Comparison of Three Methods for Measuring Factor VIII Levels in Plasma

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Abstract

We compared 1-stage clot-based, chromogenic, and immunoassay methods for measuring factor VIII in plasma with a focus on the measurement of elevated levels of factor VIII. The chromogenic assay showed the best interassay imprecision for factor VIII levels near 150 IU/dL. The best correlation was between the 1-stage clot-based and chromogenic factor VIII assays ($r^2 = 0.934$), and the lowest correlation was between the 1-stage clot-based and antigenic factor VIII assays ($r^2 = 0.821$). The presence of heparin, low-molecular-weight heparin, lepirudin, or lupus inhibitors in the sample resulted in major interference in the 1-stage clot-based assay but not the chromogenic or antigenic factor VIII assays. Overall, the chromogenic factor VIII activity assay was the optimal method, showing good precision, the best overall correlation with other assays, and no interference from heparin, low-molecular-weight heparin, lepirudin, or lupus inhibitors.

The most common reason for measuring the factor VIII level in plasma is to assess the risk of bleeding in a patient due to hereditary or acquired factor VIII deficiency. In recent years, a number of studies have shown that a persistent elevation of the factor VIII level is associated with an increased risk of venous thrombosis. The association between elevated factor VIII and thrombotic risk held whether the factor VIII level was measured using clot-based activity assays, a chromogenic activity assay, or enzyme-linked immunoassays. When a single specific gene polymorphism is discovered, like prothrombin G20210A, it can be evaluated directly. Currently, when specific polymorphisms are not known or when multiple mutations are found, the level of the factor itself must be measured to assess risk, as is done now for protein C or S. The most common method used for measuring factor VIII is the 1-stage activity assay based on the activated partial thromboplastin time (aPTT). The current generation of 1-stage clot-based assays used to measure factor VIII activity were designed to detect factor deficiencies. Risk of bleeding associated with factor VIII activity increases with roughly log decreases in the factor VIII level; therefore, sensitivity and precision at low levels of factor VIII are critical. In contrast, the difference between low risk and high risk for thrombosis may be separated by a relatively small range of moderately elevated values, more than vs less than 150 IU/dL (150% of normal). One-stage clot-based assays may not be optimal for evaluating elevated levels of factor VIII in individual patients. Other assays used to measure factor VIII include chromogenic activity assays and immunoassays. We evaluated commercially available versions of each of these assays for factor VIII—1-stage clot-based, chromogenic, and immunoassay—with respect to calibration, precision, and interference.
### Materials and Methods

#### Human Subjects

Studies on human subjects were carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants, and the study was approved by the University of Washington Human Subjects Review Committee.

#### Blood Sampling

Venous blood samples were anticoagulated by adding 4.5 mL of blood to 0.5 mL of a 0.105-mol/L concentration of citrate. All samples were centrifuged immediately at 3,600g for 2 minutes at room temperature, divided into aliquots, and frozen at −80°C until analyzed.

#### Assay Methods

Factor VIII levels in plasma were measured in 3 ways: (1) Factor VIII antigen was measured using an enzyme immunoassay, Asserachrom VIIIIC:Ag (Diagnostica Stago, Parsippany, NJ). (2) Factor VIII activity was measured using a chromogenic assay with reagents from Chromogenix (Milan, Italy) on an STA instrument (Diagnostica Stago). (3) Factor VIII activity was measured using a 1-stage clotting assay with human immunodepleted lyophilized factor VIII–deficient plasma containing low von Willebrand factor antigen, STA-PTT A reagent, and an STA instrument, all from Diagnostica Stago. The 20th British Standard for Blood Coagulation Factor VIII, Plasma, Human, 95/518 (68% or 68 IU/dL) was obtained from the National Institute for Biologic Standards and Control (NIBSC), Potters Bar, England.

