Enzyme Immunoassay for Carcinoembryonic Antigen

Adrienne M. Smith and D. J. Macdonald

In this rapid, simple enzyme immunoassay for carcinoembryonic antigen (CEA) in serum the "sandwich" system is used. The CEA is first extracted from the serum into an acetate buffer by heating at 70 °C, then incubated in a polyvinyl microtitration plate previously coated with anti-CEA. The bound CEA is then reacted with anti-CEA conjugated to horseradish peroxidase and the activity of the peroxidase bound to the wells of the plate is quantitated by adding the enzyme substrate. The assay is sensitive to 3.1 μg/L (which can be improved to 0.39 μg/L by prolonging the incubation times to 120 min), precise (between-assay CV 8.0%), and shows adequate analytical recoveries. Results compared favorably with those by a radioimmunoassay kit (Abbott Diagnostics), r = 0.99. The reference interval for serum CEA in normal adults is shown. This assay is appropriate for use in monitoring patients with colorectal tumors after surgery.

Additional Keyphrases: colorectal tumors · cancer · reference interval · monitoring therapy

Carcinoembryonic antigen (CEA) is a 200,000-Da glycoprotein produced by the gut of the human fetus. It is present in low concentrations in serum of adults, but greater concentrations are often observed in the sera of patients with colorectal cancer (1) and in patients with other types of carcinoma, such as carcinoma of the breast, lung, genitourinary tract, and lymphoid tissue (2). Increased concentrations of CEA in serum have also been observed in some patients with inflammatory nonmalignant disease. Although serum CEA was originally considered a tumor-specific marker for the diagnosis of colorectal cancer (3), its main use now appears to be in the follow-up of patients after surgery, chemotherapy, or radiotherapy. Removal of the tumor is usually followed by a decrease in the concentration of circulating CEA, whereas recurrence of the primary tumor or metastasis, or both, is accompanied by increased concentrations of the glycoprotein. Recurrence or metastasis can be detected earlier by assay for CEA in serum than by other diagnostic methods, which is important in the management and therapy of cancer patients.

Hitherto, CEA has been measured by sensitive radioimmunoassays, and in general CEA measurements have been confined to large medical centers. The National Institute of Health Consensus Development Panel recently recommended improving the clinical usefulness of the CEA assay by "the improvement of assay methods" (4). The present method is a simple, rapid, and inexpensive enzyme immunoassay sensitive enough for use with sera of both normal persons and cancer patients.

Materials and Methods

Instruments

For assay we used flexible polyvinyl microtitration plates (M29; Dynatech Laboratories, Sussex, RH14 9SJ, U.K.). Absorbances were measured in a digital concentration photometer with a drain cell attachment (MSE Scientific Instruments, Manor Royal, Crawley, Sussex, RH10 2QJ, U.K.).

Reagents

Phosphate-buffered saline (PBS). Dissolve 1.22 g of potassium dihydrogen orthophosphate and 8.77 g of sodium chloride in 800 mL of distilled water, adjust the pH to 7.4 with 4 mol/L sodium hydroxide, and dilute to 1 L with distilled water.

Chicken serum (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.).

Carbonate–bicarbonate buffer. This buffer contains 15 mmol of sodium carbonate and 35 mmol of sodium bicarbonate per liter, pH 9.6.

Wash buffer. Add 0.1 mL of Tween 20 polyoxyethylene (20) sorbitan monolaurate (ICI Americas, Inc., Wilmington, DE) to 1 L of phosphate-buffered saline.

Acetate buffer (0.2 mol/L). Dissolve 27.2 g of sodium acetate trihydrate in 800 mL of distilled water, adjust the pH to 5.0 with concentrated acetic acid, and dilute to 1 L with distilled water.

Anti-CEA and peroxidase-conjugated anti-CEA, prepared by Dako-Immunoglobulins Ltd., Copenhagen, was obtained from Mercia-Brocade Ltd., Surrey, KT14 6RA, U.K.

Chromogen. Dissolve 0.4 g of o-phenylenediamine in 1 L of a solution containing 22.5 g of disodium hydrogen phosphate and 5.6 g of citric acid (pH 6.0). The solution is stored in 30-ml aliquots at −20 °C. Immediately before use, add 20 μL of hydrogen peroxide (300 g/L).

