Improved Immunoassay of Carcinoembryonic Antigen in Serum

To the Editor:

Carcinoembryonic antigen (CEA) is present in low concentrations in the serum of adults but in greater concentration in patients with different types of carcinoma. We describe a rapid, simple enzyme immunoassay in which a one-step “sandwich” system is used, without treatment of serum. The method has the following characteristics.

The anti-CEA and anti-CEA conjugated with peroxidase (the “conjugate”) used are commercially available (Dako, Copenhagen, Denmark).

The solid phase is a polystyrene test tube, treated with a 1 mL/L glutaraldehyde solution for 3 h at 56 °C and coated with a 10 mg/L solution of anti-CEA in pH 9.6 carbonate/bicarbonate buffer for at least four days at 4 °C.

Before use, aspirate the contents of each tube and wash the tube three times with 0.15 mol/L NaCl containing 5 mL of Tween 20 polyoxyethylene (20) sorbitan monolaurate surfactant per liter.

The immune reaction is performed in one step. Incubate tubes coated with anti-CEA concurrently with antigen and conjugate. Pipet 50 μL of serum or standard and 200 μL of antibody–enzyme solution containing porcine serum (Gibco Europe, U.K.) into each tube and incubate for 45 min in a rotator at room temperature.

Assay all standards, controls, and sample in duplicate. Aspirate the contents of each tube and rinse the tubes three times with distilled water.

α-Phenylenediamine is the chromogen used for the determination of peroxidase activity. Pipet 1 mL of the 20 mmol/L chromogen solution in pH 5 acetate buffer, also containing 10 mmol of H2O2 per liter, into each tube and leave for 30 min at room temperature, in darkness. Stop the color reaction by adding 1 mL of diluent (1 mol/L HCl) to each tube and measure the absorbance in a photometer at 492 nm.

The dose–response curve is linear between 0 and 100 μg of CEA per liter of serum. The mean absorbance for a 100 μg/L solution is 1.8.

Smith and MacDonald (1) used the same antibodies in a two-step “sandwich” immunoassay. They obtained the same mean absorbance but the dose–response curve was nonlinear.

Our within-run CV for a low (6.9 μg/L) concentration is 6.8%; for a 82.8 μg/L solution it is 4.7%. Our between-run CV for a low (9.5 μg/L) concentration is 6.9%; for a 71.3 μg/L solution it is 6.0%.

The least concentration distinguishable from zero with 99.8% confidence is 3 μg/L.

This method is easy and very fast. No extraction is required. The short incubation period makes it much quicker than radioimmunoassays or two-step “sandwich”-type enzyme immunoassays.

Reference

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5,5’-Dithiobis(2-nitrobenzoic acid) Does Not Influence Isolation or Lipid Composition of Lipoproteins Obtained by Ultracentrifugation

To the Editor:

The liver, the major source of plasma lipoproteins, synthesize and secretes a lecithin–cholesterol acyltransferase (LCAT; EC 2.3.1.43). This cholesterol-esterifying enzyme utilizes nascent high-density lipoproteins (HDL) as substrate to form cholesteryl esters, thereby converting the nascent discoidal HDLs into spherical particles (1). Therefore, all blood samples used for studies of nascent HDL are collected in 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB) at 2 mmol/L to inhibit the action of LCAT (2). This reagent oxidizes free sulphhydrol groups and so prevents disulfide formation. In preliminary experiments, we observed that added DTNB, 0.5 mmol/L, caused values for enzymic determinations of triglycerides, cholesterol, and phospholipids to be low by 33%, 20%, and 9%, respectively. Thus, we made the present study to determine whether DTNB affects the isolation of the major lipoprotein classes from human serum and (or) interferes with enzymic determinations of lipid components.

Lipoproteins in 5-mL plasma samples were separated by ultracentrifugation (100,000 × g, 4 °C, 42 h) in a discontinuous KBr gradient ranging in density from 1.001 to 1.255 kg/L according to the modified procedure of Chapman et al. (3). Total cholesterol, phospholipids, and triglycerides were determined enzymically (kits from Wako, Osaka 514, Japan). Protein was measured by the method of Lowry et al. (4). Quantitative distribution of the DTNB present in the lipoprotein fractions was measured from its absorbance at 405 nm (Amax). We mixed 50 μL of sample with an aqueous pH 12.6 solution (0.5 mol/L NaOH and 0.15 mol/L NaCl, 1/8 by vol). Blanks were made by adding samples to an aqueous pH 1.4 solution (0.5 mol/L HCl and 0.15 mol/L NaCl, 1/8 by vol). Blood samples for lipoproteins studies were routinely collected in tubes containing 2.7 mmol of EDTA per liter (final concentration), to which DTNB was added to give a final concentration of 0.5 mmol/L, sufficient to prevent disulfide linkages and to inhibit LCAT activity (5).

Under these conditions, we saw no modifications of the density profile obtained after gradient ultracentrifugation. The distribution of DTNB in the density gradient seemed to be the result of a diffusion towards the highest densities, that is, over d = 1.140. The chemical composition of all the d = 1.006–1.30 kg/L fractions (VLDL, LDL, HDL1, and HDL2) was similar whether DTNB was present or not. In contrast, the presence of DTNB (final concentration from 87 to 545 mmol/L) caused values for enzymic determination of triglycerides, cholesterol, and phospholipids to be low by 5%, 3.5%, and 1.5%, respectively.

We conclude that the low values for lipids obtained with whole sera in the presence of DTNB are not found after ultracentrifugation. The only effect of the use of DTNB was on the enzymic determination of HDL4; there was no influence on the separation and yield of the other lipoproteins.

References
2. Stokke KT, Norum KR. Determination