2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight (Mr > 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 diagnosis of ovarian cancer, the assay has found widespread clinical 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 various conditions, including ovarian cancer, benign gynecologic diseases, and a range of benign and malignant diseases. Although the antigen is found in excess, immunoreactivity is lost in certain stages, and distinct epitope recognition, in excess, and native antigen. In this procedure, the immunobilization process takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and a biotinylated monoclonal anti-CA-125 antibody. Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, form to a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{Ab} \rightarrow \text{Ab}_{GUS} \rightarrow \text{Ab} \rightarrow \text{BiAb} \rightarrow \text{GUS} \rightarrow \text{GUS} \rightarrow \text{substrate} \rightarrow \text{product} + \text{H}_2\text{O}
\]

Materials Provided:

- CA-125 Calibrators - 1 ml/ivl – Icons (A-F)
- CA-125 Tracer Reagent - 12 ml/vial - Icon C
- Light Reaction Wells - 96 wells - Icon B
- Wash Solution Concentrate - 20 ml/vial - Icon A
- Wash Solution Reagent B - 0.5 ml/vial - Icon D
- Wash Solution Reagent A - 0.5 ml/vial - Icon D
- Microplate Enzymo-immunoassay, Chemiluminescence
- Microplate washers or a squeeze bottle (optional).
- Reagent Preparation Section.
- Discard the contents of the microplate by decantation or aspiration. Incubate 45 minutes at room temperature.
- Six (6) vials of references CA-125 Antigen at levels of 0( ), 15( ), 50( ), 100( ), 200( ), and 400( ) U/ml. Store at 2-8°C. A preservative has been added. Note: The standards, human serum based, were made using a >99% pure affinity purified preparation of Cancer Antigen CA-125.

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1. The Dose Response Curve should be within established ranges. The following criteria should be met:

1. It is important that the time of reaction in each well is held constant, as well as the time and temperature requirements prescribed are essential. The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

2. Pipetting of samples should not extend beyond ten (10) mm from the mouth of the pipette. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.

3. Highly lipemic, hemolyzed or grossly contaminated serum specimens were 103. The least square regression equation and coefficient of variation for each of these control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

4. The values for the CA-125 AccuLite® CLIA system were determined using the method with a population indigenous to the area in which the in-house range can be determined by the analysts using the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values for the calibrators fall within 10% of the assigned concentrations.

5. The reagents for the test system have been formulated to be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

1.10 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 ranges.

2. 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.

3. If more than one (1) plate is used, it is recommended to repeat the assay in order to obtain a baseline value for future use.

4. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor precision and spurious results.

5. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

6. It is important that the time of reaction in each well is held constant, as well as the time and temperature requirements prescribed are essential. The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

1.2. Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. 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.

3. The reagents for the test system have been formulated to eliminate maximal 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 all kinds of immunomas (Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunomas. Clin Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in conjunction with clinical examination, patient history and all other clinical findings.

1.3. Expected Ranges of Values

The serum CA-125 is elevated in % 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, pulmonary/urogenital, uterine fibrosis, acute salpingitis, hepatic diseases and inflammation of peritoneum or pericardium).

1.4. Performance Characteristics

1.4.1 precision

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

1.4.2 Sensitivity

The CA-125 AccuLite® CLIA Test System has a sensitivity of 0.11 U/ml. The sensitivity was ascertained by determining the variability of the 0 U/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

1.4.3 Accuracy

If the CA-125 AccuLite® CLIA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such biological specimens was 103. The least square regression equation and correlation coefficient were computed for the CA-125 in comparison with the reference method. The data obtained is displayed in Table 4.

1.4.4. Specificity

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.

1.5.0 REFERENCES


4. Daoud E, Bodor G, Weaver C, Landenson, JH and Scott MG. Cytotoxic drugs (10 fold the normal dose) were tested in the cytotoxicity assay. If test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. 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.

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

7. CA-125 has a low normal sensitivity and specificity somatostatin 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.

8. Patient specimens with CA -125 concentrations above 400 U/ml may be diluted in 1:10 or 1:100 normal male serum (CA -125 < 5 U/ml) and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor (10).

9. It is important that the time of reaction in each well is held constant, as well as the time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12. Risk Analytic- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.