INTRODUCTION

The most commonly performed assay for FVIII:C worldwide for many years has been the one-stage assay, described in Section 23.

There are limitations to the one-stage assay, including interference if lupus anticoagulant is present. More importantly, mild hemophilia A is not excluded by the finding of a normal FVIII:C level by one-stage assay. Several groups have reported that a subgroup of mild hemophilia A patients have discrepancy between the activity of FVIII as determined using different types of assay (Parquet-Gernez et al. 1988, Duncan et al. 1994, Keeling et al. 1999). More than 20% of mild hemophilia A patients are associated with this discrepancy, which is defined as a two-fold difference between results obtained with different assay systems (Parquet-Gernez et al. 1988).

In some cases, the one-stage assay result may be five times higher than the two-stage clotting or chromogenic assay (Parquet-Gernez et al. 1988). Most commonly, the result of the one-stage assay is more than two-fold higher than the two-stage clotting or chromogenic assay. In more than three quarters of such patients, all assay results are reduced below the lower limit of the reference range so that a diagnosis can be reliably made, irrespective of which method is employed for analysis.

However, in a small proportion of patients, the results of the one-stage assay are well within the normal range, with reduced levels with the two-stage clotting or chromogenic assay (Keeling et al. 1999, Mazurier et al. 1997). These patients have bleeding histories compatible with the lower levels obtained in the two-stage clotting or chromogenic assay.

In many cases, the genetic defect has been identified, so there is no doubt that these subjects do indeed have hemophilia (Rudzi et al. 1996, Mazurier et al. 1997). Based on the literature, about 5%–10% of genetically confirmed mild hemophilia A patients have a normal one-stage assay result. Since FVIII activity is normal in the one-stage APTT-based assay, it is not surprising that the APTT is also normal in such patients. This means that patients with a clinical history compatible with hemophilia A should have a two-stage clotting (see Section 25) or chromogenic assay, even if the APTT and one-stage assay are normal.
A number of manufacturers have commercial kits for chromogenic assay of FVIII. Many of these are suitable for diagnosis of hemophilia A in the presence of normal one-stage FVIII activity. Examples of results in such patients are shown in Figure 26.1 below. The chromogenic assay used was a commercial kit from Siemens/Dade Behring, but its inclusion does not indicate superiority over other similar assays, which could be successfully used in this context.

**Figure 26.1. Examples of patients with genetically confirmed mild hemophilia A and assay discrepancies**

<table>
<thead>
<tr>
<th>Case</th>
<th>One-stage assay (IU/dl)</th>
<th>Two-stage clotting assay (IU/dl)</th>
<th>Chromogenic assay (IU/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>101</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>88</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>C</td>
<td>63</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>D</td>
<td>55</td>
<td>24</td>
<td>40</td>
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<tr>
<td>E</td>
<td>58</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>F</td>
<td>72</td>
<td>21</td>
<td>36</td>
</tr>
<tr>
<td>G</td>
<td>84</td>
<td>19</td>
<td>45</td>
</tr>
</tbody>
</table>

There are cases of mild hemophilia A that have reduced activity by one-stage assay, but normal results by the two-stage or chromogenic assay (Mumford et al. 2002, Lyall H et al. 2008). In many (but not all) such cases, the clinical phenotype correlates with the chromogenic or two-stage clotting assay result in that there is no personal or family history of bleeding, with no requirement for FVIII replacement therapy (Lyall H et al. 2008).

Based on these results, it is advantageous for all hemophilia centres to have available a chromogenic or two-stage clotting assay. These tests should be performed on subjects with normal APTT and one-stage FVIII activity in the presence of a personal or family history consistent with mild hemophilia.

A modified chromogenic assay (modified version of the Coamatic assay, Instrumentation Laboratory Ltd.) has been described. It is suitable for assay of very low levels of FVIII (Yatuv et al. 2006). This method has been reported to allow accurate and precise measurements of FVIII in the range of 0.1–2 IU/dl (0.1–2% FVIII, or 0.001–0.02 IU/ml).
PRINCIPLE OF ANALYSIS

In some (but not all) chromogenic assays, all the FVIII in the sample is activated by thrombin. Activated FVIII then accelerates the conversion of FX to FXa in the presence of activated FIX, phospholipids, and calcium ions. The FXa activity is assessed by hydrolysis of a p-nitroanaline substrate specific to FXa. The initial rate of release of p-nitroanaline (yellow colour) measured at 405 nm is proportional to the FXa activity and thus to the FVIII activity in the sample. For a review, see Lundblad et al. (2000).

REFERENCES

Duncan EM, Duncan BM, Tunbridge LJ, Lloyd JV. Familial discrepancy between the one-stage and two-stage factor VIII assay methods in a subgroup of patients with haemophilia A. *Br J Haematol* 1994; 87: 846–848.


