3.0 PRINCIPLE
Immunoenzymometric assay (Type 3): The essential reagents required for an immunoenzymometric ligation reaction are: an enzyme conjugate, a biotinylated monoclonal antibody (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-CEA 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 for forming a sandwich complex. The interaction is illustrated by the following equation:

\[ \text{EnzAb} + \text{Ag}_{CEA} + \text{BtnAb}_{(m)} \rightarrow \text{EnzAb-}\text{Ag}_{CEA}-\text{BtnAb}_{(m)} \]

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

4.0 REAGENTS

4.1 Required but NOT provided:

1. Pipette 0.025 ml (25μl) of the appropriate serum reference, control or specimen into each well of the 96-well microplate.
2. Add 0.100 ml (100μl) of the CEA Tracer Reagent to each well.
3. It is very important to dispense all reagents close to the bottom edge of each well.
4. Swirl the microplate gently for 30-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350μl of wash buffer (see Reagent Preparation Section), decant (tall and slop) or aspirate. Repeat four (4) additional times.
8. Add 250μl of working signal reagent to all wells (see Reagent Preparation Section). Add 250μl of working signal reagent to all wells (see Reagent Preparation Section).
9. Incubate for five (5) minutes in the dark.
10. Customer and Expert Use in Humans and Animals

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 completely rule out the presence of infectious agents, 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 Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8385.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be serum, blood in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venaurecture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing of specimen. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

7.1 Quality Control Materials:

- **A. CEA standard**
- **B. Wash buffer**
- **C. Signal reagents**
- **D. Signal buffer**
- **E. Signal conjugate**

7.2 Quality Control Procedure:

1. Record the RLU’s (Relative Light Units) obtained from the preparation. Prepare the following calibrators
2. Plot the RLU’s for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit through the plotted points.
4. To determine the concentration of CEA in unknown specimens, use the best-fit standard curve.
5. Subtract the average RLU’s (10396) of the unknown intersects the calibration curve at 24.3 ng/ml CEA concentration (See Figure 1)*.

8.0 REAGENT PREPARATION

1. Wash Buffer
2. Diluate contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-8°C for up to 60 days.
3. Working Signal Reagent Solution
4. Store at 2-8°C.
5. Dilute the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B and Signal Reagent C, Signal Reagent D and Signal Reagent E. To prepare the working solution, 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 36 hours after mixing, if complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

9.0 TEST PROCEDURE

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

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

1. Format the microplates’ wells for each serum reference, control and patient 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 each well of the 96-well microplate.
3. Add 0.100 ml (100μl) of the CEA Tracer Reagent to each well.
4. It is very important to dispense all reagents close to the bottom edge of each well.
5. Swirl the microplate gently for 20-30 seconds to mix and cover.
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 absorbent paper.
8. Add 350μl of wash buffer (see Reagent Preparation Section), decant (tall and slop) or aspirate. Repeat four (4) additional times.
9. Add 250μl of working signal reagent to all wells (see Reagent Preparation Section).
10. Add always reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION.

11. Incubate for five (5) minutes in the dark.
12. Read the relative light units in each well for 0.2 - 1.0 seconds.
13. The result should be read within thirty (30) minutes of adding the signal solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CEA in unknown specimens.

1. Record the RLU’s (Relative Light Units) obtained from the preparation. Prepare the following calibrators
2. Plot the RLU’s for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit through the plotted points.
4. To determine the concentration of CEA in unknown specimens, use the best-fit standard curve.
5. Subtract the average RLU’s (10396) of the unknown intersects the calibration curve at 24.3 ng/ml CEA concentration (See Figure 1)*.

Note: Computer data reduction software designed for CLIA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained
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. Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).

12.2 Interpretation

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

2. Laboratory results 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. For valid test results, adequate quality control parameters must be within the listed ranges and assay requirements.

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.

5. 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.

6. CEA has a low clinical sensitivity and specificity as a tumor diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

11.0 RANGED EXPECTED VALUES

Nearly 99% of non-smokers have CEA concentrations less than 5.0 ng/ml. Similarly 99% of smokers have concentrations less than 10 ng/ml. 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.

14.5 Linearity & Hook Effect: Three different lot preparations of the CEA AccuBind™ ELSIA reagents were used to assess the linearity and hook effect. Linear regression analyses of CEA (>60,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 92.0 to 111.4%.

15.0 REFERENCES


