Serum inhibin A measurements have become increasingly common in clinical use, and their inclusion in prenatal screening for Down syndrome has been recently advocated. Two methods are commercially available for the specific measurement of dimeric inhibin A in human serum or plasma (1). Given the absence of a gold standard and the evolving clinical utility of inhibin A, a detailed comparison of the two inhibin A ELISAs is warranted. In the studies reported here, we undertook the evaluation of these methods and a direct comparison of their performance. The results obtained support the use of both methods and provide a basis for comparing values across these methods. Furthermore, we recommend that laboratories adopt a reporting unit of IU/mL based on the recombinant human Inhibin A International Reference Preparation (IRP) distributed by the National Institute for Biological Standards and Control on behalf of the WHO (91/624).

Inhibin A is a dimeric glycoprotein hormone belonging to the transforming growth factor-β superfamily of cytokines. In addition to its numerous local, tissue-specific regulatory or “cytokine-type” actions (2–4), ovary-produced inhibin A is an important negative feedback hormone that suppresses pituitary secretion of follicle-stimulating hormone during the late follicular and luteal phases of the menstrual cycle (5,6). Furthermore, circulating concentrations of inhibin A appear to reflect tumor mass for certain forms of ovarian cancer (7), particularly granulosa cell adenocarcinomas, and measurement of inhibin A also may be useful in assessment of gestational trophoblastic disease (8,9). In addition, the measurement of serum inhibin A has recently been demonstrated as useful in the clinical setting as part of the “quadrupele” antenatal screen for Down syndrome (10–12).

The only analytical methods developed to date for measuring the picomolar concentrations of inhibin A found in peripheral blood have been immunoassays (13). It has been difficult to design assays that distinguish inhibin A from other, structurally similar transforming growth factor-β superfamily proteins, particularly the free α subunits, inhibin B and activin A. As a result, there are only two established methods for specifically measuring dimeric inhibin A in human serum. Both of these methods are commercially available two-site ELISAs that depend on the same pair of monoclonal antibodies developed against synthetic peptide epitopes of the two subunits of dimeric inhibin A (13, 14). The first to be developed is manufactured by Serotec, Inc. This method is not been cleared by the US Food and Drug Administration for clinical use but has been used in an investigational context to measure inhibin A in various clinical conditions (15, 16). Because of the difficulties in assay development, the Serotec inhibin A ELISA represents the method on which much of our current knowledge concerning dimeric inhibin A concentrations in human blood is based. In late 1999, a second ELISA became available. This method, manufactured by Diagnostic System Laboratories (DSL), although using the same monoclonal antibody pair, includes several technical improvements with respect to specimen handling and test performance. The DSL assay has been cleared by the Food and Drug Administration for the measurement of inhibin A in human serum for use as an endpoint equivalent to estradiol measurement during the menstrual cycle or during ovarian stimulation.

Blood samples were taken from volunteers participating in research studies in the Reproductive Endocrine Unit of the Massachusetts General Hospital. All specimens were obtained after volunteers gave their informed consent to participate in the Institutional Review Board-approved study protocols. Serotec assays (Ultra Sensitive Inhibin A Dimer Assay Kit; cat. no. MCA950KZZ; Oxford BiInnovation, Inc.) were purchased from Serotec, Inc. DSL assays (Inhibin A ELISA; cat. no. DSL-10-28100-1) were provided by Diagnostic Systems Laboratory (Webster, TX). All reagents were used according to the manufacturers’ procedures described in the package inserts. Of note, the Serotec assay requires a sample pretreatment step (including boiling) not required in the DSL assay. Recombinant human inhibin A (cat. no. 624-IN) was purchased from R/D Systems. Lyophilized recombinant human inhibin A was reconstituted as recommended by the manufacturer, diluted in human serum, and stored at <20°C before assay. Pooled serum from men was used for these studies because male serum contains essentially no inhibin A (16, 17), which minimizes the potential for nonlinearity attributable to mixtures of inhibin A isoforms. The reference preparation of inhibin A used in these experiments was a recombinant human inhibin A IRP (91/624) distributed by the National Institute for Biological Standards and Control on behalf of the WHO (18). SigmaPlot statistical software (SPSS) was used to process the data and for statistical analysis.