The factor VIII standard from NIBSC was used to calibrate the standard curves of the factor VIII activity assays. It also was used as a comparison against the kit standard for factor VIII antigen. The NIBSC factor VIII activity standard has not been calibrated for factor VIII antigen. These standard curves were used to determine all of the factor VIII results reported in this study. Lupus inhibitors were evaluated using the Staclot LA assay (Diagnostica Stago). This assay meets the International Society on Thrombosis and Haemostasis standard for identification of lupus inhibitors, including a sensitive screening step (aPTT) to detect lupus inhibitors, a mixing step with normal plasma, and a specific confirmation step using higher levels of hexagonal phase phospholipids. Unfractionated porcine heparin was obtained from Elkins-Sinn, Cherry Hill, NJ. Enoxaparin was obtained from Aventis Pharmaceuticals, Bridgewater, NJ. Lepirudin was obtained from Berlex Laboratories, Montville, NJ.

#### Results

The assigned level of factor VIII activity in the kit standards for the 1-stage clot-based and chromogenic factor VIII assays agreed well with the expected level in NIBSC standard 95/518 Table 1. The level of factor VIII in the antigen kit standard was approximately 20% less than that expected from the equivalent activity level in the NIBSC standard, but the NIBSC standard was not calibrated for measurement of factor VIII antigen. For all subsequent results in this study, the assigned level of factor VIII in the kit standards for the 1-stage clot-based and antigen assays were set to the level measured against NIBSC standard 95/518; the chromogenic assay already matched the standard and, thus, did not need correction.

Table 1 

<table>
<thead>
<tr>
<th>Assay Assessed</th>
<th>Assigned Level of Kit Standard (IU/dL)</th>
<th>Measured Level Against NIBSC Standard 95/518 (IU/dL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clot-based factor VIII activity</td>
<td>100</td>
<td>106</td>
</tr>
<tr>
<td>Chromogenic factor VIII activity</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Factor VIII antigen</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

* Assigned factor VIII activity in National Institute for Biologic Standards and Control (NIBSC) standard = 68 IU/dL (68% [0.68]). The NIBSC standard was not calibrated for factor VIII antigen but was used as a comparison with the kit standard.

Table 2 shows the intra-assay and interassay imprecision for each assay using plasma pools containing either low factor VIII activity (approximately 5% of normal) or high factor VIII activity (approximately 150% of normal). All 3 assays measured similar levels of factor VIII in the low factor VIII pool. Intra-assay imprecision on the low factor VIII plasma pool was lowest for the immunoassay method (coefficient of variation [CV], approximately 7%), with similar CVs for the 1-stage clot-based and chromogenic assays (CV, approximately 13%). Interassay imprecision on the low factor VIII plasma pool was lowest for the 1-stage clot-based assay (CV, approximately 18%). Intra-assay imprecision for the high factor VIII plasma pool was similar for all 3 assays (CV, approximately 6%-8%). The interassay imprecision on the high factor VIII plasma pool was lowest for the chromogenic assay (CV, approximately 13%).

A set of 45 samples including high, normal, and low factor VIII activities were compared using the 3 different factor VIII assays Figure 1. Samples from 29 healthy subjects (mean ± SD, 37 ± 10 years; 14 women, 15 men) were run by each method to compare the reference ranges for each assay: (1) factor VIII chromogenic assay: mean ± SD, 94.3 ± 23.8 IU/dL; 95% confidence interval (CI) = 46.8 to 141.8 IU/dL; (2) factor VIII clotting assay: mean ± SD,
Three Factor VIII Assays

The addition of unfractionated heparin, up to 2 U/mL, had no effect on factor VIII activity measured using the chromogenic and immunoassay methods. Unfractionated heparin levels of more than 0.7 U/mL led to an apparent reduction in factor VIII activity measured using the 1-stage clot-based method (Figure 2).

The addition of low-molecular-weight heparin, up to 2 anti-Xa U/mL, had no effect on factor VIII activity measured using the chromogenic and immunoassay methods. Unfractionated heparin levels of more than 0.7 U/mL led to an apparent reduction in factor VIII activity measured using the 1-stage clot-based method (Figure 2).