Sulfuric acid, 4 mol/L. Carefully add 100 mL of concentrated sulfuric acid to 350 mL of distilled water.

CEA standard was obtained from the National Institute for Biological Standards and Control, Holly Hill, London, NW3 6RB, U.K. Dissolve the contents of each ampoule (coded 73/601, and containing 10 μg of CEA (5)) in 100 mL of chicken serum, divide into 1-mL aliquots, and store at −20 °C. Re-dilute each aliquot, which contains 100 μg of CEA per liter, in chicken serum to prepare calibrators over the range 3.1–100 μg/L.

Procedure

Pipet 40 μL of anti-CEA solution into 24 mL of carbonate buffer, mix, and pipet 200 μL of this solution into each well of a microtitre plate. Cover the plate and leave overnight at room temperature. Before use, decant the plate and wash it three times with wash buffer.

Dilute 0.5 mL of standards and test serum with 1 mL of acetate buffer, heat to 70 °C for 15 min, cool, and centrifuge.
Pipet 200 µL of supernate from each standard and test sample, in duplicate, into the wells of the microtitre plate at timed intervals, cover the plate with parafilm, and incubate for 60 min at 37 °C. Aspirate the contents of each well at the appropriate time interval and wash the plate three times with wash buffer. Pipet 200 µL of antibody–enzyme solution (containing 11 mL of PBS, 11 mL of heat-inactivated chicken serum, and 40 µL of anti-CEA–peroxidase conjugate) into each well and incubate for 60 min at 37 °C. Aspirate and discard the contents of each well and wash the plate three times with wash buffer.

Pipet 200 µL of chromogen into each well and incubate the plate at room temperature. After 30 min add 50 µL of diluted sulfuric acid, then read the absorbance of each well in the photometer, using the 492-nm filter. Plot a dose–response curve on two-cycle semi-logarithmic paper.

Results

Assay conditions. The assay conditions were investigated as described previously (6), and preliminary report of the results of optimizing the assay conditions has been presented in a previous communication. A typical dose–response curve is illustrated in Figure 1.

Sensitivity. The least amount distinguishable from zero at the 99% confidence level was 3.1 µg/L. The sensitivity of the assay could be increased by extending the first and second incubations to 120 min and the incubation with chromogen to 45 min; the sensitivity then at the 99% confidence limit was 0.39 µg/L.

Recovery. The analytical recovery of the assay was assessed by two experiments. First, aliquots of serum from a patient with a high concentration of serum CEA were added to a serum sample from a patient with a normal concentration of CEA, which had been diluted threefold with (CEA-free) chicken serum. The CEA concentrations were measured and compared with the estimated concentrations. Analytical recovery (Table 1) ranged from 91 to 100%.

Second, a serum specimen with an above-normal concentration of CEA was diluted with chicken serum in the following proportions (by vol): 7/8, 3/4, 2/3, 1/2, 1/3, 1/4, and 1/8; the CEA was measured and compared with the estimated concentrations. Analytical recovery ranged from 89 to 109%. We have not found any effects on linearity of response to concentration, with samples containing high CEA concentrations.

Precision. To assess the precision of the assay, we used three different pools of human serum, some of which had been diluted with chicken serum to give appropriately low values. The within-assay precision was estimated by 60 replicate analyses of CEA concentrations in two of the pools. The between-assay precision was estimated by performing duplicate measurements on the other two pools in 25 consecutive assays on different days. The precision of the more sensitive assay, i.e., with the 120-min incubation, was assessed with four other pools. All the results are shown in Table 2.

Correlation with radioimmunoassay. We assessed the correlation between the enzyme immunoassay (y) and the Abbott radioimmunoassay procedure (Abbott Diagnostics, Basingstoke, Hampshire, U.K.), using the National Institute for Biological Standards and Control preparation 73/601 as the standard. In 100 samples from different patients, including smokers, the correlation coefficient was 0.99 and the equation of linear regression was y = 0.96 x + 0.8.

