Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chémiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA) is comprised of a heterogeneous family of glycoproteins with a molecular weight ranging from 175 to 200 kDa due to variations in its heterogeneous nature. CEA is the first of the so-called heterogeneous family of glycoproteins that was discovered in 1965 by Gold and Freeman. Even though its biological function is not very well defined CEA is the most widely used marker for colorectal cancer.

Although CEA is primarily associated with colorectal cancers (CRC), other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign conditions. CEA is the test of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman. Even though its biological function is not very well defined CEA is the most widely used marker for colorectal cancer.

3.0 PRINCIPLE

Immunoenzymometric assay (Type III):
The essential reagents required for an immunoenzymometric luminescence 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 plate. This process is dependent on the well and exogenously added biotinylated monoclonal anti-CEA antibody.

After mixing monoclonal biotinylated antibody, the enzyme-labeled antigen 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:

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

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 and controls to room temperature (20 - 27°C).

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

1. Format the microwells' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the container. **Do not stack the microwell strips.**

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 100 µl (100 µl of the CEA Tracer Reagent to each well.

4. It is very important to dispense all reagents close to the bottom of the coated well. Avoid air bubbles.

5. Swirl the microwell 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 0.350 ml (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 sponge bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

9. Add 0.100 ml (100µl) of washing signal reagent to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

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

11. Prepare the RLU's (Relative Light Units) obtained from the printout of the microlate reader as outlined in Example 1.

12. 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 for plotting purposes).

13. Draw the best-fit curve through the plotted points.

14. To determine the concentration of CEA for an unknown, locate the average RLU's for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU's (10396) of the unknown intersects the calibration curve at (24.3ng/ml) CEA (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.
Accurate and precise pipetting, as well as following the exact patient specimens with CEA concentrations above 250 ng/ml is important. The addition of signal reagent initiates a kinetic reaction, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

In order for the assay results to be considered valid, the following criteria should be met:

1. The Dose Response curve (50% & 200% intercepts) should be within established parameters.
2. Four out of six quality control pools should be within the listed ranges and assay requirements.
3. Highly lipemic, hemolyzed or grossly contaminated specimens should not extend beyond ten (10) minutes to avoid assay drift.
4. 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.

TABLE 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This method (y)</td>
<td>5.67</td>
<td>y = 1.0324(x) - 0.1164</td>
<td>0.985</td>
</tr>
<tr>
<td>Reference (x)</td>
<td>5.75</td>
<td></td>
<td></td>
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</tbody>
</table>

14.5 Linearity & Hook Effect:

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 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>Acetylcholinesterase</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Caffeine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>AFP</td>
<td>10 µg/ml</td>
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<tr>
<td>PSA</td>
<td>1 µg/ml</td>
</tr>
<tr>
<td>CA-125</td>
<td>10,000 U/ml</td>
</tr>
<tr>
<td>nGCG</td>
<td>11 U/ml</td>
</tr>
<tr>
<td>nLH</td>
<td>10 U/ml</td>
</tr>
<tr>
<td>tHSH</td>
<td>100 U/ml</td>
</tr>
<tr>
<td>NPLR</td>
<td>100 U/ml</td>
</tr>
</tbody>
</table>

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