The activity of the enzyme present on the surface of the well is a dose response curve of activity and concentration. From the employment of several serum references of known unbound enzyme-CEA conjugate by aspiration or decantation.

Between the various CEA antibodies and native CEA forms a indicative of malignancy. Also, values seen in malignant and non-malignant cases exceeding 5 times the normal reference range are taken as

Although CEA is primarily associated with colorectal cancers and benign liver cancer. Heavy Smokers, as a group, have serum levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer. Heavy Smokers, as a group, have values, a dose response curve can be generated from which the comparison to the dose response curve, an unknown specimen’s interaction is illustrated below:

D. Wash Solution Concentrate – 20 ml/vial – Icon

ENZAb - AgCEA - BtnAb(m) = Antigen-Antibodies Sandwich Complex

ENZAb = Enzyme labeled Antibody (Excess Quantity)

AgCEA = Native antigen (Variable Quantity)

BtnAb(m) = Rate Constant of Association

k-a = Rate Constant of Dissociation

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

A. CEA Calibrators – 1 ml/vial Icons A-F

B. CEA Tracer Reagent – 13 ml/vial – Icon

C. Kit and component stability are identified on the package in an aluminum bag with a drying agent. Store at 2-8 °C.

One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8 °C.

F. Signal Reagent B – 7ml/vial – Icon CB

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 microwell through the interaction of streptavidin-coated ELISA plate and exogenously added biotinylated monoclonal anti-CEA antibody.

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

\[
 k_a = \frac{d[ENZab]}{dt} = \frac{d[A]}{dt} = \frac{d[AgCEA]}{dt}
\]

Add Ab – CEA, Ab – Streptavidin-CEA – Immobilized complex

Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity is performed in a plan label renal plate using appropriate washes with anti-digoxigenin or antibody-conjugated secondary antibody.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Individual laboratory should be read within thirty (30) minutes of adding the signal solution.

A. 4mmol/L sodium bicarbonate and 4mmol/L potassium dihydrogen phosphate (pH 9.25), 3.0 ml/vial

B. 10 mmol/L sodium phosphate and 140 mmol/L sodium chloride, 1 ml/vial

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. Dispense 2.0 ml (250µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of the CEA Tracer Reagent to each well. This is very important to dispense all reagents close to the bottom of the coated well.

4. Swirl the microplate gently for 20-30 seconds and mix.

5. Incubate 45 minutes at room temperature.

6. Discard the contents of the microwell by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) 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 squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to displace the wash.

The results should be read within thirty (30) minutes of adding the signal solution.

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 luminometer’s printout of the microplate reader as outlined in Example 1.

2. Plot the RLU’s for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper. Match the duplicates of the serum references before plotting.

3. Draw a best fit curve through the plotted points.

4. To determine the concentration of CEA for an unknown, locate the average RLU’s for each unknown on the vertical axis of the graph, and draw a horizontal line from that point to the curve, and read the CEA concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged if indicated). In the results section, the average RLU’s (10396) is the x-axis.

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.

Cellulose acetate membra
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 the automated instruments used with this device, and to perform routine preventative maintenance.

12. Risk Analysis: As required by CE Mark IVD Directive 98/79/EC, this and other devices made, by Monobind, can be requested via email at Monobind@monobind.com.

12. Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional. 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. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problematic for all kinds of immunoassays (Boscardin, LM; Stuart, MC; "Heterophilic antibodies: a problem for all immunoassays" Clin. Chem. 1988; 34:27-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

3. For valid test results, adequate controls and other 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 test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

6. The CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated CEA value alone is not diagnostic of disease and should not 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. Some reagents can demonstrate a higher range of baseline values than nonsmokers.

13. EXPECTED RANGES OF VALUES

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

14.4 Specificity:

Highly specific antibodies to CEA molecules have been used in the CEA AccuLite® CLIA Test System. No interference was detected with the performance of CEA AccuLite® CLIA upon addition of substantial amounts of the following substances to a human serum pool.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsaliclyc Acid</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Atp</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>CA 125</td>
<td>10,000 U/ml</td>
</tr>
<tr>
<td>Caffeine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>PSA</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>NCG</td>
<td>1000 UI/ml</td>
</tr>
<tr>
<td>NLH</td>
<td>10 UI/ml</td>
</tr>
<tr>
<td>tHST</td>
<td>100 UI/ml</td>
</tr>
<tr>
<td>HFRP</td>
<td>100 µg/ml</td>
</tr>
</tbody>
</table>

15. Linearity & Hook Effect:

Three different lot preparations of the CEA AccuLite® CLIA reagents were used to assess the linearity and hook effect. Mass concentrations of CEA (> 60,000 ng/ml) were used for linear dilutions in pooled normal human sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 96.0 to 108.2%.

15. REFERENCES


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MP1875

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