Estimation of Nuwiq® (simoctocog alfa) activity using one-stage and chromogenic assays—Results from an international comparative field study

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Abstract

Background: Accurate determination of coagulation factor VIII activity (FVIII:C) is essential for effective and safe FVIII replacement therapy. FVIII:C can be measured by one-stage and chromogenic substrate assays (OSAs and CSAs, respectively); however, there is significant interlaboratory and interassay variability.

Aims: This international comparative field study characterized the behaviour of OSAs and CSAs used in routine laboratory practice to measure the activity of Nuwiq® (human-cl rhFVIII, simoctocog alfa), a fourth-generation recombinant human FVIII produced in a human cell line.

Methods: FVIII-deficient plasma was spiked with Nuwiq® or Advate® at 1, 5, 30 and 100 international units (IU)/dL. Participating laboratories analysed the samples using their routine procedures and equipment. Accuracy, inter- and intralaboratory variation, CSA:OSA ratio and the impact of different OSA and CSA reagents were assessed.
1 | INTRODUCTION

The mainstay of current haemophilia A treatment is coagulation factor VIII (FVIII) therapy, that is replacement of the absent endogenous FVIII by either plasma-derived (pdFVIII) or recombinant FVIII (rFVIII).1 As underdosing of exogenous FVIII may result in insufficient FVIII cover and increase patients’ bleeding risk, accurate assignment of the potency of FVIII concentrates is paramount. Likewise, accurate measurement of FVIII activity (FVIII:C) in plasma during treatment is essential for monitoring FVIII:C trough levels, assessment of FVIII pharmacokinetics and perioperative management of patients with haemophilia A.2

FVIII:C in patients receiving FVIII replacement therapy can be measured by either one-stage or chromogenic substrate assays (OSAs and CSAs, respectively). The OSA is based on a modified activated partial thromboplastin time (aPTT) assay that is calibrated to a series of FVIII concentrations.3 The CSA employs a two-stage design. In the first step, FVIII acts as a cofactor in the generation of activated coagulation factor X (FXa). The amount of FXa reflects the amount of FVIII in the sample and is determined by measuring the extent of FXa-mediated hydrolysis of a chromogenic substrate.3,4

While the number of commercially available CSAs is relatively small, a large number of different aPTT reagents are available.5 This can lead to considerable variation in FVIII:C results, as OSA results are affected by the choice of phospholipid4 and surface activator/aPTT reagent,7,8 as well as by the von Willebrand factor content of the FVIII-deficient plasma.9 These variations are typically observed with rFVIII concentrates6,9 or modified FVIII products,7,8 but not normally with pdFVIII concentrates.6,9 Inter-reagent variability is lower for CSAs, which represent the reference method of the European Pharmacopoeia for the potency assignment of products used for FVIII replacement therapy.10 However, the majority of laboratories use OSAs for monitoring in the clinical setting.11

Discrepancies between FVIII:C determined by OSAs and CSAs have been observed for rFVIII concentrates, with CSA:OSA ratios typically between 1.1 and 1.3.2 However, for ReFacto®, a B-domain-deleted (BDD) rFVIII, OSA results were found to be 20%-50% lower than those obtained with the CSA.6,12 The use of a product-specific reference standard (reference laboratory standard, RLS) reduced this discrepancy and allowed for more accurate estimates using the OSA.13 FVIII:C results for Afstyla®, a single-chain BDD rFVIII, were approximately 50% lower with OSAs than with CSAs.14 In light of these observations, the factor VIII and factor IX Subcommittee of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (SSC/ISTH) recommends full characterization of both OSAs and CSAs for any new FVIII product.15

Nuwiq®, simoctocog alfa, human-cl rhFVIII; Octapharma) is a fourth-generation BDD rFVIII16 produced in a human cell line with human-like post-translational protein processing and without chemical modification or fusion to any other protein.17,18 Nuwiq® is effective in the prevention and treatment of bleeds and for bleeding management during surgery in adults and children with haemophilia A19-21 and has demonstrated low immunogenicity in previously untreated patients.22

The aim of this field study was to assess whether Nuwiq® activity can be accurately measured with a variety of OSAs and CSAs used in routine laboratory practice.

