PATHOZYME®BREAST CANCER ANTIGEN 15-3

Ref OD297 Enzyme-Immunoassay (EIA) for the Quantitative Determination of CA15-3 In human serum. Store at 2°C to 8°C. DO NOT FREEZE For in-vitro diagnostic use only.

INTRODUCTION

Breast cancer is the most frequent form of cancer (excluding non-melanoma skin cancers) amongst women and is the leading cause of cancer death in women between the ages of 40 and 55. One of the key markers for this disease is the **Cancer Antigen 15-3**, other markers also include CA549.

CA15-3 is a mucin-type glycoprotein with a high molecular weight. It is localised to the apical side of alveoli and ducts on mammary glands and is present as a circulating antigen. CA15-3 is found in greater than 80% of all cases of metastatic Breast Cancer. CA15-3 can be elevated in benign conditions, especially those hepatic in origin, however these elevated CA15-3 levels are rarely above 100 U/ml. Elevated CA15-3 is only found in 5% of healthy controls.

There is no correlation between serum CA 15-3 levels, disease stage and prognosis. However very high CA15-3 levels (5 – 10 times the normal) tend to indicate advanced disease and possibly the presence of metastatic disease. Studies have suggested that there is a good general correlation between changing CA 15-3 levels and response to therapy in metastatic cancer, but that it cannot be relied on in the absence of confirming clinical data.

Cancer Antigen 15-3 levels are also increased in Colon, Lung and Hepatic Tumours.

INTENDED USE

PATHOZYME BREAST CANCER ANTIGEN 15-3 is an Enzyme Immunoassay (EIA) for the the quantitative determination of Breast Cancer Antigen 15-3 in human Serum.
For professional use only.

PRINCIPLE OF THE TEST

The CA 15-3 ELISA test is based on the principle of a solid phase enzyme linked immunosorbant assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant (on the microtitration plate). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme linked antibodies. After two separate incubation steps the wells are washed with distilled water to remove unbound labelled antibodies. A solution of TMB Reagent is added, a colour will develop only in those wells in which enzyme is present, indicating the presence of Cancer Antigen 15-3. The colour development is stopped with the addition of Stop Solution changing the colour to yellow. The concentration of CA15-3 is directly proportional to the colour intensity of the test sample. Absorbance is then measured spectrophotometrically at 450nm.
This test has been calibrated against in house standards.
There is no International standard for this test.

CONTENTS

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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 U/ml**	**2 ml**
Reference Standard: Human serum free of Cancer antigen 15-3. Ready to use. (Colourless)			
Cal	**B**	**15 U/ml**	**2 ml**
Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)			
Cal	**C**	**30 U/ml**	**2 ml**
Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)			
Cal	**D**	**60U/ml**	**2 ml**
Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)			
Cal	**E**	**120U/ml**	**2 ml**
Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)			
Cal	**F**	**240U/ml**	**2 ml**
Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)			
Conj	**22X**		**1 ml**
Anti-Cancer Antigen 15-3 HRP Conjugate Concentrate: Anti-Cancer Antigen 15-3 concentrate conjugated to Horseradish Peroxidase. (colourless)			
DIL	**SPEC**		**2 X 50ml**
Sample Diluent: Phosphate based buffer containing stabilising proteins. Working strength. (Yellow)			
DIL	**Conj**		**21 ml**
Conjugate Diluent: Phosphate based buffer containing stabilising proteins. Working strength. (Pink)			
Subs	**TMB**		**11 ml**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)			
Soln	**Stop**	**HCl**	**1M** **11 ml**
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
Vortex Mixer
Incubator: Temperature of 37°C +/- 1°C
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME BREAST CANCER ANTIGEN 15-3 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 BREAST CANCER ANTIGEN 15-3 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 BREAST CANCER ANTIGEN 15-3 Stop Solution is diluted Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME BREAST CANCER ANTIGEN 15-3 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.

To prepare working strength Anti-Cancer Antigen 15-3 HRP Conjugate, add one part concentate conjugate to 21 parts conjugate diluent (1/22 dilution): 200µl is required per well. Diluted reagent is stable at 2°C to 8°C for four months.

