Determination of Hemoglobin A1c by Liquid Chromatography

To the Editor:

Schreiber and Wadsworth inquired (1) about methemoglobin formation in the liquid-chromatographic (FPLC) method of Jeppsson et al. for quantifying glycated hemoglobin (Hb A1c) (2).

We also used the method of Jeppsson et al., and eliminated the labile glycated fraction by the method of Ellis et al. (3). We noticed that including 0.2 g of sodium azide per liter of the 10 mmol/L malonic acid buffers (A and B), pH 5.7, eliminated the methemoglobin peak. When we analyzed 55 blood samples by FPLC with and without 0.02% sodium azide, we obtained the correlation between results shown in Figure 1.

The presence of azide in the buffers prevents the formation of methemoglobin, which thus affects the reference values for Hb A1c as determined by the FPLC method.

References

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Heat inactivation of Serum prior to Measurement of HIV Antibody by Enzyme Immunoassay

To the Editor:

In a recent Technical Brief, Montani and Vemicrati (1) recommended that serum be heated at 56 °C for 1 h, to inactivate HIV. Others have supported this recommendation (2, 3). Several studies have demonstrated that heating serum reduces the infectivity of HIV. However, complete inactivation of the virus has not been confirmed in these studies, owing to variable results based on the time of heating, temperature, concentration of the virus, and method of measuring the virus (4–9).

It has been shown that heat inactivation of serum at 56 °C for 30 min to 1 h does not alter results obtained for specimens that are positive for HIV antibody, hepatitis B surface antigen and antibody, and reaginic and treponemal antibody (1, 4, 10). However, heat inactivation of serum obtained from normal individuals has been shown to give false positive results for HIV antibody (11–13). In one study in which 15 sera from normal individuals were tested for HIV antibody with the Abbott HTLV III enzyme immunoassay kit, all 15 sera tested positive after heat inactivation of the sera at 56 °C for 30 min (13).

It has been emphasized in earlier communications (11–14) that heat inactivation of serum before it is tested for HIV antibody is not recommended unless the method of assay has been shown to give the same results before and after heating. This criterion should be applied to any immunological method involving assay of antibodies in potentially infectious specimens and is especially important for the assay of HIV antibody, where a false positive could result in serious psychological, social, or economic effects on the individual involved.

Until further studies demonstrate that heating serum at 56 °C for 30 min to 1 h completely inactivates HIV and does not produce false-positive results for normal sera, I think that specimens for assay should be unheated and appropriate measures for their safe handling should be adopted by the laboratory as outlined by the CDC (9).

References