2 | METHODS

2.1 | Questionnaire

All participating laboratories completed a questionnaire to document in-house standard procedures for all assays routinely used, including information on analysers, reagents, standards, dilutions and calibration techniques.
2.2 | Sample preparation

Samples were prepared centrally by spiking congenital FVIII-deficient plasma (Helena Biosciences) with Nuwiq® or Advate® at 4 concentrations (1, 5, 30 and 100 international units [IU]/dL) based on nominal potencies determined by a CSA. SSC/ISTH Secondary Coagulation Standard Lot #4 (NIBSC code: SSCLOT4) was provided in lyophilized form. Samples were shipped frozen on dry ice. Each laboratory received triplicate aliquots of each sample. Laboratories were blinded as to the identity of the concentrates and the target concentrations.

Advate®, a full-length rFVIII of hamster cell line origin, was chosen as a comparator as it was the most widely used rFVIII at the time the study was conceived, and it has been used as a comparator in field studies with other rFVIII products.7,14,23-26

2.3 | Performance of assays

Participating laboratories were asked to determine FVIII:C on three separate days by means of whichever assay(s) they used routinely, following their standard procedures and using their in-house equipment. Results were reported as final calculated activities.

2.4 | Data analysis

All questionnaires and FVIII:C data were submitted to a central processing site that reviewed, edited and compiled the data. Compilation records were checked against the source files for 100% accuracy. In case of missing or implausible data, laboratories were contacted for clarification.

Statistical analysis was descriptive and performed by Ergomed CDS, Cologne, Germany using SAS version 9.3 (SAS Institute). Parameters analysed included absolute FVIII:C, relative recovery and inter- and intra-laboratory coefficients of variation (CV). Values were calculated separately for OSAs and CSAs. Recovery was calculated as absolute FVIII:C measured divided by the target concentration and expressed as a percentage of the target. Sensitivity analyses were performed to assess the impact of specific analysers, reagents or calibration methods on FVIII:C results. The CSA:OSA ratio was determined based on both the overall mean values and on the individual ratios for all laboratories performing both assays.

For all analyses, except intralaboratory precision, one mean value per sample, assay and laboratory was calculated from the triplicate results provided. Intralaboratory precision was determined based on the three results for each sample.

Outlier detection was performed based on robust regression diagnostics with a cut-off equal to four times the mean.

3 | RESULTS

3.1 | Participating laboratories

Of 59 laboratories invited, 50 agreed to participate in the study. One laboratory was subsequently excluded for logistical reasons and did not receive a study kit. The majority of participating laboratories were located in Europe (Austria [n = 2], Belgium [n = 3], France [n = 11], Germany [n = 18], the Netherlands [n = 2], Switzerland [n = 6] and the UK [n = 5]), one was in Saudi Arabia and one in the USA.

3.2 | Methodologies used

Most laboratories (40/49; 81.6%) performed OSAs and CSAs, while 8/49 (16.3%) performed the OSA only, and 1/49 (2.0%) performed the CSA only. As several laboratories performed more than one assay of a given type, the final data set consisted of 51 OSAs and 43 CSAs.

The most common aPTT reagents for the OSA were Synthasil (Instrumentation Laboratory; n = 12), Pathromtin SL (Siemens; n = 11), C.K. Prest (Stago; n = 8) and Actin FS (Siemens; n = 8). The most frequently used platforms were ACL TOP (Instrumentation Laboratory; n = 16), BCS/BCS XP (Siemens; n = 13) and STA/STA R (Stago; n = 11). For the majority of OSAs, immunodepleted FVIII-deficient plasma (n = 46) and commercial calibrators (n = 47) were employed. Single calibration curves (median number of points 8; range 6-11) and dual calibration curves (median number of points 12; range 8-13) were used in 27 and 19 assay set-ups, respectively. Most laboratories (31/48; 64.6%) performed OSAs 3-5 times per week.

For the CSA, the most common kits were Biophen FVIII:C (Hyphen BioMed; n = 20), factor VIII chromogenic assay (Siemens; n = 12) and Coamatic Factor VIII (Chromogenix; n = 5). The most frequently used platforms were BCS/BCS XP (Siemens; n = 15) and those from the ACL/ACL TOP (Instrumentation Laboratory; n = 10) and STA/STA R families (Stago; n = 10). Almost all laboratories employed commercial calibrators; dual and single calibration curves were used in 23 and 19 cases, with a median (range) number of 10 (8-17) and 6 (4-9) points, respectively. Most laboratories (22/41; 53.7%) performed CSAs 1-3 times per month.