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. Test serum should be diluted before use. Mix 20µl of test serum with 1000µl of sample diluent.. The CA 15-3 standards are pre-diluted and are ready for use.
5. Dispense 200µl of Standards and diluted sample into the assigned wells and mix for 10 seconds.
6. Incubate for 60 minutes at 37°C.
7. 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.
8. 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.
9. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. 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.
11. Dispense 200µl 1/22 Anti-Cancer Antigen 15-3 HRP Conjugate into each well and mix for 10 seconds.
12. Incubate the plate for 60 minutes at 37°C.
13. Wash plate as above.
14. Dispense 100µl Substrate Solution into each well and mix gently for 10 seconds.
15. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
16. Stop the reaction by adding 100µl Stop Solution to each well.
17. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
18. 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 U/ml on graph paper. Use the mean absorbance values for each specimen to determine the corresponding concentration of Cancer Antigen 15-3 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 0.5 and the OD of calibrator F greater than 1.1 for the assay results to be valid.

Healthy women are expected to have Cancer Antigen 15-3 assay values below 35U/ml. The minimum detectable concentration of Cancer Antigen 15-3 by PATHOZYME BREAST CANCER ANTIGEN 15-3 is estimated to be 5U/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of **PATHOZYME BREAST CANCER ANTIGEN 15-3** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme CA 15-3 kit and the Abbott AxSym Kit for samples with levels between 8.0 U/ml and 2000 U/ml the following data was generated.

Number of Samples	63
Correlation Co-efficient	0.97
Slope	0.93
Intercept	10.2
Omega Mean	209.9 U/ml
Abbott Mean	197 U/ml

These kits were shown to give good correlation.

REFERENCES

1. **Aziz D. C**. Quantitation of estrogen and progesterone receptors by immunocytochemical and image analysis. AJ. Clin. Pathol. 1992;98:105-11.
2. **Aziz DC. Peter J. B**. DNA ploidy and cell cycle analysis. Tools for assessment of cancer prognosis. J. Clin. Pathol. 1991;5:422-38.
3. **Clark GM, Dressler LG, Owens MA, Dounds G, Oldaker T, McGuire W.L.** Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. N. Engl. J. Med. 1989;320:627-33.
4. **Elledge RM, McGuire W. L**. Prognostic factors and therapeutic decisions in axillary node-negative breast cancer. Annu. Rev. Med. 1993;44:201-10.
5. **Foekens JA, Rio C, Seguin P. et al.** Prediction of relapse and survival in breast cancer patients by pS2 protein. Cancer. Res. 1990 50-3832-7.
6. **Isola H, Visakorp T, Holli K, Kallionieml D**. Association of p53 expression with other prognostic factors and long term survival in node-negative breast cancer. J. Cell. Biochem. 1992;(Suppl 16D):101.
7. **Kute TE, Shao ZM, Snugg NK, Long RT, Russell GB, Case L. D. Cathepsin D** as a prognostic indicator for node-negative breast cancer patients using both immunoassays and enzymatic assays. Cancer. Res. 1992;52-198-203.
8. **McGuire WL, Tandon AK, Allred D, Chamnes G.C., Clark, G. M.** How to use prognostic factors in axillary node-negative patients. J. Natl. Cancer Inst. 1990;82:1006-7.
9. **Nicholson S, Richard J, Sainsbury C. et al**. Epidermal growth factor receptor (EGFr):results of a 6 year follow up study in operable breast cancer with emphasis on the node-negative subgroup. Br. J. Cancer 1991;63:146-50.
10. **Somerville JE, Clarke LA, Biggart J. D**. C-erb B-2 overexpression and histological type of in-situ and invasive breast carcinoma. J. Clin. Pathol. 1992;45-16-20.
11. **Ueronese S, Gambocorta M**. Detection of Ki-67 rate in breast cancer. Am. J. Clin. Pathol. 1991;95:30-4.
12. **Lotnicker M, Pavesi F, Scarabelli M.** Tumour associated antigens CA-15-3 and CA-125 in ovarian cancer. Int. J. Biolog. Markers 1991;6:115.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 200µl of Standards or diluted sample into each well and gently mix for 10 seconds.
2. Incubate for 60 minutes at 37°C.
3. Discard the well contents and wash 5 times with distilled water.
4. Dispense 200µl of 1/22 Anti-Cancer Antigen 15-3 HRP Conjugate into each well. Gently mix for 10 seconds.
5. Incubate for 60 minutes at 37°C.
6. Discard the well contents and wash 5 times with distilled water.
7. Dispense 100µl of Substrate. Gently mix for 10 seconds.
8. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
9. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
10. Read Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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