Radioimmunoassay

A CEA Kit with Second Antibody Procedure: Modifications to Permit Small Tube Assay

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An improved CEA kit using a second antibody suspension to replace the Z-gel separation was recently introduced. Both the improved and the original methods require use of a counting tube greater than 13 × 100 mm, which some instruments may not be able to count. A simple technique that allows the completed assay to be counted in small tubes (12 × 75 mm) without adverse effects on the assay results is described.

Microprocessor counting equipment and limited laboratory working space have led to the purchase of smaller, compact, bench-type gamma counting equipment. These instruments for the most part do not allow tubes larger than 12 × 75 mm to be counted. The volume of solutions required by the Roche Diagnostics method for carcinoembryonic antigen (CEA) assay does not permit this assay to be performed in small 12 × 75 tubes, therefore making it difficult for some laboratories to assay patient samples for CEA. We report modifications in the CEA-ROCHE® second antibody procedure that enable the assay to be counted in 12 × 75 tubes.

Materials and Methods

The CEA-Roche methodology provides the option of dialysis or gel filtration chromatography for removal of perchloric acid and electrolytes from the samples; we use the gel filtration chromatography. To do this, we use the CEA Acrylamide column and reagent buffer pack (Clinetics Corp., Tustin, CA). We follow the manufacturer's protocol without modification.

For the radioimmunoassay of the gel filtration eluates, we use CEA-Roche with second antibody suspension and follow the protocol for preparation of the standard inhibition curve without modification. The manufacturer's protocol for the primary assay for eluates of CEA content is as follows:

1. Add 25 μl of the anti-CEA serum to each of the tubes (except the nonspecific binding and total count tubes, if included) and mix contents with a vortex mixer.
2. Place tubes in a 45°C ± 1° circulating water bath for 30 ± 1 min.
3. Remove tubes from the water bath and add 25 μl of I-125 CEA reagent to each of the tubes. Mix contents with a vortex mixer. Set aside the total count tubes (if included) at room termperature until they are to be counted.
4. Return all tubes (except total count tubes) to the water bath for 30 ± 1 min.
5. Remove tubes and immediately add 1.0 ml of second antibody suspension to each. It is important that the second antibody suspension be thoroughly suspended during use (do not shake; use a magnetic stirrer to resuspend for at least 10 min prior to use) and added rapidly with a semiautomatic dispensing instrument so the reaction is kept constant in all tubes.
6. Mix tubes with vortex mixer.
7. Incubate at room temperature for 15 min.
8. Centrifuge at 1000 × g for 10 min.
9. Decant the supernatants into an isotope waste container. Invert the tubes, blot, and drain for 5 min on absorbent paper. Blot the rims again at the end of the draining period.
10. Determine the amount of bound I-125 CEA with a gamma scintillation spectrometer by counting each tube for 1 min. Duplicates should be within 5% of the mean CPM. If not, re-extract plasma specimen and repeat assay.
11. Construct a standard inhibition curve by plotting the mean CPM of I-125 CEA bound to the second antibody polymer against the antigen dose in each of the tubes. Other methods of data reduction (such as %B/T) can be used if desired.
12. The CEA content of each of the extracts is then determined from the graph. Values must be multiplied by two to convert to ng of CEA/ml of plasma. CPM values greater than the 0 antigen dose are reported as 0 ng of CEA/ml plasma. Values that are greater than 50 ng/ml should not be reported and the specimens

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should be reassayed using the dilution assay (Hoffmann-LaRoche, Inc., Nutley, NJ).

So that we could satisfactorily count the assay in small tubes, we modified steps 8 and 9 of the protocol as follows:

8. Label a corresponding series of 12 × 75 tubes.
9. (a) Decant approximately 4.0 ml of each tube's solution into the corresponding 12 × 75 tube (except T.C.).
   (b) Centrifuge the 12 × 75 tubes at 1000 × g for 10 min.
   (c) Decant the supernatants of the 12 × 75 tubes.
   (d) Gently resuspend the remainder of solution in the large tubes and decant into the respective 12 × 75 tube.
   (e) Centrifuge the 12 × 75 tube at 1000 × g for 10 min.
   (f) Decant the supernatants of the 12 × 75 tubes and let drain for approximately 5 min.

The rest of the original protocol is followed without further modification.

Discussion

The Roche method calls for large volumes of reagents and eluates per tube. Because our laboratory has a small tube capability, we modified the method to enable us to count the bound-CEA fraction in 12 × 75 mm tubes. Two options were available: a complicated process of decreasing volumes of reagents and sample eluates; or a "two-shift" centrifugation technique. We chose the latter because of time and cost considerations.

We decant approximately 4.0 ml of the completed CEA assay solutions into respectively labeled 12 × 75 tubes, centrifuge and decant, then decant the remainder of each solution (approximately 3.0 ml) into the 12 × 75 tubes and centrifuge again. We do not resuspend the pellet from the first centrifugation before the second centrifugation; we allow the pellet from the second centrifugation to be packed directly on top of the first.

In reviewing our results with those of a reference laboratory (Table 1), we determined the coefficient of variation (CV) between each sample. We also determined the upper limit of 95% confidence level of these CVs at 16.3%. Of 13 patients, ten had less than the 16.3 upper limit of confidence. Two patients had CVs between 25–30%, a result that is unexplained. However, the variation of these results did not affect clinical decision making in the patients involved. One patient had a CV of 17.5%, but this was considered clinically insignificant, as both values were less than 1.0 ng/ml.

TABLE 1. Comparison of Results

Patient no.	Reference laboratory results	In-house results	CV
1	0.9	0.7	17.5
2	5.7	3.8	28.5
3	13.2	12.9	1.5
4	41.0	37.8	5.7
5	1.8	2.2	10.0
6	3.2	3.3	2.1
7	1.0	1.1	9.5
8	2073.0	2022.0	1.8
9	892.0	718.0	15.0
10	3.5	2.3	27.7
11	2.9	2.9	-0-
12	1.4	0.9	10.9
13	3.0	2.7	1.6

Conclusion

The modification described above in a manufacturer's protocol to accommodate the use of small tubes does not have any clinically significant effect on the results of the assay. This modification is simple, easy to do, and only requires 15–20 min of additional time. One-fourth of this time involves direct technologist participation. Our protocol allows the CEA-ROCHE® assay to be done in institutions with gamma counting instruments that only accept smaller counting tubes.

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