1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Chemiluminescence Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight (M₉ > 200,000). High concentrations of this antigen are associated with ovarian cancer and a range of benign and malignant diseases, although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early detection of ovarian cancer. However, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. Published literature has shown that elevated serum CA-125 levels can be observed in patients with serous, endometrioid, clear cell and undifferentiated ovarian carcinoma. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, cancerrinoma, endometriosis, ureteral fibrosis, acute salpingitis, hepatic diseases and inflammation of peritoneum or pericardium).

In this method, CA-125 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against a distinct and different epitopes of CA-125) are added and the reactants mixed. Reaction between the various CA-125 antibodies and native CA-125 forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CA-125 antibody bound conjugate is separated from the unbound enzyme-CA-125 conjugate by decantation or aspiration. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light. The employment of several serum reference concentrations of known Cancer Antigen 125 (CA-125) conjugates for construction of a dose response curve, response curve activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CA-125 concentration.

3.0 PRINCIPLE

Chemiluminescence Immuno-assay (Type 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a specific enzyme activity is generated. The activity of the enzyme is without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{ENZ} + \text{ENZAb} + \text{AgCA-125} + \text{BtnAb(m)} \rightarrow \text{Immobilized complex}
\]

Where: 
- ENZ: Enzyme 
- ENZAb: Enzyme labeled Antibody 
- AgCA-125: Antigen 
- BtnAb(m): Biotinylated antibody

The interaction is illustrated below:

\[
\text{Ab} - \text{AgCA-125} - \text{BtnAb(m)} + \text{StreptavidinC.W.} \rightarrow \text{Immobilized complex}
\]

As the interaction proceeds, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
\text{Ab} - \text{AgCA-125} - \text{BtnAb(m)} + \text{StreptavidinC.W.} \rightarrow \text{Immobilized complex}
\]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. The interaction is illustrated below:

\[
\text{Ab} - \text{AgCA-125} - \text{BtnAb(m)} + \text{StreptavidinC.W.} \rightarrow \text{Immobilized complex}
\]

4.0 REAGENTS

Materials Provided:

- A. (250) Calibrators 1ml/vial – Icon A
- B. CA-125 Tracer Reagent – 13ml/vial - Icon B
- C. Light Reaction Wells 96 wells - Icon C
- D. Wash Solution Concentrate – 20ml - Icon D
- E. Signal Reagent A – 7ml/vial - Icon E
- F. Signal Reagent B – 7ml/vial - Icon F
- G. Product Insert.

2.8°C. Kit and component stability are identified on the

Note: Do not use reagents that are contaminated or have bacteria growth.

4.1 Required But Not Provided:

1. Pipette capable of delivering 25µl volumes with a precision of better than 1.5%.
2. Pipette of sufficient capacity and volume for the test to be performed.
3. Pipette capable of delivering 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
4. Microplate washer
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microscope slides.
7. Vacuum aspirator (optional) for wash steps.
8. Pipette tip rack.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Note: Do not use reagents for which the expiry date has passed.

6.0 SPECIMEN COLLECTION AND PREPARATION

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control. National Institute of Health. “Biosafety in Microbiological and Biological Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8598.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the production of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed contamination or degradation of reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container.
   Store diluted buffer at 2-8°C for up to 60 days.

   2. Working Signal Reagent Solution - Store at 2-8°C

   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is added to reduce the possibility of bacteria growth. The plate should be used within 24 hours after mixing.

   **Test procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference, patient and control specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the CA-125 Tracer Reagent to each well.
4. It is very important to dispense all reagents close to the bottom of the coated well.
5. Swirl the microplate gently for 20-30 seconds to mix and incubate at 2-8°C.
6. Incubate 45 minutes at room temperature.
7. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with a sterile absorbent paper.
8. Aspirate (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plate washer should be used. Avoid repetitive or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.
9. Incubate for five (5) minutes in the dark.
10. Read the relative light units in each well for 0.2 – 1.0 seconds.

Strategy 1: Read the absorbance within thirty (30) minutes of adding the substrate solution.

8.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CA-125 in unknown specimens.

1. Record the RLUs (Relative Light Units) obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding CA-125 concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting). Draw the best-fit curve through the plotted points. The dose response curve for CA-125 for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (30388) of an unknown intersects the calibration curve at 114(U/ml) CA-125 concentration (See Figure 1).

Note 1: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CA-125 AccuLite™ CLIA assay were determined by analyses on two different levels of control sera. The number, minimum value, standard deviation (s) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

14.5 High Dose Hook-Effect:

The test will not be affected by CA-125 concentrations up to 10,000 U/ml in serum, plasma or urine. However, samples expected to be over 400 U/ml should be diluted 1:10 and 1:100 in normal pooled human serum and the normal pool assayed along side to obtain a base value. The base value and dilution factors should be taken into account to get the corrected concentration of CA-125 in the sample.

15.0 REFERENCES


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