3.3 | Activity results

3.3.1 | Absolute FVIII:C and mean recovery

Mean absolute FVIII:C values were comparable for Nuwiq® and Advate® at all concentrations for both OSAs and CSAs (Table 1). The interproduct ratios (Nuwiq®:Advate®) based on overall mean FVIII:C ranged from 1.03 to 1.13 for the OSAs and from 1.02 to 1.11 for the CSAs. Mean recoveries in the OSA ranged from 97.3% to 183.8% for Nuwiq® and from 92.7% to 161.3% for Advate®; in the CSA, ranges were 112.4% to 190.8% for Nuwiq® and 108.5% to 172.0% for Advate®. For both OSAs and CSAs, higher recoveries were observed at lower concentrations. All reported recoveries for the SSC/ISTH standard were within 100 ± 25% (Table 1).

3.3.2 | Distribution of recovery

Figure 1 shows the distribution of recovery values for the OSAs and CSAs. Results were comparable for Nuwiq® and Advate® at all
concentrations. As observed for the mean values, median recovery decreased with increasing concentrations for both products.

3.3.3 Inter- and Intralaboratory Precision
In accordance with the distribution patterns, interlaboratory CVs were high for low concentrations and decreased with increasing potency (Table 2). For the OSA, they were 44.2% and 41.8% for the 1 IU/dL samples and 15.9% and 11.8% for the 100 IU/dL samples for Nuwiq® and Advate® respectively. For the CSA, values were 53.5% and 55.5% for the 1 IU/dL samples and 9.8% and 8.6% for the 100 IU/dL samples for Nuwiq® and Advate® respectively.

Mean intralaboratory CVs were lower overall and tended to be higher at lower concentrations. Values for the OSA ranged from 5.6% to 11.2% for Nuwiq® and from 4.8% to 12.7% for Advate®. For the CSA, ranges were 6.0% to 17.5% for Nuwiq® and from 4.8% to 19.2% for Advate®. A re-calculation of inter- and intralaboratory CVs excluding outliers led to reductions for most values. The differences were mostly minor for interlaboratory CVs, whereas several interlaboratory CVs increased by 30% or more of their initial value, indicating the substantial effect of outliers on these values.

3.3.4 Analyses by reagents
An analysis of FVIII:C recovery by aPTT reagent showed nearly twofold differences in mean recovery between reagents for the 1 IU/dL samples; however, standard deviations and ranges were large (Figure 2). Comparable patterns were observed for Nuwiq® and Advate®, with the highest recoveries for SynthASil and C.K. Prest and the lowest recoveries with Pathromtin. Inter-reagent differences decreased with increasing concentrations and were almost non-existent for the 100 IU/dL samples. Similar results were obtained when the recoveries were analysed by activator (Table S1).

Figure 3 presents CSA results by assay kit. Mean recoveries differed slightly for the 1 IU/dL samples, with the factor VIII chromogenic assay kit showing the highest recovery for both products and both concentration ranges. For both subgroup analyses, results for median recoveries were comparable to those for the means (data not shown).

3.3.5 Analyses by instruments
FVIII:C recovery by instrument for the OSA is displayed in Figure S1. As in the analysis by aPTT reagent, differences of nearly twofold in mean recovery were observed for the 1 IU/dL samples, with higher recoveries with ACL TOP and STA/STA R and lower recoveries with BCS/BCS XP. Patterns were comparable for Nuwiq® and Advate®, and differences diminished with increasing concentrations. Figure S2 shows CSA results by instrument. A much lower degree of variability was observed at low sample concentrations, with a tendency towards a more uniform distribution for both products.
towards higher recoveries with STA/STA R, but differences in mean recovery were small compared with the standard deviations.

3.3.6 | CSA:OSA ratios

CSA:OSA ratios based on the overall means ranged from 0.99 to 1.17 for Nuwiq® and from 1.01 to 1.17 for Advate®. When ratios were calculated based on the mean of individual ratios from laboratories performing both assays, results ranged from 1.05 to 1.19 for Nuwiq® and from 1.06 to 1.25 for Advate®. In both cases, there were no concentration-dependent trends (Table 3).