Reference interval. The frequency distribution of CEA content in 200 normal adults is shown in Figure 2. The reference interval for adults, 8 to 14 µg/L, agrees with the findings of Booth et al. (7), who noted an upper limit of normal of 15 µg/L in adults, and of Laurence et al. (8), who reported an upper limit of normal of 12.5 µg/L. Our reference interval, however, does not agree with those provided by the Abbott radioimmunoassay kit. The most likely explanation for this is that the calibrator provided by the Abbott kit is not equivalent to the British Standard (73/601), which has already been reported in the literature (9).

Discussion

The CEA enzyme immunoassay described in this report has a sensitivity, reproducibility, and specificity comparable with those of radioimmunoassay procedures available commercially and is suitable for either the routine clinical laboratory or the research laboratory.

This procedure for CEA has some distinct advantages over currently available procedures. Firstly, it involves a simple and rapid extraction with a noncorrosive reagent. This extraction procedure is much simpler to perform than perchloric acid extraction, which requires subsequent neutralization, dialysis, and ultrafiltration or gel chromatography to remove the excess acid. Secondly, because of the short incubation period, the assay itself can be performed in less than 4 h, which is much quicker than the assay times usually associated with double-antibody separation. Thirdly, because all the reagents are commercially available, the time required to develop a local procedure is minimal. Fourthly, the assay is inexpensive to operate: at the time of this writing, the cost of reagents was 7 p (U.S. 11¢) per pair of (duplicate) tests.

Table 1. Analytical Recovery of CEA

<table>
<thead>
<tr>
<th>Added µg/L</th>
<th>Recovered µg/L</th>
<th>% recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.6</td>
<td>100</td>
</tr>
<tr>
<td>3.3</td>
<td>9.4</td>
<td>94</td>
</tr>
<tr>
<td>6.6</td>
<td>12.0</td>
<td>91</td>
</tr>
<tr>
<td>9.9</td>
<td>16.0</td>
<td>97</td>
</tr>
<tr>
<td>13.2</td>
<td>18.5</td>
<td>93</td>
</tr>
<tr>
<td>16.5</td>
<td>22.1</td>
<td>95</td>
</tr>
<tr>
<td>19.8</td>
<td>24.3</td>
<td>92</td>
</tr>
<tr>
<td>23.1</td>
<td>28.5</td>
<td>99</td>
</tr>
<tr>
<td>28.4</td>
<td>33.0</td>
<td>100</td>
</tr>
</tbody>
</table>


Fig. 1. Standard curve for CEA
Table 2. Precision Studies

<table>
<thead>
<tr>
<th></th>
<th>60-min incubation</th>
<th></th>
<th>120-min incubation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEA, µg/L</td>
<td>Mean</td>
<td>SD</td>
<td>CEA, µg/L</td>
</tr>
<tr>
<td>Within assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool 1</td>
<td>10.8</td>
<td>0.58</td>
<td>5.4</td>
<td>Pool 5</td>
</tr>
<tr>
<td>Pool 2</td>
<td>39.8</td>
<td>2.22</td>
<td>5.6</td>
<td>Pool 6</td>
</tr>
<tr>
<td>Between assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool 3</td>
<td>10.7</td>
<td>0.74</td>
<td>6.9</td>
<td>Pool 7</td>
</tr>
<tr>
<td>Pool 4</td>
<td>42.0</td>
<td>2.11</td>
<td>5.0</td>
<td>Pool 8</td>
</tr>
</tbody>
</table>

In conclusion, this CEA method will be of interest to both the clinician and the clinical chemist interested in the post-operative monitoring of patients with malignant disease.

We thank Mr. A. McBain for providing samples from patients, the laboratory staff in Rutherford Maternity for their invaluable cooperation, and Mrs. A. Russell for typing the manuscript. We also thank the National Institute for Biological Standards and Control for providing the 1st British Standard for CEA (73/801).

References

Fig. 2. Frequency distribution of CEA concentrations in normal adults.

The most important role for CEA measurements appears to be as a prognostic indicator pre-operatively, and as a post-operative monitor to detect recurrence of colorectal cancer. It is not totally reliable as a diagnostic aid in colorectal cancer, given that between 15 to 20% of patients with proven malignancies fail to have an increase in serum CEA and that increases in serum CEA concentrations sometimes accompany smoking and non-neoplastic disease. Serial CEA measurements, however, are of value in assessing the therapeutic response to various chemotherapeutic agents in the post-operative stage, increasing concentrations indicating a poor response to treatment.