Shaking Polystyrene Beads during Coating with Antibody: Effect on the Precision Profile of Enzyme Immunoassay of Triiodothyronine

To the Editor:

Evaluating an enzyme immunoassay (EIA) procedure based on antibody coated on solid-phase materials for assay of triiodothyronine (T3) (1), we observed a considerable improvement in the precision of this assay when the antibody coating on solid-phase matrix was carried out with shaking.

In the EIA procedure, the coated beads are incubated with 30 pg of horseradish peroxidase-labeled T3 and T3 standards in concentrations ranging from 0.25 to 4 μg/L. After 2 h, the beads are washed and kept in contact with 1 mL of substrate/chromogen solution containing H2O2 and o-phenylenediamine. The reaction is stopped by addition of 4 mol/L H2SO4 after 40 min, and the absorbance is measured. For this study, we performed the assay in 10 replicates and constructed precision profiles (2).

Rabbit antiserum to T3 was absorbed onto the surface of 6-mm polystyrene beads (Precision Balls Co., 3000 N. Cicero Avenue, Chicago, IL 60641) by incubating the beads with antiserum diluted 20 000-fold in bicarbonate buffer (100 mmol/L, pH 9.6) for 3 h at room temperature without disturbing the mixture. The beads were kept immersed in the antiserum in single layer (in a beaker) to get uniform coating.

After this incubation, we washed the beads with distilled water, then saturated any remaining adsorption sites on the beads by further incubation with bovine serum albumin (5 g/L) for 20 min. After again washing the beads with distilled water, we stored them at 4°C.

This same coating procedure was repeated, this time with the beaker rotated gently at 60 rpm. We also individually coated the beads with antibody in a uniform 12 × 75 mm glass tube.

The precision profile (Figure 1) was considerably improved when the coating was done with gentle shaking. Also, the precision obtained with beads coated individually in glass tubes was superior to that obtained for coating in a beaker without shaking.

When the beads are incubated in antibody solution, after a time the concentration of antibody around each bead varies considerably; i.e., there is non-uniform adsorption of antibody. Shaking during the coating process avoids this, and maximum adsorption is attained more quickly (coating time, 20 min; 40% binding of T3 tracer).

Without shaking, the optimum time was 3 h and the binding 30%. Evidently, shaking plays an important role in the kinetics of antibody adsorption and improves the precision of the assay.

References

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Differential Determination of HDL-Subfractions in Clinical Laboratories

To the Editor:

In a recent report, Dias et al. (1) evaluated a dual-precipitation method for determining cholesterol in high-density lipoprotein (HDL) and its subfractions HDL2 and HDL3. The method resulted in values for HDL2- and HDL3-cholesterol that correlated highly with and did not differ significantly from those obtained by density-gradient ultracentrifugation or by a combined precipitation-ultracentrifugation method (correlation coefficients for the comparison of results in 52 samples were between 0.91 and 0.95). Given the fact that the method was also precise (CVs of 8.6% and 8.8% for HDL2- and HDL3-cholesterol, respectively) and because of its simplicity, the authors concluded that the dual-precipitation method is suited for routine hospital laboratories.

In our opinion, however, more epidemiological and case-control studies are needed before introducing methods for subfractionation of HDL to clinical laboratories. At present, the published data on the clinical significance of this measurement are still scarce or controversial (2–7), and it remains doubtful whether the differential measurement of HDL2- and HDL3-cholesterol adds much to the measurement of total HDL-cholesterol. In a study in 250 sera covering a large range in total HDL-cholesterol, we found that the values of HDL2- and HDL3-cholesterol determined by density-gradient ultracentrifugation correlated highly with total, unfractionated, HDL-cholesterol. The following polynomial relations were found: HDL2-cholesterol = 0.20(HDL-cholesterol)2 + 0.02(HDL-cholesterol) + 0.03 mmol/L, and HDL3-cholesterol = -0.20(HDL-cholesterol)2 + 0.98HDL-cholesterol - 0.03 mmol/L, with correlation coefficients of 0.93 and 0.95, respectively.

