Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

\[ \text{Ag} \times \text{Ab} \rightarrow \text{Ag} \times \text{Ab} \]

where \( \text{Ag} \) is antigen, \( \text{Ab} \) is antibody, \( \text{Ag} \times \text{Ab} \) is antigen-antibody complex, \( \text{Ag} \times \text{Ab} \) is antigen-antibody complex, and \( \text{Ag} \times \text{Ab} \) is antigen-antibody complex.

1. **INTRODUCTION**

   Although several serum based tumor markers have been described for breast cancer, such as CA 15-3, BR 27-29, carcinoembryonic antigen (CEA), tissue polyepipeptide antigen (TPE), tissue polyepipeptide antigen (TPA), tissue polypeptide antigen (TPA), and anti-HER-2 (membrane bound glycoprotein of extracellular domain), the most widely used are CA 3-5 and CEA. CA 3-5 is considered to be one of the first circulating prognostic antigens. Sensitivity and specificity concentrations might be combined with prognostic factors for predicting outcome in patients with newly diagnosed breast cancer. At present, the most important clinical application of CA 3-5 is in monitoring therapy in patients with advanced breast cancer that is not accessible by existing clinical or radiologic procedures. 3

   The CA 3-5 assay measures the protein product of MUC1 gene. MUC1 protein is a large transmembrane glycosylated molecule containing three main domains, a large extracellular region, a membrane spanning region, and a cytoplasmic domain. Although the physiological function of MUC1 is unclear, the glycoprotein has been implicated in cell adhesion, immunity and metastasis.

   4. **REAGENTS**

   Materials Provided:
   1. CA 53 Calibrators - 1.0 ml/vial - Icons A-F
   2. Dispenser(s) for repetitive deliveries of 0.100 ml (100 µl) and 0.025 ml (25 µl)
   3. Patient Sample Dilution (1:21)
   4. Signal Reagent A - 7mL/vial - IC
   5. Signal Reagent B - 7mL/vial - IC
   6. Working Signal Reagent A
   7. Working Signal Reagent B
   8. Wash Buffer
   9. Wash Buffer
   10. Quality control materials.

   **Note 1:** The calibrators are provided prediluted.
   **Note 2:** The calibrators, human serum bases, were made using a purified preparation of CA 3-5. The preparation was calibrated against Centocor CA 3-5 IRMA test.

   **A. CA 3-5 Calibrators:** 1.0 ml/vial - Icons A-F
   2. Dispenser(s) for repetitive deliveries of 0.100 ml (100 µl) and 0.025 ml (25 µl)
   3. Patient Sample Dilution (1:21)
   4. Signal Reagent A - 7mL/vial - IC
   5. Signal Reagent B - 7mL/vial - IC
   6. Working Signal Reagent A
   7. Working Signal Reagent B
   8. Wash Buffer
   9. Wash Buffer
   10. Quality control materials.

   **Note 3:** Each laboratory should assay controls at levels in the low, normal and upper range. Therefore, standards/controls/kit controls should be measured at least twice in every test procedure performed. Quality control charts should be maintained for each test procedure. Quality assurance should be used to monitor the performance of the supplied reagents. Pivotal statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate the need to change the conditions or discard of kit reagents. Fresh reagents should be used to determine the reason for the variations.

   **14. Read the Relative Light Units (RLUs) in each well for 0.2 – 1.0 minute.**

2. **WORKING SIGNAL REAGENT SOLUTION - Store at 2 – 8 ºC.**

   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 1 ml of B per two (2) eight well strips (a slight excess of solution is made). Discard the unused portion if not used within 30 minutes after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of the vials of Signal Reagent B into Signal Reagent A and label accordingly.

   **3. PATIENT SAMPLE COLLECTION (1:21):**

   Dispense 0.025 ml (25 µl) of each control and or patient specimen into 0.500 ml (50 µl) of CA 15-3 dilution matrix appropriately labeled, clean container(s) and mix thoroughly before the specimen(s) are refrigerated at 2-8ºC for up to 48 hours.

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

   9.0 **TEST PROCEDURE**

   Before proceeding with the assay, bring all reagents, serum references, controls, and reagents to room temperature (20-27°C). **Note:** Test procedure should be performed by a skilled individual or trained professional.

   1. Format the microtubes' wells for each serum reference calibrator, control and patient specimen to assay. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
   2. Pipette 0.025 ml (25 µl) of the appropriate diluted serum reference calibrator, control or specimen into the assigned well.
   3. Add 0.100 ml (100 µl) of the biotinylated labeled antibody to each well. It is very important to dispense all reagents close to the bottom of the microtubule to prevent air bubbles.
   4. Swirl the microtubes gently for 20-30 seconds and mix.
   5. Incubate 45 minutes at room temperature.
   6. Dispense all reagents into the appropriate container by decantation or aspiration. If decanting, tap and blot the plate dry with paper towel.
   7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times.
   8. Add 0.100 ml (100 µl) of the CA 15-3 Tracer Reagent to each well.
   9. DO NOT SHAKE THE PLATE AFTER TRACER ADDITION
   10. Cover and incubate 45 minutes at room temperature.
   11. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
   12. Add 0.350 ml (350 µl) of wash buffer (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 can be used. Follow the manufacturer’s instruction for proper usage. If a squeegee bottle is used, add 0.050 ml (5 µl) of air bubbles to disperse the wash. Discard the wash and repeat four (4) additional times.
   13. Add 0.001 ml (0.01 µl) of the CA 15-3 Tracer Reagent to each well.

