The employment of several serum references of known CEA levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CEA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (Type 3): An immunoenzymometric luminescence assay includes high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in either direct or indirect sandwich forms. In this procedure, the immobilization takes place during the assay at the surface of a microwell through the interaction of streptavidin coated on the well and exogenously 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, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{Ab}_{\text{BA}} + \text{Ab}_{\text{EA}} + \text{Ag}_{\text{CEA}} \rightarrow \text{Ab}_{\text{EA}} \cdot \text{Ag}_{\text{CEA}}
\]

\(\text{Ab}_{\text{BA}}\) = Biotinylated Monoclonal Antibody (Excass Quantity)
\(\text{Ab}_{\text{EA}}\) = Native Antibiot (Variable Quantity)
\(\text{Ag}_{\text{CEA}}\) = Enzyme Labeled Antibody (Excass Quantity)

4.0 REAGENTS

Materials Provided:
A. Next Generation Calibration Reagent -1ml/ vial - Icons A-F
Six (6) vials of serum references CEA Antigen at levels of 0(A), 5(B), 10(C), 25(D), 100(E) and 250(F) mg/L. A preservative has been added. Store at 2-8°C.
B. CEA Next Generation Tracer Reagent -1ml/microwell - Icon C
One (1) vial contains enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
C. Light Reaction Wells - 96 wells - Icon U
One (1) well contains a microwell coated with streptavidin. Each well contains hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.
D. Product Insert
Note 1: Do not use reagents beyond the kit expiration date.
reduction. If such software is utilized, the validation of the software should be ascertained.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The Dose Response Curves (50%, 20% & 40% intercepts) should be within established parameters. 
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available and on request from Monobind Inc.

12.1 Assay Performance

It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly Ispenic, hemolysed or grossly contaminated samples should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent should be added in the same sequence to eliminate any time delay during reaction.
6. Failure to remove stress solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the lot. No intermingling of reagents from different batches.
8. 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).

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements described are essential. Any deviation from Monobind’s 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.

1. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and other instrumentation used with this device, and to perform routine preventative maintenance.

12.2 Interpretation

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 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 problems for all kinds of immunoassays.
4. Measurements and interpret ation of results must be performed by a skilled individual or trained professional. The within and between assay precision of the CEA Next Generation AccuLite® CLIA test system . No interference was detected with the performance of the test system when CEA concentrations were determined by the CLIA method with a population indigenous to the area in which the test was performed. Measurements and interpret ation of results must be performed by a skilled individual or trained professional.

14.3 Accuracy

The CEA Next Generation AccuLite® CLIA test system was compared with a reference method. Biological specimens from normal and elevated concentrations were assayed. The total number of such specimens was 70. The values ranged from 0.01 – 251ng/ml. The least square regression equation and the correlation coefficient were computed for the CEA-Next Generation AccuLite® CLIA method in comparison with the reference method. The data obtained is displayed in Table 4.

14.4 Specificity

Highly specific antibodies to CEA molecules have been used in the Next Generation AccuLite® CLIA test system. No interference was expected with the performance of the test system upon addition of massive amounts of the following substances to a human serum pool:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsalicylic Acid</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Caffeine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>PSA</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>CA-125</td>
<td>10,000 U/ml</td>
</tr>
<tr>
<td>HCG</td>
<td>1000 U/ml</td>
</tr>
<tr>
<td>HLP</td>
<td>10 U/ml</td>
</tr>
<tr>
<td>HBS</td>
<td>100 U/ml</td>
</tr>
<tr>
<td>HAP</td>
<td>100 µg/ml</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Well</th>
<th>RLU (A)</th>
<th>RLU (B)</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.2</td>
<td>20.8</td>
<td>209</td>
<td>0</td>
</tr>
<tr>
<td>A2</td>
<td>0.1</td>
<td>20.8</td>
<td>209</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1: Expected Values for the CEA Next Generation AccuBind® ELISA Test System

Non-smokers: 0 < CEA < 10 ng/ml

Smokers: < 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: 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 values that they expect to encounter 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 precision of the CEA Next Generation AccuLite® CLIA test system were determined by analyses on three different levels: control sera, specimen(s) mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

Table 2: Within Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>4.2</td>
<td>0.30</td>
<td>7.1%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>22.0</td>
<td>1.11</td>
<td>5.0%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>53.2</td>
<td>3.85</td>
<td>7.2%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

Table 3: Between Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>4.0</td>
<td>0.37</td>
<td>9.3%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>21.6</td>
<td>1.30</td>
<td>6.0%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>54.5</td>
<td>4.12</td>
<td>7.6%</td>
</tr>
</tbody>
</table>