A

B

C

Table 2
Imprecision of Three Factor VIII Assays

<table>
<thead>
<tr>
<th></th>
<th>Clot-based Assay</th>
<th>Chromogenic Assay</th>
<th>Antigenic Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within Run</td>
<td>Between Run</td>
<td>Within Run</td>
</tr>
<tr>
<td>Low Mean, IU/dL</td>
<td>5.8</td>
<td>5.0</td>
<td>7.9</td>
</tr>
<tr>
<td>SD, IU/dL</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>CV, %</td>
<td>12.9</td>
<td>17.5</td>
<td>12.6</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>High Mean, IU/dL</td>
<td>173</td>
<td>160</td>
<td>141</td>
</tr>
<tr>
<td>SD, IU/dL</td>
<td>13.7</td>
<td>35</td>
<td>8.3</td>
</tr>
<tr>
<td>CV, %</td>
<td>7.9</td>
<td>21.9</td>
<td>5.9</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.

98.3 ± 24.3 IU/dL; 95% CI = 49.7 to 146.8 IU/dL; and (3) factor VIII antigenic assay: mean ± SD, 92.6 ± 29.9 IU/dL, 95% CI = 32.9 to 152.4 IU/dL.

Figure 1 Comparison of factor VIII measured by chromogenic activity (Chr), 1-stage clot-based activity (Clot), and antigenic immunoassay (Anti) methods. The factor VIII activity assays (1-stage clot-based and chromogenic) were calibrated using National Institute for Biologic Standards and Control standard 95/518. Solid lines indicate the Deming regression fit\(^1\) (n = 45). Regression constants and correlation coefficients are as follows: A, Clot-based assay vs chromogenic assay, Clot = 1.28(Chr) – 10.0; \(r^2 = 0.934\). B, Immunoassay vs chromogenic assay, Anti = 1.23(Chr) – 24.3; \(r^2 = 0.839\). C, Immunoassay vs clot-based assay, Anti = 0.98(Clot) – 15.6; \(r^2 = 0.821\). Values are given in conventional units; to convert to Système International units (proportion of 1.0), multiply by 0.01.
methods. Low-molecular-weight heparin levels of more than 0.7 anti-Xa U/mL led to an apparent reduction in factor VIII activity measured using the 1-stage clot-based method \( \text{Figure 3L} \).

The addition of lepirudin, up to 5 µg/mL, had no effect on factor VIII activity measured using the chromogenic and immunoassay methods. Lepirudin levels of 0.1 µg/mL or more led to an apparent reduction in factor VIII activity measured using the 1-stage clot-based method \( \text{Figure 4L} \).

Factor VIII activity and antigen were measured in 27 samples containing weak to strong lupus inhibitors (as determined by the Staclot LA assay). For the 1-stage clot-based factor VIII activity assay, 4 samples showed evidence of an inhibitor effect with higher dilutions of the plasma showing increasing recovery of factor VIII activity \( \text{Figure 5L} \). Lupus inhibitors had no apparent effect on the chromogenic and immunoassay methods based on analysis of increasing dilutions.

**Discussion**

The underlying premise for measuring factor VIII is that if the average level is increased in the patient long-term, then the patient may be at increased risk of thrombosis long-term. Care must be taken in selecting a factor VIII assay type and
acute phase factor such as C-reactive protein simultaneously and recommend repeating the assays if the C-reactive protein level is elevated substantially.

At this time, the optimal assay for evaluating elevated factor VIII activity as a risk factor for thrombosis is the chromogenic method. The chromogenic assay correlates well with the 1-stage clot-based assay for patient samples, its precision is typically better than the 1-stage clot-based assay at high factor VIII levels, and it does not suffer from interference related to heparins, direct thrombin inhibitors, or lupus anticoagulants. Its precision and sensitivity to low levels of factor VIII activity are similar to the 1-stage clot-based assay. It may be possible to replace the 1-stage clot-based assay entirely with a chromogenic method. Each laboratory contemplating running factor VIII assays for thrombosis risk evaluation and considering the use of chromogenic assays should evaluate the assay against its existing method. Most medium to large automated coagulation analyzers and some chemistry analyzers have the ability to perform chromogenic assays (1977-1978).


References


