Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight entity (M_w > 200,000). High concentrations of this antigen are associated with ovarian cancer and a range of other cancers. Although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of ascites 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 serious endometroid, clear cell and exfoliated cancers, 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 serious endometroid, clear cell and exfoliated cancers.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

4.0 REAGENTS

A. CA-125 Calibrators - 1/mlvial - Ions A-F
B. CA-125 Enzyme-Reagent – 13mlvial - Ion C
C. Streptavidin Plate – 96 wells - Ion U
D. Wash Solution Concentrate – 20ml/vial
E. Substrate A – 7mlvial - Ion A
F. Substrate B – 7mlvial - Ion B
G. Stop Solution – 8mlvial - Ion G
H. Product Instructions

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

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7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and elevated 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 performance of the supplied reagents. Pertinent statistical monitoring data should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, sera reference calibrators and controls to room temperature (20-27°C).

2. Pipette 0.025ml (25 µl) of the appropriate serum reference and control to wells.
3. Add 0.100ml (100 µl) of the CA-125 Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Pipette the sample gently for 20-30 seconds to mix and cover.
5. Incubate at room temperature for 60 minutes.
6. Discard the contents of the microtiter plate by decantation or aspiration. If decanting, tap and blot the plate dry with an absorbent paper.
7. Add 0.350ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blit) or aspirate. Repeat two to three additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash.
8. Deactivate the wash solution twice (2) additional times.
9. Incubate at room temperatures for 15 minutes.
10. Add 0.050ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

12.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CA-125 in the sample. The absorption obtained from the printout of the microplate reader as outlined in Example 1.
1. Plot the absorbance versus the concentration of CA-125 using a computer program to determine the concentration of CA-125.
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of CA-125 for an unknown, locate the absorbance on the y-axis for the concentration of the standard curve. The absorbance of the standard curve compared to the absorbance obtained from the unknown will be similar. The concentration of CA-125 in the unknown can be determined by interpolation on the standard curve.
4. Note: Computer data reduction software designed ELISA assays may also be used for the data reduction, if such software is utilized, the validation of the software should be ascertained.

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12.0 RISK ANALYSIS

12.1 Assay Performance

1. The absorbance (OD) of calibrator F should be > 1.8 only and following criteria should be met:
   - Use components from the same lot. No intermixing of reagents from different batches.
   - Plate readers measure vertically. Do not touch the bottom of the wells.
   - Use clean pipettes, and use three quality control pools should be within the established ranges.
   - Dilution factor (10).

2. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

3. If computer controlled data reduction is used to interpret the results, the test is impertinent that the predicted values for the calibrators fall within 10% of the assigned concentrations.

4. CA-125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated CA-125 value alone is not of diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

12.2 Interpretation

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

3. The reagents for the test system have been formulated to eliminate matrix interference, however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for some types of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" Clin. Chem. 1988;34:372-373). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and findings.

4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

12.3 Expected Range of Values

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 limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salpingitis, hepatic diseases and inflammation of peritonitis or pericardium).

12.4 Specificity

In order to test the specificity of the antibody pair used, massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. In addition some widely used, over-the-counter, drugs and some cytostatic drugs (10 fold the normal dose) were tested in the assay. No cross reaction was found. Percent recoveries for some of these additions are listed below in Table 5.

12.5 Accuracy

1. The 2-CA-125 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was determined by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

12.6 Precision

The CA-125 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 121. The least square regression equation and the correlation coefficient were computed for CA-125 in the in-house range to determine the reference method. The data obtained is displayed in Table 4.

13.0 REFERENCES