PATHOZYME® PROGESTERONE Ref OD487
Enzyme Immunoassay for the quantitative determination of Progesterone in human serum or plasma.
Store at 2°C to 8°C. DO NOT FREEZE.
For in-vitro use only.

INTRODUCTION
Progesterone is a C21 steroid which is synthesised from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolised in the liver to pregnandiol conjugated as a glucuronide prior to excretion by the kidneys.
Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak.

MEASUREMENT PRINCIPLE
Progesterone measurements are thus performed to determine ovulation as well as to characterise luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluation comprises the remaining uses of progesterone assays.

INTENDED USE
PATHOZYME PROGESTERONE is an Enzyme Immunoassay (EIA) for the quantitative determination of total Progesterone in human serum or plasma.

PRINCIPLE OF THE TEST
The PATHOZYME PROGESTERONE is based on the principle of competitive binding between Progesterone in the test specimen and Progesterone-HRP Conjugate for a constant amount of rabbit anti-Progesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with Progesterone standards, controls, patient samples, Progesterone-HRP Conjugate Reagent and rabbit anti-Progesterone Reagent. During the incubation, a fixed amount of HRP-labelled Progesterone competes with the endogenous Progesterone in the standard and sample or quality control serum for a fixed number of binding sites of the specific Progesterone antibody. Thus, the amount of Progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Progesterone in the specimen increases. Unbound Progesterone peroxidase conjugate is then removed and the wells washed. The Substrate (TMB) is then added, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of rabbit anti-Progesterone reagent remaining to react with the standard and sample or quality control serum for a fixed number of binding sites of the specific Progesterone antibody.

CAPTURE REAGENT
Coated wells are incubated with Progesterone-HRP Conjugate for a constant amount of rabbit anti-Progesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with Progesterone standards, controls, patient samples, Progesterone-HRP Conjugate Reagent and rabbit anti-Progesterone Reagent. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of rabbit anti-Progesterone reagent remaining to react with the standard and sample or quality control serum for a fixed number of binding sites of the specific Progesterone antibody. Thus, the amount of Progesterone peroxidase conjugate immunologically bound to the well is obtained by plotting the concentration of the standard versus the absorbance. The Progesterone concentration in the specimen can be calculated from the standard curve.

The test has been calibrated against in-house standards. There is no international standard for this test.

CONTENTS

MATERIAL REQUIRED BUT NOT PROVIDED

Microtitre Plate: 12 x 8 wells x 1
Breakable wells coated with Goat anti Rabbit IgG contained in a nonaqueous film bag with a desiccant.
Cat. A 0.5 mg/ml
Reference Standard: Human serum free of Progesterone. Ready to use. (Colourless)
Cat. B 0.5 mg/ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)
Cat. C 25 mg/ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)
Cat. D 10 mg/ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)
Cat. E 25 ng/ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)
Cat. F 0 ng/ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)

Control 1 1 Level as stated on vial
Known level of Progesterone diluted in human serum. Ready to use. (Colourless)

Control 2 2 Level as stated on vial
Known level of Progesterone diluted in human serum. Ready to use. (Colourless)

Ad: HEG  Progesterone
Ready to use. (Pink)

1.3 ml
Cat. G 11X
Progesterone reagent to horseradish Peroxide Ready to use. (Blue)

13 ml
Cat. H 100X
Progesterone coated buffer containing stabilising proteins. Ready to use. (Blue)

11 ml
Substrate Solution: 3.7, 5.5 Tetramethyl Benzidine in a citrate buffer. Ready to use. (Yellow)

1 ml
Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)

Graph paper
Micropipettes: 100, 200, 1000 and 5000µl
Disposable pipette tips
Absorbent paper
Micropipette reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME PROGESTERONE contains materials of human origin which have been tested and confirmed negative for HIV 1 and 2 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 PROGESTERONE 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 PROGESTERONE Stop Solution is dilute 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 preservative which may be toxic if ingested. In case of contact, rinse thoroughly with water and seek medical advice.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Serum: 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.

Plasma: Obtain a sample of venous blood from the patient and add to EDTA blood collection vial. Centrifuge sample and collect clear plasma. Fresh plasma samples are required.

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

For in-vitro use only.

The use of samples other than serum and EDTA plasma have 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 essay.
ASSAY PROCEDURE
1. Bring all the kit components and the test sample 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 sample. Secure the desired number of coated wells in the holder. Record the position of the standards and the test samples on the EIA Data Recording Sheet provided.
3. Unused strips should be sealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dilute 25μl of standards, test samples and controls into the appropriate wells.
5. Dispense 100μl working solution of Progesterone-HRP Conjugate Reagent to each well.
6. Dispense 50μl of rabbit anti-Progesterone Reagent to each well. Thoroughly mix for 30 seconds.
7. Incubate at room temperature (20°C to 25°C) for 90 minutes.
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. Wash the empty wells 5 times.
11. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense a 100μl volume of Substrate solution into each well. Gently mix for 5 seconds.
13. Incubate in the dark at room temperature (20°C to 25°C) for 20 minutes.
14. Stop the reaction by adding 100μl of Stop Solution to each well.
15. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour immediately.
16. Read the absorbance at 450nm with a microtitre well reader within 10 minutes.

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.

Sensitivity
The lowest detectable level of Progesterone in this test is approximately 0.0625 ng/ml.

SPECIFICITY
The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Progesterone.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Progesterone</th>
<th>Androstenedione</th>
<th>Corticosterone</th>
<th>Cortisol</th>
<th>Cholesterol</th>
<th>Oestradiol</th>
<th>Oestrone</th>
<th>Estriol</th>
<th>Prednisolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>100%</td>
<td>0.08%</td>
<td>0.74%</td>
<td>0.11%</td>
<td>&lt;0.09%</td>
<td>&lt;0.01%</td>
<td>0.69%</td>
<td>0.034%</td>
<td>0.075%</td>
</tr>
<tr>
<td>Cross-reactivity</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
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<td>10%</td>
</tr>
</tbody>
</table>

These kits were shown to give good correlation.

REFERENCES

QUICK REFERENCE TEST PROCEDURE
1. Dispense 25μl of standards, test samples, controls.
2. Add 100μl working solution Progesterone HRP conjugate to each well.
3. Add 50μl of Rabbit anti-Progesterone into each well. Gently mix for 30 seconds.
4. Incubate for 90 minutes at room temperature (20°C to 25°C).
5. Discard the well contents and wash 5 times with distilled water.
6. Add 100μl of substrate solution into each well and gently mix for 5 seconds.
7. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
8. Add 100μl Stop Solution to each well and gently mix for 30 seconds.
9. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.