To the Editor:

In previously reported studies, factor VIII (FVIII) inhibitors are quantitatively determined by laboratory mixing tests that measure the capacity of the inhibitor to neutralize FVIII:C activity and may not recognize nonactivating antibodies. However, these Iggs may significantly influence plasma clearance, survival times, and circulating levels of infused FVIII:C protein. Both the Oxford and the
Bethesda tests were developed to assign unitage to known inhibitors so that the course of a given patient could be observed over time and so that one patient could be compared, roughly, with another. However, inhibitor “units” are related arbitrarily to the amount of residual FVIII:C and wide discrepancies of quantitative results exist among laboratories in collaborative multicenter studies. Several reasons were reported for conflicting data on the frequency of development of FVIII inhibitor and it has been pointed out that the Bethesda assay commonly used tended by an experimental artifact to overestimate antibody activity and, therefore, the incidence of FVIII inhibitors. Reasonably good agreement is obtained when these assays are used either to rank inhibitor activity as low or high titer or to distinguish between low and high responders. Variability of data may arise for many reasons: the assay is insensitive to low titer inhibitors; results of inhibitor assays vary according to levels of FVIII:C activity in normal plasma; unavailability of universal FVIII:C inhibitor standard; or the variable kinetics of inactivation of FVIII:C activity by the inhibitor according to its time- and temperature-dependent binding affinity for the coagulation protein.

To improve the detection of FVIII inhibitors, we have developed a sensitive enzyme immunoassay that measures immunoglobulins reacting either with functional epitopes or antigenic sites. A micro–enzyme-linked immunosorbent assay (micro-ELISA) method including 4 layers has been previously described for the immunologic characterization of FVIII inhibitors. The recent availability of highly purified FVIII concentrates, the evidence of the IgG nature, the prevalence of the IgG class, and the IgG4 subclass restriction of the inhibitors have prompted us to measure FVIII antibodies as the amount of IgG and IgG4 directed against the FVIII molecule. The immunoassay included only 3 layers, minimizing nonspecific background phenomenon; plates coated with purified FVIII prepared by ion-exchange chromatography were first incubated with the sample containing the inhibitor and then with conjugated anti-IgG or anti-IgG4 monoclonal antibodies. We have tested plasma samples of 15 hemophilia A patients (inhibitor activity from 2 to 5,500 Bethesda units [BU/mL]), 3 patients with autoantibodies (1 to 90 BU/mL), and 5 pools of normal human subjects. At dilutions ranging from 1/100 to 1/10,000, none of the normal plasma results were positive. All 15 of the plasmas of hemophiliacs and the 3 plasmas of patients with autoantibodies exhibited clear positive reactions. Additionally, similar shapes of the curves were observed for all samples by plotting serial dilutions of each sample against absorbance. Inhibitor activity as low as 0.1 BU/mL would be clearly detected by means of this test. Variations in the antibody level were measured for samples observed over time.

Purified anti-FVIII antibodies (only one band in sodium dodecyl sulfate-polyacrylamide gel) were obtained from a plasma containing an IgG4 subclass inhibitor (90 BU/mL) by immunoabsorption on immobilized purified FVIII. These antibodies were measured for FVIII inhibitors, IgG, and IgG4. Analysis of the plasma samples using these purified antibodies as standards resulted in the calculation of the amount of IgG or IgG4, directed against FVIII. In this preliminary study, 1 BU is related to 100 to 500 ng of IgG depending on the IgG or IgG4 nature of the conjugated antibody.

Our data suggest the possibility of measurement of functional as well as nonactivating antibody concentrations and the possibility of easy standardization of the assay. A further prospective study will be conducted using the immunoassay in parallel with biologic assays to confirm the reliability of the method for the detection of developing antibodies and the quantitation of alloantibodies and autoantibodies.

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REFERENCES
Quantitation of factor VIII antibodies by an enzyme-linked immunoassay method [letter]

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