Enzyme Immunoassay for the Quantitative Determination of Carcinoembryonic Antigen (CEA) in Human Serum

Carcinoembryonic antigen (CEA) is a cell-surface 200-kd glycoprotein. It was discovered in 1969 by Kingston and Day while they were studying the development of the rabbit. The first report of circulating CEA in the serum appeared in 1970 at a conference. Since the initial report, CEA has been shown to have a variety of functions in normal and diseased tissue. CEA is a tumor marker, and it is found in normal tissue of the colon, bladder, cervix, and prostate. It is also found in patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, and the site of metastasis. CEA is also found in normal tissue. Increased levels of CEA are observed in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, and the site of metastasis. CEA is also found in normal tissue. Increased levels of CEA are observed in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, and the site of metastasis. CEA is also found in normal tissue.

**Materials and components**

1. Antibody-coated microtiter plate, 96 wells
2. Enzyme Conjugate Reagent, 12 mL
3. TMB Substrate, 12 mL
4. Stop Solution, 12 mL
5. Reference standards, containing 0, 3, 12, 30, 60, and 120 ng/mL of CEA, in liquid form (ready to use) or lyophilized Form
6. Wash Buffer Concentrate (50X, 15mL)
7. Precision pipettes: 40µL-200µL, 200-1000µL
8. Disposable pipette tips
9. Distilled water
10. Vortex mixer or equivalent
11. Absorbent paper or paper towel
12. Graph paper
13. Microtiter plate reader

**Preparation of standards and specimens**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required is recommended.

**Calculation of results**

Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/mL, on graph paper, with absorbance values on the vertical Y-axis and concentrations on the horizontal X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CEA in ng/mL from the standard curve.

**Example of standard curve**

<table>
<thead>
<tr>
<th>CEA (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.019</td>
</tr>
<tr>
<td>3</td>
<td>0.105</td>
</tr>
<tr>
<td>12</td>
<td>0.362</td>
</tr>
<tr>
<td>30</td>
<td>0.814</td>
</tr>
<tr>
<td>60</td>
<td>1.390</td>
</tr>
<tr>
<td>120</td>
<td>2.032</td>
</tr>
</tbody>
</table>

**Important Note**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required is recommended.
This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.

**Expected values and sensitivity**

The most complete study of CEA is a compilation of collaborative studies in which CEA values in 35,000 samples from more than 10,000 patients and controls were analyzed. Of 1425 normal persons who did not smoke, 98.7% had values less than 5.0 ng/mL. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of CEA by this assay is estimated to be 1.0 ng/mL.

**Performance characteristics**

1. **Accuracy:** Comparison between Our kits and commercial available kits provide the following data

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Replicates</th>
<th>Mean</th>
<th>S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>24</td>
<td>5.07</td>
<td>0.173</td>
<td>3.4</td>
</tr>
<tr>
<td>Level I</td>
<td>24</td>
<td>20.3</td>
<td>0.744</td>
<td>3.7</td>
</tr>
<tr>
<td>Level II</td>
<td>24</td>
<td>35.44</td>
<td>1.09</td>
<td>3.1</td>
</tr>
</tbody>
</table>

2. **Inter-Assay:**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Replicates</th>
<th>Mean</th>
<th>S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>20</td>
<td>4.94</td>
<td>0.243</td>
<td>4.9</td>
</tr>
<tr>
<td>Level I</td>
<td>20</td>
<td>19.82</td>
<td>1.24</td>
<td>6.3</td>
</tr>
<tr>
<td>Level II</td>
<td>20</td>
<td>35.36</td>
<td>1.33</td>
<td>3.8</td>
</tr>
</tbody>
</table>

3. **Linearity**

   A patient serum were serially diluted with 0 ng/mL standard. In a linearity study. The average recovery was 99.7 %.

4. **Recovery**

   Various patient serum samples of known CEA levels were mixed and assayed in duplicate. The average recovery was 100.7 %.

<table>
<thead>
<tr>
<th>Expected Concentration</th>
<th>Observed Concentration</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.28</td>
<td>53.21</td>
<td>98.0</td>
</tr>
<tr>
<td>28.42</td>
<td>29.13</td>
<td>102.5</td>
</tr>
<tr>
<td>23.85</td>
<td>22.98</td>
<td>96.4</td>
</tr>
<tr>
<td>16.53</td>
<td>15.99</td>
<td>96.7</td>
</tr>
<tr>
<td>20.90</td>
<td>21.36</td>
<td>102.2</td>
</tr>
<tr>
<td>18.62</td>
<td>19.65</td>
<td>105.5</td>
</tr>
<tr>
<td>28.77</td>
<td>29.78</td>
<td>103.5</td>
</tr>
</tbody>
</table>

   Average Recovery: 100.7 %

5. **Sensitivity**

   The minimum detectable concentration of this assay is estimated to be 1.0 ng/mL.

6. **Cross-reactivity**

   The following human materials were tested for crossreactivity of the assay:

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Concentration</th>
<th>Equivalent CEA</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG</td>
<td>400 IU/mL</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>PAP</td>
<td>1,000 ng/mL</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>PSA</td>
<td>1,000 ng/mL</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AFP</td>
<td>1,000 ng/mL</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

7. **Hook Effect**

   No hook effect was observed up to 40,000 ng/mL CEA in this Assay.

**Limitations of the Procedure**

There are some limitation of the assay. We should let our customers know about that.

1. **As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.**

2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3. The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

**References**


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