PATHOZYME®OVARIAN CANCER ANTIGEN 125 Ref OD287

Enzyme Immunoassay (EIA) for the quantitative determination of Ovarian Cancer Antigen 125 in human serum.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro diagnostic use only.

INTRODUCTION

Ovarian cancer is the sixth most frequent form of cancer (excluding non-melanoma skin cancers) amongst women and the fifth most common cause of cancer deaths. One of the key markers for this disease is the Cancer Antigen 125.

CA 125 is a glycoprotein with a molecular weight of 200000 – 1000000 Da. It is produced only by epithelial ovarian carcinoma cells and not by normal cells. CA125 is found in greater than 80% of all cases of Ovarian Cancer. 6% of cases with elevated serum CA125 can be attributed to non-gynaecological malignancies such as pancreas or lungs. A selection of non-malignant conditions can also result in an increase in CA125; these conditions include menstruation, endometriosis and pregnancy (during the first trimester). Elevated CA125 is only found in 1% of healthy controls.

When the CA125 levels are monitored with a patient an increase in CA125 indicates progressive malignant disease and poor therapeutic response. A decrease in CA125 shows a favourable prognosis and good therapeutic response.

INTENDED USE

PATHOZYME OVARIAN CANCER ANTIGEN 125 is an Enzyme Immunoassay (EIA) for the the quantitative determination of Ovarian Cancer Antigen 125 in human serum. For professional use only.

PRINCIPLE OF THE TEST

Specific monoclonal anti-Cancer Antigen 125 antibodies are coated on to microtitre wells. Test sera is added to each well. Anti-Cancer Antigen 125 antibody, labelled with Horseradish Peroxidase enzyme (Conjugate) is added to each well and then incubated at 37°C. This results in the Cancer Antigen 125 molecules being sandwiched between the solid phase and the enzyme linked antibodies.
After incubation, the wells are washed with 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 Cancer Antigen 125. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of Cancer Antigen 125 is directly proportional to the colour intensity of the test samples.
This test has been calibrated to in house standards. There is no International Standard for this test.

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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 0 U/ml — **1ml**
Reference Standard: Human serum free Cancer Antigen 125. Ready to use.(Colourless)

Cal B 15 U/ml — **1ml**
Reference Standard: Cancer antigen 125 diluted in Human serum. Ready to use. (Colourless)

Cal C 50 U/ml — **1ml**
Reference Standard: Cancer antigen 125 diluted in human serum. Ready to use. (Colourless)

Cal D 100 U/ml — 1ml
Reference Standard: Cancer antigen 125 diluted in human serum. Ready to use. (Colourless)

Cal E 200 U/ml — **1ml**
Reference Standard: Cancer antigen 125 diluted in human serum. Ready to use. (Colourless)

Cal F 400 U/ml — **1ml**
Reference Standard: Cancer antigen 125 diluted in human serum. Ready to use. (Colourless)

Conj — **11 ml**
Anti-Cancer Antigen 125 HRP Conjugate: Anti-Cancer Antigen 125 conjugated to HRP. Ready to use (Purple)

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
Incubator: Temperature of 37°C +/- 1°C
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME OVARIAN CANCER ANTIGEN 125 contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by approved procedures 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 OVARIAN CANCER ANTIGEN 125 reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. Final disposal must be in accordance with local legistlation.

PATHOZYME OVARIAN CANCER ANTIGEN 125 Stop Solution is diluted Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME OVARIAN CANCER ANTIGEN 125 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 from 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.

LIMITATIONS OF USE

The use of samples other than serum have not been validated in this test. There is no reuse protocol for this product.
When making an inter-pretation 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 100µl of Standards and test serum into the assigned wells and **mix for 10 seconds**.
5. Dispense 100µl of Anti-cancer Antigen 125 HRP Conjugate into each well.
6. Thoroughly mix for 30 seconds. It is very important to have a complete mixing at this stage.
7. incubate plate for 90 minutes at 37°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 10 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 mean absorbance value (A_{450}) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in units/ml on graph paper. Use the mean absorbance values for each specimen to determine the corresponding concentration of Cancer Antigen 125 in U/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 0.2 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid.Healthy women are expected to have Cancer Antigen 125 assay values below 35 U/ml. The minimum detectable concentration of Cancer Antigen 125 by **PATHOZYME OVARIAN CANCER ANTIGEN 125** is estimated to be 5 U/ml.

EVALUATION DATA

Calibrated to major competeitors and in house standards.
The co-efficient of variation of Pathozyme Ovarian Cancer Antigen 125 is less than or equal to 10%.

In an evaluation between the Omega Pathozyme CA 125 kit and the Abbott AxSym CA 125 Kit for samples with levels between 2.0 and 1344 U/ml the following data was generated.

Number of Samples	153
Correlation Co-efficient	0.94
Slope	0.97
Intercept	- 0.372
Omega Mean	114.9 U/ml
Abbott Mean	119.2 U/ml

These kits were shown to give good correlation.

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QUICK REFERENCE TEST PROCEDURE

1. Dispense 100µl of samples or Standards into each well and mix for 10 seconds.
2. Dispense 100µl of Enzyme Conjugate into each well and gently mix for 30 seconds.
3. Incubate for 90 minutes at 37°C.
4. Discard the well contents and wash 5 times with distilled water.
5. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.
6. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
7. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
8. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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