We first studied the measurement of recombinant inhibin A in human serum to determine whether the Serotec and DSL ELISAs performed with different specificities, which would be apparent as a nonlinearity of the response curve generated by serum dilutions of recombinant human inhibin A compared with the linear response generated with the calibrators provided with the assays. For each assay method, the recombinant human inhibin A and the assay calibrators showed parallel dose–response curves (data not shown). These results indicate that, as expected, the two assay systems demonstrate similar specificities for the detection of inhibin A.
We then performed a method comparison of the two assay systems in regard to their ability to detect inhibin A in human serum. For this analysis we used both assay systems with the calibrators provided by the manufacturers to measure inhibin A concentrations in ng/L in 1172 serum specimens. As shown in Fig. 1A, the concentrations of inhibin A obtained using the DSL assay were ~1.6-fold higher than those obtained for the same specimens with the Serotec assay (intercept, 8.64 ng/L; slope, 1.61; \( r = 0.908 \)), a finding similar to that seen previously (1).

Determining the accuracy of inhibin A measurements is not currently possible because there is no gold standard measurement method and there is considerable heterogeneity in purified preparations of inhibin A proteins derived from natural sources or produced by recombinant methods. The Serotec method uses for calibration an affinity-purified mixture of inhibin proteins isolated from follicular fluid recovered from women undergoing in vitro fertilization/assisted reproduction procedures. This mixture is calibrated relative to a preparation of purified \( M_r 32\,000 \) recombinant human inhibin A. The DSL assay uses a purified \( M_r 32\,000 \) recombinant human inhibin A, and the calibration method is based on the protein mass of this calibrator. In both cases the units reported are mass based (ng/L), but the mass units are derived either from immunoassay calibration (Serotec) or by weight using an in-house recombinant protein whose immunoreactivity relative to other standards/calibrators is unknown (DSL).

It has been demonstrated that different preparations of recombinant or native inhibin A of comparable purity may have very different immunoreactivities with respect to individual antibodies or with respect to complex two-site ELISAs or RIAs (19). Thus, accuracy must currently be defined based on a reference preparation.

We used the First IRP for inhibin A (WHO 91/624) as the reference preparation to compare the accuracies of the Serotec and DSL inhibin A immunoassays and as the basis for comparing results obtained with the two different methods. The method comparison was repeated with the IRP for inhibin A (WHO 91/624) used to calibrate each assay. As shown in Fig. 1B, when the IRP was used to calibrate the assays, the results obtained were statistically indistinguishable (slope, 0.87; intercept, 0.34 IU/mL; \( r = 0.87 \)). This result suggests that differences in the calibrators provided by the assay manufacturers are contributing to the differences in inhibin A values obtained for the same serum samples observed with the two assays.

To facilitate comparisons between inhibin A values expressed in units of IU/mL and values expressed in units of ng/L, we performed a dose–response analysis for each assay, using both the calibrator provided with the assay and the IRP for inhibin A. A plot of the ng/L values vs the extrapolated IU/mL values generated a linear plot (slope, 0.0744; \( r = 0.99 \); see Fig. 2A in the Data Supplement that accompanies the online version of this Technical Brief at http://wwwclinchemorg/content/vol50/issue4/), permitting the calculation of a conversion factor for ng/L to IU/mL for the Serotec assay (13.4 pg/IU). A plot of the ng/L values vs the extrapolated IU/mL values

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**Fig. 1.** Comparison of inhibin A measurements in serum samples tested by each assay after calibration with the manufacturer-provided calibrators (A) and after calibration against the IRP for inhibin A (B). (A), slope, 1.61; intercept, 8.64 ng/L; \( r = 0.908 \); \( n = 1172 \). (B), slope, 0.87; intercept, 0.34 IU/mL; \( r = 0.908 \); \( n = 1172 \).
generated a linear plot (slope, 0.0383; \( r = 0.99 \); see Fig. 2B in the online Data Supplement), permitting calculation of a conversion factor for ng/L to IU/mL for the DSL assay (26.1 pg/IU).

The performance of the two available inhibin A immunoassays was similar with respect to sensitivity, specificity, and precision (data not shown). However, we observed significant differences in the absolute values of inhibin A detected by the two assays. These differences were attributable to differences in the calibrators used in the two assay systems. In light of these issues, the use of a standardized, reference preparation of inhibin A was evaluated. When we used the reference preparation, we obtained identical results for the two assay systems. Given the importance of accurate inhibin A results for antenatal screening and other tests of reproductive physiology, we recommend the use of the WHO IRP for inhibin A for the calibration of inhibin A measurements and for inhibin A concentrations to be reported in IU/mL.

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References


