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 CEA and CA125.

CA15-3 is a mucin-type glycoprotein with a high molecular weight. It is located 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 quantitative determination of Breast Cancer Antigen 15-3 in human Serum.

For professional use only.

PRINCIPLE OF THE TEST

The CA 15-3 ELSA test is based on the principle of a solid phase enzyme linked immunosorbent 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 validated against in house standards. There is no International standard for this test.

CONTENTS

- ELSA Plate: 12 x 8 wells x 1
- Reference antigen Human serum free of Cancer antigen 15-3. Ready to use. (Colourless)
- Calibration A 1 U/ml
- Calibration B 15 U/ml
- Calibration C 30 U/ml
- Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)
- Calibration D 100 U/ml
- Calibration E 300 U/ml
- Reference Standard: Cancer antigen 15-3 diluted in human serum. Ready to use. (Colourless)
- Calibration F 1000 U/ml
- Anti-Cancer Antigen 15-3 HRP Conjugate Concentrate: Anti-Cancer Antigen 15-3 concentrate conjugated to horseradish peroxidase (colourless)
- Dil 1 ml
- SPEC Dil 2 X 50ml
- Sample Diluent: Phosphate based buffer containing stabilising proteins. Working strength. (Yellow)
- ULC Dil 21 ml
- Control Dil: Phosphate based buffer containing stabilising proteins. Working strength. (Pink)
- TMB 11 ml
- Substrate solution: 3 ml Tetramethyl Benzidine in a 2ml bottle. Ready to use. (Colourless)
- Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)

MATERIAL REQUIRED BUT NOT PROVIDED

- Micropipettes: 10ul, 200ul and 1000ul
- Disposable pipette tips
- Vortex Mixer
- Incubator: Temperature of 37°C +/- 1°C
- Absorbent paper
- Microtitre reader fitted with a 450nm filter
- Graph paper
- Thoroughly clean laboratory glassware.

PRECAUTIONS

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 and seek medical advice.

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 lipemic 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 Azide as a preservative as this may cause irreversible damage.

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

Do not freeze make-up 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 concentrate conjugate to 2 parts conjugate diluent (1:22 dilution): 200ul 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. Diagnoses 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 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. Test serum should be diluted before use. Mix 20μl of test serum with 100μl of sample diluent. The CA 15-3 standards are pre-diluted and are ready for use.
5. Dispense 20μl 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 20μl of 1/2 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.

Duplicate all of 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 may cause pipetting of all Standards and specimens, although not required, is recommended.

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 (A450) 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 less than 15U/ml. The minimum detectable concentration of Cancer Antigen 15-3 by PATHOZYME BREAST CANCER ANTIGEN 15-3 is estimated to be 5.0U/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 Pathzyme 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.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Kit</th>
<th>Correlation Coefficient</th>
<th>Slope</th>
<th>Intercept</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abbott</td>
<td>0.83</td>
<td>0.98</td>
<td>-0.22</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Omega</td>
<td>0.90</td>
<td>0.82</td>
<td>-0.35</td>
<td>95%</td>
</tr>
</tbody>
</table>

These kits were shown to give good correlation.