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:

\[ \text{Enzyme} \cdot \text{Ab} + \text{Ag} \cdot \text{CEA} + \text{Streptavidin} \rightarrow \text{Enzyme} \cdot \text{Ab} \cdot \text{Ag} \cdot \text{CEA} \cdot \text{Streptavidin} \]

Required But Not Provided:
1. Pipette(s) capable of delivering 25µl & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a commercial washing unit.
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or 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 offer complete assurance that infectious agents are absent, 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, "Biologicals in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, US Department of Health and Human Services.}

TEST PROCEDURE

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

1. Format the microplate 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 the assigned well.
3. Add 0.100 ml (100µl) of the CEA Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 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 0.0050 ml (50µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) 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 dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) of working substrate solution 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 SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.0500 ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference and control against the known concentration. POLYCHROMATIC methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticeable change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the range for the variations.

REAGENT PREPARATION:

1. Wash Buffer
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.

2. Working Substrate Solution
   Pour contents of the vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note: Do not use the working substrate if it looks blue.

Note 3:

1. Format the microplate 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.
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Note 3:
in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility and spurious data.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. 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).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
11. 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.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

3. Accuracy
The CEA AccuBind™ ELISA method was compared with a reference Chemiluminescence method. Biological specimens from normal and elevated concentrations were assayed. The total number of such specimens was 64. The values ranged from 0.4 to 125 ng/ml. The least square regression equation and the correlation coefficient were computed for the CEA AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

4. Linearity & Hook Effect:
Three different lot preparations of the CEA AccuBind™ ELISA reagents were used to test the linearity and hook effect. Massive concentrations of CEA (> 80,000 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 within a dose recovery of 92.0% to 111.4%.

5. Specificity:
Highly specific antibodies to CEA molecules have been used in the CEA AccuBind™ ELISA test system. No interference was detected with the performance of the CEA AccuBind™ ELISA up to an addition of massive amounts of the following substances to a human serum pool.

- Acetylsalicylic Acid
- Ascorbic Acid
- Caffeine
- AFP
- PSA
- CA-125
- HCG
- LH
- NTSH
- hPRL

TABLE I

**Expected Values for the CEA Elisa Test System**

<table>
<thead>
<tr>
<th>Type</th>
<th>Absorption</th>
<th>Non-smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5ng/ml</td>
<td>0.025</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>&lt;10ng/ml</td>
<td>0.05</td>
<td>0.18</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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 is to 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.

**EXPECTED RANGES OF VALUES**

- Nearly 99% of non-smokers have CEA concentrations less than 5ng/ml. Similarly 99% of smokers have concentrations less than 10ng/ml.

**REFERENCES**


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Please visit our website to learn more about our other interesting products and services.