Alternative ELISA for Sex Hormone-Binding Globulin in Plasma

To the Editor:
Bioavailable testosterone is often derived from the free androgen index (1), which is reliant on measurements of both testosterone and sex hormone-binding globulin (SHBG). Although testosterone assays are widely available in various formats, assays for SHBG are more restricted partly because of the absence of automated procedures and the relatively high expense of kits. Dako (Denmark) has a rabbit polyclonal antibody to SHBG as well as, until recently, a peroxidase-labeled rabbit polyclonal antibody to SHBG. These two antibodies can be used together with pooled human pregnancy plasma as a calibrator to provide a direct inexpensive ELISA for SHBG in plasma, as detailed by Dako in their package insert and their “general ELISA procedure” guide. Briefly, microtiter plates are coated with antibody diluted 1:350 in bicarbonate buffer overnight at 4 °C, followed by “blocking” and the addition of either the calibrator (diluted pregnancy plasma) or diluted plasma samples for an overnight incubation at 4 °C. The next day, the plate is washed, and bound SHBG is detected with a 6-h incubation using the polyclonal peroxidase-labeled SHBG antibody (1:3000 dilution). The plates are finally washed, and o-phenylenediamine substrate is added.

Recently this peroxidase-labeled polyclonal antibody has become unavailable, requiring the use of alternatives. We have obtained a mouse monoclonal antibody to SHBG, and its use in a modified ELISA shows excellent agreement to the Dako method (Fig. 1). The modification uses microtiter plates coated with Dako SHBG polyclonal diluted (1:700) in phosphate-buffered saline (PBS) and identical initial incubation conditions. The method differs in that the bound SHBG is detected using this mouse monoclonal SHBG antibody supernatant diluted 1:20 in PBS containing 1 mL/L Tween 20 and 1 g/L gelatin (assay buffer) for 30 min at room temperature, followed by washing and the addition of anti-mouse Ig peroxidase from Amersham (1:1000 in assay buffer) for an additional 30-min incubation at room temperature. The plate is finally washed, and o-phenylenediamine substrate is added.

The modification described uses “assay buffer” as a common reagent for all steps except coating, which is carried out in PBS with no additives. The method offers a shorter and immediate alternative to those laboratories currently determining SHBG by ELISA using both Dako antibodies.

References

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Hemoglobin D [β 121(GH4)Glu→Gln] Causing Falsely Low and High HbA₁c Values in HPLC

To the Editor:
Despite advances in the standardization of methods for glycohemoglobins (1), hemoglobinopathies may falsely lower glycohemoglobin values and may cause falsely high hemoglobin (Hb)A₁c values and/or additional peaks in HPLC chromatograms (2, 3). We report a 66-year-old female Caucasian diabetic patient, treated with diet, whose HbA₁c was repeatedly 3.2% (reference interval in non-diabetics, 4.2–6.1%) as measured with the Diamat HPLC (Bio-Rad Laboratories). The chromatogram contained no anomalous peaks, and neither low blood glucose values nor symptoms of hypoglycemia were detectable. The Diamat HPLC uses a borate-containing buffer and a step gradient of three phosphate buffers with increasing ionic strength.

The patient’s HbA₁c value with a second HPLC method (Hi-Auto A₁c, HA-8140, Menarini Diagnostics) was repeatedly 8.4% (reference values, 4.5–5.7%), and the HPLC chromatogram showed an additional peak at

**Fig. 1. Comparison of plasma SHBG concentrations determined by ELISA using Dako polyclonal antibodies with the modification using the monoclonal antibody.**
HbA\(_{1c}\). This analyzer system denoted the chromatogram as abnormal separation. This HPLC method uses cation-exchange and reversed-phase chromatography on a solid phase of methacrylic acid and methacrylate ester. The hemoglobin fractions are eluted by varying the pH of the mobile phase.

The mean blood glucose of our patient was near the upper limit if the reference interval is at 7 mmol/L (126 mg/dL), based on a mean of 3–4 daily home measurements during 1 week before HbA\(_{1c}\) evaluation. The serum fructosamine was 256 mmol/L (reference values, 285 mmol/L).

Electrophoretic analysis on cellulose acetate membranes (Helena Laboratories) of a blood sample from our patient revealed an abnormal hemoglobin anodal to HbS. Citrate agar electrophoresis of hemoglobin using citrate buffer at pH 6.2 demonstrated a hemoglobin variant co-migrated with HbA. Identification of HbD was performed with the \(\beta\)-Globin StripAssay (Vienna Lab) using reverse-hybridization with the patient’s amplified DNA and probes for HbD. Routine hematological indices were within reference values.

We conclude that HbD, a common hemoglobinopathy (4), may cause diagnostic confusion because of falsely low and high HbA\(_{1c}\) values when measured by these two HPLC methods. This supports the suggestion that fructosamine measurement is a suitable alternative to control diabetes in patients with hemoglobin variants (5).

References