Carcinoembryonic Antigen (CEA) Test System

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Immunoassay test.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDa. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freidberg. CEA is the most widely used marker for gastrointestinal cancer.

Although CEA is primarily associated with colorectal cancers (CRC), other malignancies that can cause elevated levels of CEA include breast, lung, stomach, ovary, and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver disease. In smokers, as a group, have higher than normal baseline concentration of CEA. Serum values in healthy adults are normally ≤ 5.0 ng/ml however, serum values exceeding 5.0 ng/ml are taken as indicative of malignancy. Also, values seen in malignant and non-malignant conditions can overlap thus making CEA a not very specific marker. Also, values seen in malignant and non-malignant conditions can overlap thus making CEA a not very specific marker. CEA values seen at non-CRCs, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, ovary, and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver disease. In smokers, as a group, have higher than normal baseline concentration of CEA. Serum values in healthy adults are normally ≤ 5.0 ng/ml however, serum values exceeding 5.0 ng/ml are taken as indicative of malignancy. Also, values seen in malignant and non-malignant conditions can overlap thus making CEA a not very specific marker.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include highly specific 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 microplate well through the interaction of streptavidin coated on the well and the enzyme-CEA antibody bound conjugate is separated from the well.

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:

\[
E_{na} + A_{g} + CEA + \text{Enzyme-CEA antibody bound conjugate} \Rightarrow \text{Immobilized complex}
\]

Enzyme activity can be correlated with CEA concentration. After equilibrium is attained, the antibody-bound fraction is separated from the serum by centrifugation or filtration. The antibody-bound fraction is separated from the serum by centrifugation or filtration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. Using different assay systems, the enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

4.0 REAGENTS

Materials Provided:

A. Carcinoembryonic antigen (CEA) – 2ml/vial Icons A-F

Six (6) vials of references CEA Antigen at levels of 0(A), 5(B), 10(C), 25(D), 50(E) and 250(F) ng/ml. Store at 2-8°C. A preservation solution has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 2nd International Reference Preparation (IRP 73h 81001).

B. CEA Enzyme Reagent – 1ml/vial - Icon E

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

C. Streptavidin Coated Plate - 96 wells - Icon D

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate - 20 ml - Icon B

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon A

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon B

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

G. Stop Solution – 8ml/vial - Icon D

One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

5.0 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 the assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory practice and guidelines for handling should be found in the Center for Disease Control / National Institute of Health, “Biotechnology in Medicine and Biomedical Laboratories,” 2nd Edition, 1984, pp. 88-89.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in vials and urine in vials. The concentration of vascupathic 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 citrate/EDTA venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high bio-doses (i.e. >2mg/kg), no sample should be taken until at least 8 hours after the last bio-dose administration, preferably overnight to ensure fasting sample.

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 after the last bio-dose. Avoid repetitive freezing and thawing. Once assayed, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These tests controls should be treated as unknowns and values determined 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 0.100 ml (100µl) of 25µl of the appropriate serum reference, control or specimen into the assigned well.

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.

10.0 CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance obtained from the assay to 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 curve through the plotted points.

4. To determine the concentration of CEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the CEA concentration in ng/ml, then project horizontally from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the

indicate unchanged notice in experimental conditions or degradation of kit reagents. Fresh reagents should be done to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-8°C for up to 60 days.

2. Working Substrate Solution

Pour the contents of the amber 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 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C). Controls should be performed by a skilled individual or trained professional.”

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Remove the microplate cover and wipe down the microplate surfaces.

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 2-8°C.

6. Discard the contents of the microplate by decantation or aspiration. Decant and tap and blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat two (2) additional times for a total of three (3) washes.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

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.

12. Subtract the absorbance at 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.

13. Calculate the concentration of CEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the CEA concentration in ng/ml, then project horizontally from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the
average absorbance (0.391 Abs) intersects the dose response curve at (22.5 ng/ml) CEA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data 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 absorbance (OD) of diluent F should be > 1.3.
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 on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve best performance.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) 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 substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added 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 replication and spurious results.
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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

12.2 Interpretation

1. 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. 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 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 marker. Clinically an elevated CEA alone is not of 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 who do not exhibit elevated CEA concentrations 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.

13.0 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 [5].

<table>
<thead>
<tr>
<th>Method Mean</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Use (Y)</td>
<td>5.67 y = -0.1164+1.0324x</td>
<td>0.935</td>
</tr>
<tr>
<td>Reference (Y)</td>
<td>5.75</td>
<td></td>
</tr>
</tbody>
</table>

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