4 | DISCUSSION

This comparative international field study examined FVIII:C results obtained for simulated postinfusion samples of Nuwiq®, a BDD rFVIII produced in a human cell line, and Advate®, a full-length rFVIII produced in a hamster cell line, using a broad range of OSAs and CSAs in routine laboratory practice. The data show comparable accuracy and precision for both products across a range of concentrations and assay reagents for both assays, with CSA:OSA ratios below 1.3 in all cases.

The observed ranges in FVIII:C results are large for both products, particularly at low concentrations. The results were similar for Advate® and Nuwiq®, which suggests that the variability is not related to differences in the products, but rather to methodological differences between the assays or to differences between individual laboratories. The greater variability in values at lower concentrations has also been observed in previous field studies with other rFVIII products, with measured values more than twice as high as expected for individual samples at the lowest concentrations assayed.14,23,26 Of note, the lowest concentration used in this study, 1 IU/dL, is lower than that reported in previous studies, which generally ranged from 3 to 5 IU/dL.14,24,26 and was even 20 IU/dL in one case.

For the OSA, high variability of assay results and overestimation of FVIII:C at low concentrations have been described previously.11,27
The CSA is generally assumed to be more sensitive at low concentrations. However, results from previous field studies are inconsistent and recent data from proficiency testing indicate that both OSAs and CSAs show high variability at low concentrations. It has been speculated that high variability at low target concentrations in the CSA may be due to the absence of very low concentration samples in calibration curves, and in fact, according to the questionnaire data, the lowest concentration used for calibration was ≥2.5 IU/dL for almost one-third of CSAs in this study (data not shown). Independently of this, it is important to note that for samples

### Table 2: Inter- and intralaboratory precision

| Product   | Target concentration (IU/dL) | OSA (n = 50)<sup>a</sup> | CSA (n = 43) |
|-----------|-------------------------------|---------------------------|--------------|
|           | Interlaboratory CV (%)        | Mean intralaboratory CV (%)<sup>b</sup> | Interlaboratory CV (%) | Mean intralaboratory CV (%)<sup>b</sup> |
| Nuwiq®   | 1                             | 44.2<sup>c</sup>          | 11.2<sup>c</sup>/10.3<sup>d</sup> | 53.5<sup>c</sup>/45.7<sup>d</sup> | 17.5<sup>c</sup>/12.9<sup>d</sup> |
|            | 5                             | 42.9/22.7<sup>d</sup>     | 7.9/7.2<sup>d</sup>          | 35.1<sup>c</sup>/24.7<sup>d</sup> | 12.3<sup>c</sup>/10.8<sup>d</sup> |
|            | 30                            | 34.9/14.4<sup>d</sup>     | 5.6/5.2<sup>d</sup>          | 10.8/9.2<sup>d</sup>            | 6.1                          |
|            | 100                           | 15.9/11.7<sup>d</sup>     | 5.7/5.2<sup>d</sup>          | 9.8                            | 6.0/4.6<sup>d</sup>          |
| Advate®   | 1                             | 41.8<sup>c</sup>          | 12.7/11.7<sup>d</sup>        | 55.5<sup>c</sup>/47.7<sup>d</sup> | 19.2<sup>c</sup>/14.5<sup>d</sup> |
|            | 5                             | 26.7                       | 8.9                         | 32.0<sup>c</sup>/23.3<sup>d</sup> | 11.9<sup>c</sup>/7.7<sup>d</sup> |
|            | 30                            | 17.6/11.8<sup>d</sup>     | 5.2/4.3<sup>d</sup>          | 10.6                           | 4.8/4.5<sup>d</sup>          |
|            | 100                           | 11.8/9.2<sup>c</sup>      | 4.8/4.3<sup>d</sup>          | 8.6                            | 5.2                          |
| Standard  | 88                            | 8.5                        | 5.1/3.9<sup>d</sup>          | 7.4                            | 4.1                          |

Note: Data are shown as CVs calculated based on all available data/CVs calculated excluding outliers (where different; otherwise not indicated). Abbreviations: CSA, chromogenic assay; CV, coefficient of variation; IU, international unit; OSA, one-stage assay.

<sup>a</sup>Results from one of the 51 assays were excluded due to a calculation error affecting all reported FVIII:C values.

