 seconds.
5. Dispense 100µl of Zero Buffer into each well.
6. Thoroughly mix for 10 seconds. It is very important to have a complete mixing at this stage.
7. Incubate the plate for 30 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 wash buffer 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 wash buffer 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 150µl Anti-hCG HRP Conjugate into each well. Gently mix for 5 seconds.
13. Incubate for 15 minutes at room temperature (20°C to 25°C).
14. Wash plate as described above.
15. Dispense 100µl of Substrate Solution into each well and gently mix for 5 seconds.
16. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
17. Stop the reaction by adding 100µl Stop Solution to each well.
18. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
19. 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 mean absorbance value (A^{450}) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance values for each specimen to determine the corresponding concentration of hCG in mIU/ml from the standard curve.
If levels of Calibrators or users known samples do not give expected results, test results must be considered invalid.
If using a sofware 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. hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of hCG in the serum of pregnant women increases to 5-50 mIU/ml one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100,000-200,000 mIU/ml at the end of the first trimester.
The minimum sensitivity of **PATHOZYME hCG** test is 2.0mIU/ml.
Concentrations of 1, 000, 000 mIU/ml have been observed using Pathozyme hCG with no prozone (Hook) effect.

EVALUATION DATA

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

In an evaluation of samples between the Omega Pathozyme hCG kit and the BioRad CoTube Hcg IRMA Kit and the Serono hCG MAIAclone Kit the following data was generated.

Number of Samples	53
Correlation coefficient	0.994
Slope	0.992
Intercept	-0.781
Omega Mean	267.5 mIU/ml
BioRad Mean	264.5 mIU/ml

In an evaluation of samples between the Omega Pathozyme hCG kit and the Serono hCG MAIAclone Kit the following data was generated.

Number of Samples	53
Correlation coefficient	0.996
Slope	0.902
Intercept	4.970
Omega Mean	267.5 mIU/ml
Serono Mean	246.2 mIU/ml

These kits were shown to give good correlation.

REFERENCES

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(3) **Dipietro, D. L.** *Laboratory Management.* 1981;19:1.

(4) **Uotillia, M., Ruoslahti, E. and Engvall, E.** *J. Immunol. Methods*. 1981;42:11-15.

(5) **Messeyeff, R. and Malolini, R.** *J. Immunol. Methods*. 1975;8:233

QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of test serum or Standards into appropriate wells. Gently mix for 30 seconds.
2. Dispense 100µl of Zero Buffer into each well. Thoroughly mix for 10 seconds.
3. Incubate for 30 minutes at room temperature (20°C to 25°C).
4. Discard well contents and wash five times with wash buffer.
5. Add 150µl of Anti-hCG HRP Conjugate into each well and gently mix for 5 seconds.
6. Incubate for 15 minutes at room temperature (20°C to 25°C).
7. Discard well contents and wash five times with wash buffer.
8. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.
9. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
10. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
11. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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