Carcinoembryonic Antigen Assayed by Column Chromatography on Polyacrylamide Gel

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I evaluated a commercially available polyacrylamide column (Clinetics Corp.) for use in assaysing for carcinoembryonic antigen. The procedure eliminates the need for dialysis and 50 samples, assayed in duplicate, may be completed in 5 h. In general, results by this technique are 1.0 µg/L (range 0.5–2.0) lower than by the conventional dialysis method (r = 0.9876). Sensitivity approximates 0.5 µg/L. Within-day precision for two pools of patients’ plasma (n = 10) was \( \bar{x} = 2.29 \) (SEM 0.05) µg/L with CV = 6.55% and \( \bar{x} = 10.61 \) (SEM 0.10) µg/L with CV = 2.92%, respectively; between-day precision (n = 6) was \( \bar{x} = 3.90 \) (SEM 0.16) µg/L with CV = 10.0% and \( \bar{x} = 10.1 \) (SEM 0.37) µg/L. CV = 8.91%, respectively. The percentage analytical recovery of added carcinoembryonic antigen was about 80% at concentrations of approximately 8 and 12 µg/L, 73% at 18 µg/L.

Carcinoembryonic antigen (CEA) was first described by Gold and Freeman (1). Its laboratory measurement in human plasma involves extraction with perchloric acid, followed by extensive dialysis to eliminate excess acid before radioimmunoassay by various procedures (2–6). A commercial assay based upon the above work, marketed in kit form (Roche Diagnostics, Nutley, NJ 07110), is widely used for CEA quantitations. The method is tedious, in that each plasma extract must be dialyzed for 12–16 h vs de-ionized water, then dialyzed for 3 h vs buffer, with changes of water every 3 h. Moreover, the pH of the dialyzing buffer must be carefully controlled (7).

Other techniques have been proposed involving the use of Sephadex G-50 columns (8) to eliminate the need for dialysis. A commercial column based on this technique (Isolab, Inc., Akron, OH 44321) did not work well in my hands: it was cumbersome for large analytical runs and gave poor results. A recently introduced method, in which heat treatment of serum or plasma is followed by solid-phase radioimmunoassay (9, 10) appears to have potential, but is not yet licensed for use in this country.

I used the conventional Roche assay, but substituted the use of polyacrylamide gel columns for dialysis to remove excess perchloric acid. The method compares favorably with dialysis, but is much faster and its greater cost is justified by the labor savings.

Materials and Methods

Columns: Polyacrylamide gel columns (12 x 1.6 cm) were obtained from Clinetics Corp., Tustin, CA 92680.

Reagents: All reagents were the same as described in the Roche1 procedure, except that the pH of the 10 mmol/L acetate buffer was 6.60 (±0.10).

Assay Procedure

To 0.5-mL plasma samples and controls add 2.0 mL of NaCl solution (8.5 g/L) and mix. Add 2.5 mL of perchloric acid (1.2 mol/L) to each sample and control and mix; it is best to do this step one tube at a time. After centrifuging for 20 min at 1000 x g, decant the supernates onto polyacrylamide columns pre-equilibrated with 10 mmol/L acetate buffer, pH 6.60 (±0.10), allow to drain, and add 6.0 mL of the acetate buffer to each column. Collect the eluate for use in the radioimmunoassay for CEA. Six milliliters of ethylenediaminetetraacetate-containing buffer (included in the kit) is used for the 10, 25, 50, and 100 µL CEA standards used in preparing the standard curve. Immediately recharge the column by adding at least 36 mL of the acetate buffer. Store the columns with 5 to 10 mL of the acetate buffer in them and with top and bottom closures in place.

Results

Perchloric acid elution profile. Label 40 test tubes (dimensions: 16 x 125 mm). Extract one plasma sample as in the assay procedure. Select a column at random and allow it to drain. Add 1.0 mL of the perchloric acid reagent to the polyacrylamide gel column. Over 97% of the activity elutes between the 5th and 8th milliliter of effluent. A secondary peak is seen (Figure 1) between the 18th and 26th milliliters of effluent, which probably represents CEA fragments and inorganic iodide. Figure 1 also shows the pH elution profile, demonstrating that the perchloric acid elutes after intact 125I-labeled CEA, between the 14th and 35th milliliters of column effluent. The columns are regenerated with 36 mL of acetate buffer to assure complete removal of perchloric acid.

Precision studies. Perchloric acid extracts of human plasma pools were placed on the column and the eluates analyzed for CEA. Within-day precision for two different pools (n = 10) was \( \bar{x} = 2.29 \) (SEM 0.05 µg/L), CV = 6.55%, and \( \bar{x} = 10.61 \) (SEM 0.10 µg/L), CV = 2.92%. Between-day precision for two different pools (n = 6) was \( \bar{x} = 3.9 \) (SEM 0.16 µg/L), CV = 10.0%, and \( \bar{x} = 10.1 \) (SEM 0.37 µg/L), CV = 8.91%.

Analytical recovery. I analyzed 0.5-mL plasma samples, either without added CEA or 10, 25, or 50 µL of Roche CEA standard (125 µg/L) added. Table 1 depicts the recoveries for both the dialysis and column methods.

Comparison of column vs dialysis. Figure 2 compares values for the polyacrylamide gel separation method with those by the dialysis separation technique. With few exceptions the column procedure yielded results 0.5–2.0 µg/L lower than the dialysis method for values <8 µg/L. At higher values, results were closer to the line of identity. Results of 0.5 µg/L or less, for either the dialysis or column procedure, were assigned a value of 0.5 µg/L. Likewise, results exceeding 20 µg/L were designated as 20 µg/L.

Discussion

CEA has not been assayed in many laboratories because of
the dialysis step and the required changes of the water tank every 3 h. The method I describe involves a commercially available polyacrylamide gel column, which removes perchloric acid without the need for dialysis. The manufacturer specifies that the same column may be re-used at least 20 times. I confirmed this by assuring that all controls were within assayed limits at the 20th pass.

The polyacrylamide gel column may bind some CEA-like substances, yielding slightly lower values than the dialysis method. Moreover, recovery studies show that with the column method about 20% less is accounted for than with the dialysis method. There was closer agreement at higher values, with equal recoveries for both methods. All results that exceeded the highest standard in the dialysis method also exceeded the highest standard in the column procedure. Many samples that dialysis indicated contained CEA in the 0.5 to 2.0 µg/L range gave undetectable results with the column, i.e., higher counts than the zero standard. The reasons for this are not known.

Table 1. Analytical Recovery

<table>
<thead>
<tr>
<th>CEA added, µg/L</th>
<th>Expected</th>
<th>Found</th>
<th>Recovery, %</th>
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<tbody>
<tr>
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<tr>
<td>Plasma (base)</td>
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<td>5.2</td>
<td>100.0</td>
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<td>6.2</td>
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<td>Dialysis method</td>
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<td>Plasma (base)</td>
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<td>Plasma + 12.5</td>
<td>17.1</td>
<td>12.5</td>
<td>73.0</td>
</tr>
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</table>

* CEA was added to a control plasma (<2.0 µg/L) and all samples were diluted to the same volume with de-ionized water.

Fig. 1. Elution profile for 125I-labeled CEA applied to a column of polyacrylamide gel.

Fig. 2. Correlation between CEA values as obtained by column elution and by equilibrium analysis. Coincident points not shown.

The current cost of each assay on a column is about $0.75, based on 20 uses per column. The time saved in labor should enable a laboratory to justify use of the polyacrylamide column. Moreover, results are available to the clinician the same day that the sample is submitted to the laboratory.

References