<sup>b</sup>CV calculated based on triplicate measurements per sample, method and laboratory.

<sup>c</sup>Some values were excluded from the analysis because exact FVIII:C values were not provided; therefore, n is less than the n listed in the column title (OSA: n = 48 for Nuwiq® 1 IU/dL and n = 47 for Advate® 1 IU/dL; CSA: n = 37 for both 1 IU/dL samples for interlaboratory CVs, n = 36 for both 1 IU/dL samples for intralaboratory CVs, and n = 40 for both 5 IU/dL samples).

<sup>d</sup>Due to exclusion of outliers, n is less than the n for the calculation based on all available values (OSA: n = 49 for both 30 IU/dL and 100 IU/dL samples and n = 48 for Nuwiq® 5 IU/dL for interlaboratory CVs; n = 49 for Nuwiq® 5 IU/dL and 30 IU/dL and for both 100 IU/dL samples, n = 47 for Nuwiq® 1 IU/dL and Advate® 30 IU/dL and n = 46 for Advate® 1 IU/dL for intralaboratory CVs; CSA: n = 40 for Nuwiq® 5 IU/dL, n = 38 for both 5 IU/dL samples and n = 36 for both 1 IU/dL samples for interlaboratory CVs; n = 42 for Advate® 30 IU/dL, n = 41 for Nuwiq® 100 IU/dL, n = 38 for Nuwiq® 5 IU/dL, n = 35 for Advate® 5 IU/dL and n = 34 for both 1 IU/dL samples for intralaboratory CVs).

### Figure 2: Target recovery (%) as determined by OSAs, analysed by aPTT reagent. Data are presented as mean ± SD (range). Only aPTT reagents used by at least five laboratories were included in the analysis. Results from one of the 51 assays were excluded due to an obvious calculation error affecting all reported FVIII:C values. aPTT, activated partial thromboplastin time; IU, international unit; OSA, one-stage assay; SD, standard deviation. Some values were excluded from the analysis because no exact FVIII:C values were provided; therefore, for the columns with footnotes, n is less than the n listed in the legend:

- <sup>b</sup>n = 7
- <sup>c</sup>n = 6
- <sup>d</sup>n = 4
with a concentration as low as 1 IU/dL, large percentage differences correspond to very small differences in absolute FVIII:C values.

To decrease variation in FVIII:C, particularly when measuring high-purity FVIII concentrates, recommendations for the standardization of assays have been issued.29 These include the use of FVIII-deficient plasma for predilution of samples and the use of 1% of albumin in all assay buffers. However, in our study using the assays routinely employed in the participating laboratories, diluents other than FVIII-deficient plasma were used for predilution of samples in 46 of the 50 evaluated OSAs, and buffers without albumin were used for sample preparation in 15 of the 43 CSAs. For the OSA, it is hard to assess the effect of diluents other than FVIII-deficient plasma on assay results in our study as only two laboratories confirmed the use of FVIII-deficient plasma (the other two did not provide any information). However, published data demonstrate that diluting all samples and standards in FVIII-deficient plasma improves the accuracy of the OSA by decreasing the overestimation for samples with low FVIII:C.30 Regarding the CSA results obtained in this study, the ranges of actual FVIII:C values for samples with low concentrations (ie 1 and 5 IU/dL) were indeed wider for assays in which buffers without albumin were used, compared with those employing albumin-containing buffers (data not shown).

The interlaboratory and intralaboratory precision for Advate® in our study were similar to previously reported values. Published results for interlaboratory CVs range from 10% to 34%24 for the OSA and from 5% to 51% for the CSA, with the highest values consistently observed for the lowest concentrations. High interlaboratory CVs at low sample concentrations have also been reported in proficiency testing, with values of up to approximately 120% for samples with concentrations below 20 IU/dL.28,31 For intralaboratory precision, previously reported values for Advate® range from 5% to 13% for the OSA and from 2% to 15% for the CSA.

Our study showed inter-reagent differences in FVIII:C of approximately twofold for both the OSA and the CSA at low concentrations. Differences of a similar magnitude between different aPTT reagents have been reported in a study using low concentrations of Afstyla®.32 Another study reported differences between aPTT reagents even at higher FVIII:C, with higher values observed for SynthASil vs Actin FS,33 consistent with our results. Analyses by CSA reagents did not show any major inter-reagent differences but were exclusively based on samples with FVIII:C of >20 IU/dL,34,35 in which marked differences are less likely to be found. In the present study, differences were less pronounced in the 30 and 100 IU/dL samples.