   **DO NOT SHAKE THE PLATE AFTER TRACER ADDITION**

   14. Cover and incubate 45 minutes at room temperature.

   15. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

   16. Add 0.350 ml (350 µl) of wash buffer (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 can be used. Follow the manufacturer’s instruction for proper usage. If a squeegee bottle is used, fill each well by depressing the container (avoiding air bubbles) to disperse the wash. Discard the wash and repeat four (4) additional times.

   17. Add 0.100 ml (100 µl) of working signal reagent A. Repeat four (4) additional times for a total of five (5) washes. An automatic plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeegee bottle is used, fill each well by depressing the container (avoiding air bubbles) to disperse the wash. Discard the wash and repeat four (4) additional times.

   18. Add 0.100 ml (100 µl) of working signal reagent B. Repeat four (4) additional times for a total of five (5) washes. An automatic plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeegee bottle is used, fill each well by depressing the container (avoiding air bubbles) to disperse the wash. Discard the wash and repeat four (4) additional times.

   19. Incubate for five (5) minutes in the dark.

   20. Read the Relative Light Units (RLUs) in each well for 0.2 – 1.0 seconds. The results should be read within thirty (30) minutes of adding the signal solution.

2. **CALCULATION OF RESULTS**

   A dose response curve is used to ascertain the concentration of the antigen in the sample. The curve is plotted using the data obtained from the assay. Each data point is plotted on the graph, and a curve is drawn through the points. The concentration of the antigen in the sample is determined by comparing the curve to the standard curve.

   **Example:**
   Suppose the concentration of the antigen in the sample is 100 µg/ml. The curve is compared to the standard curve, and the concentration of the antigen is determined to be 100 µg/ml.

   **Graph:**
   [Graph of a dose response curve showing the concentration of the antigen in the sample and the standard curve.]

   **Conclusion:**
   The concentration of the antigen in the sample is 100 µg/ml.
2. Four out of six quality control pools should be within the levels determined by the within-assay variability in order to avoid assay drift.

3. Connect the points with a best-fit curve.

5. 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.

6. Failure to remove adhesion solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

7. Use components from the same lot. No intermixing of reagents from different lots should occur, only and have been normalized to 100,000 RLUs for the A calibrator (111/100 higher) with CA-15-3 diluted serum diluent and re-assayed. The sample concentration is obtained by multiplying the result by the dilution factor.

8. Patient specimens (diluted) with CA-15-3 concentrations above 400 U/ml may be assayed in duplicate (111/2 higher) with CA-15-3 diluted serum diluent and re-assayed. The sample concentration is obtained by multiplying the result by the dilution factor.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.


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

12. Risk Analysis - as required by CE Mark IV Directive 93/42/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 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 determinations.

3. The reagents for AccuLite® CLIA procedure 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 kits lacking the antibodies (Boscato, LM, Stuart, MC, "Heterophilic antibodies: a problem for all immunoassays?" Clin. Chem. 1988;34:227-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid 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 incompletely interpreted, Monobind shall have no liability.

6. If computer controlled dilution is used to interpret the results of the test, it is imperative that the predicted values for the patient be calculated after dilution and not before. The calibration curve used in conjunction with clinical observations (measurements) and diagnostic parameters.

13.0 EXPECTED RANGES OF VALUES:

The serum CA-15-3 is elevated in 2% of normal healthy women and 7% of patients with non-neoplastic conditions. Also, it has been reported to be elevated for conditions of liver, lung, ovarian and colorectal cancers. No definitive ranges have been reported for these conditions.

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 variability of the CA 15-3 AccuLite® CLIA Test Procedure was determined by analyses on three different levels of control sera. The mean level, standard deviation (σ), and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

14.2 Sensitivity

The CA-15-3 AccuLite® CLIA Test System procedure has an LLOQ of 0.2 U/ml at three (3) SD from the zero calibrator. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to: calculate the functional sensitivity (20% CV) was found to be 1.71U/ml.

14.3 Accuracy

The CA-15-3 AccuLite® CLIA Test System procedure was compared with reference methods for specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 43. The least square regression equation and the correlation coefficient computed for the CA 15-3 in comparison with the reference method. The data obtained is displayed in Table 4.

14.4 Specificity

In order to test the specificity of the antibody pair used in the CA-15-3 AccuLite® CLIA Test System, massive concentrations of well-characterized proteins were assayed in parallel with the base sera. No cross reaction was observed. Possible cross-reactants were added to known serum pools and no cross reaction was observed. Possible cross-reactants were added to known serum pools and no cross reaction was observed.

14.5 REFERENCES


Revision: 3 Date: 2019-Jul-16 DCO: 1353 MP5675 Product Code: 5873-30