These results indicate that, at a low total HDL-cholesterol concentration (< 0.75 mmol/L), almost all cholesterol is present in the HDL2-cholesterol. When total HDL-cholesterol increases from 0.75 to 1.25 mmol/L, the increase in HDL2-cholesterol (from 0.16 to 0.37 mmol/L) becomes more important, but that in HDL3-cholesterol (from 0.60 to 0.88 mmol/L) is still larger in absolute terms, as can be calculated with the above-mentioned formulas. An increase at above-normal HDL-cholesterol concentrations is caused almost exclu-
sively by an increase of HDL₃-cholesterol. Assuming that the risk of coronary heart disease is linearly related to the inverse of the HDL-cholesterol concentration, our findings indicate that an increase in HDL₃-cholesterol may be as favorable as an increase in HDL₂-cholesterol, which makes a differential determination superficial (8).

Besides, measurement of HDL₂- and HDL₃-cholesterol by the dual-precipitation method is two to three times less precise than measurement of total HDL-cholesterol (1,9,10). Furthermore, we found that HDL₂- and HDL₃-cholesterol fractions obtained by the dual-precipitation method are slightly cross-contaminated (10). Therefore, it seems premature to introduce subfractionation of HDL into HDL₂ and HDL₃ in clinical laboratories. However, for new epidemiological and case-control studies, the dual-precipitation method is preferable to one of the ultracentrifugation methods. In such studies, the significance of other HDL-subfractions composition (i.e., apo A-I, apo A-II, or both) should be evaluated as well (11).

References

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Alkaline Phosphatase of Possible Renal Origin Identified in Plasma after Colchicine Overdose

To the Editor:

A single gene is thought to control the production of the "unspecific" forms of alkaline phosphatase (EC 3.1.3.1) found in liver, bone, and kidney, and to account for their similar immunological reactivity. Despite this similarity, the "usual" liver isoenzyme can be differentiated from the high-molecular-mass form of alkaline phosphatase of liver origin by use of naturally occurring enzyme-binding immunoglobulin autoantibodies (1) or artificially produced monoclonal antibodies (2). Also, the bone and liver isoforms can to some extent be immunologically differentiated by using murine monoclonal antibodies (2,3).

We observed that alkaline phosphatase-binding immunoglobulins in some sera can readily bind the liver and bone isoforms, but poorly bind added alkaline phosphatase derived from kidney. We used this observation to make a tentative identification of an alkaline phosphatase of unusual mobility found in the serum of a patient who was recovering from an overdose of colchicine.

The patient, a 27-year-old woman, was admitted 10 h after swallowing 25 0.5-mg tablets of colchicine. On the day of admission, the patient's value for alkaline phosphatase in serum was 135 U/L (upper reference limit: 100). It increased to 639 U/L the next day and declined rapidly thereafter. These changes were paralleled by changes in serum urea and creatinine concentrations.

Evaluation of alkaline phosphatase isoenzymes in the second serum sample by electrophoresis on cellulose acetate (4) demonstrated the presence of a principal band with mobility somewhat slower than that usually observed with bone but anodal to intestinal alkaline phosphatase, together with a normal amount of alkaline phosphatase with liver-type mobility and some streaking between the two. Enzyme activity was also present at the origin (Figure 1, track I). The principal band was labile to heat (56 °C, 10 min), resistant to inhibition by L-phenylalanine, and remained at the origin after the sample was incubated with sitagliptinase (EC 3.2.1.18). Lectin affinity electrophoresis (5) demonstrated a liver-type band, some enzyme in the bone isoenzyme position, and a marked increase in activity at the origin. The acrallylase gel electrophoretogram (6) was similar to that for cellulose acetate, with faint activity in the liver position, a predominant staining band of mobility slower than the bone isoenzyme but in advance of intestinal alkaline phosphatase, and marked activity at the origin.

Knowing that kidney-extract alkaline phosphatase migrates on cellulose acetate in the approximate position of the principal band we observed in the patient's sample, and that colchicine toxicity may result in kidney damage, we thought it possible that the band was of renal origin. We used serum containing an IgG kappa alkaline-phosphatase-binding immunoglobulin to provide support for this suggestion.

Fig. 1. Binding of added alkaline phosphatase (ALP) by serum containing alkaline phosphatase-binding immunoglobulin (ALP-lg)

Patient's serum (track 1, top); ALP-lg serum with added patient's serum (track 2); serum from patient with increased liver (L) and high-molecular-mass (s1) ALP (track 3); ALP-lg serum with added liver/s1 serum (track 4); serum from patient with increased bone (B) ALP (track 5); ALP-lg serum with added bone serum (track 6); ALP-lg serum (track 7)