The influence of other methodological parameters has rarely been studied. A possible minor impact of the analyser on FVIII:C results has been reported in two small studies,34,35 with one of them showing a tendency towards higher recovery with ACL TOP compared with Sysmex for the OSA,35 in line with our findings.

CSA:OSA ratios were comparable for both products and below or within the expected range of 1.1 to 1.3,2 indicating that the deletion of the B-domain per se does not result in CSA-OSA discrepancies, which confirms observations from other field studies involving BDD rFVIII.24,26

In previous studies, usually only a low proportion of laboratories (one-third or below) provided results for CSAs.7,14,23-26 The present field study is the first such study with over 80% of laboratories routinely using CSAs and providing corresponding FVIII:C results.

This study has some limitations. First, although samples mimicked postinfusion samples from FVIII-deficient patients as closely as possible, it cannot be excluded that actual patient postinfusion samples may behave differently.27 However, it was recently demonstrated for Advate® that both types of samples yield comparable results in OSAs and CSAs.35 Second, only one lot of each product and of FVIII-deficient plasma was used to prepare the samples, and as most laboratories performed the three assays within 2 weeks, the use of different reagent lots is unlikely. Third, the influence of some assay parameters was not analysed, because certain reagents were either not used in the study (eg FVIII-deficient plasma containing low levels of von Willebrand factor) or were used by one or two laboratories only, precluding reliable statistical analysis.

In conclusion, comparable FVIII:C results were obtained for Nuwiq® and Advate® across a range of concentrations and using a variety of OSA and CSA reagents. Therefore, both OSAs and CSAs

![Graph](image-url)

**FIGURE 3** Target recovery (%) as determined by CSAs, analysed by assay kit. Data are presented as mean ± SD (range). Only kits used by at least five laboratories were included in the analysis. CSA, chromogenic assay; IU, international unit; SD, standard deviation. Some values were excluded from the analysis because no exact FVIII:C values were provided; therefore, for the columns with footnotes, n is less than the n listed in the legend: n = 18; 2n = 19; 3n = 9; 4n = 11
Table 3: Mean ratios of values obtained by CSAs and OSAs

| Product  | Target concentration (IU/dL) | CSA:OSA ratio based on means for CSA (n = 43) and OSA (n = 50) | Mean ± SD of CSA:OSA ratios at individual laboratories (n = 43) | Range of CSA:OSA ratios at individual laboratories (n = 43) |
|----------|-----------------------------|-------------------------------------------------|-------------------------------|----------------------------------|
| Nuwiq®  | 1                           | 1.13                                            | 1.19 ± 0.704                  | 0.33-3.66                         |
|          | 5                           | 0.99                                            | 1.05 ± 0.452                  | 0.09-2.37                         |
|          | 30                          | 1.10                                            | 1.13 ± 0.223                  | 0.36-1.71                         |
|          | 100                         | 1.17                                            | 1.17 ± 0.189                  | 0.83-1.59                         |
| Advate® | 1                           | 1.16                                            | 1.25 ± 0.709                  | 0.37-3.56                         |
|          | 5                           | 1.01                                            | 1.06 ± 0.428                  | 0.09-2.44                         |
|          | 30                          | 1.12                                            | 1.12 ± 0.168                  | 0.63-1.55                         |
|          | 100                         | 1.17                                            | 1.17 ± 0.134                  | 0.81-1.46                         |
| Standard | 88                          | 1.02                                            | 1.03 ± 0.104                  | 0.85-1.37                         |

Abbreviations: CSA, chromogenic assay; IU, international unit; OSA, one-stage assay; SD, standard deviation.

*Results from one of the 51 assays were excluded due to a calculation error affecting all reported FVIII:C values.

Only 40 laboratories performed both assays; however, three of these performed more than one assay of a given type and all possible combinations were included in the analysis.

Some values were excluded from the analysis because exact FVIII:C values were not provided; therefore, n is <43 (n = 36 for both 1 IU/dL samples and n = 40 for both 5 IU/dL samples).
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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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