Measurement of FVIII Extended Half-Life Products

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Technical Director, Vice President
Colorado Coagulation, LabCorp
<table>
<thead>
<tr>
<th>Conflict</th>
<th>Disclosure</th>
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<tr>
<td>Research Support</td>
<td>No conflict of interest to disclose</td>
</tr>
<tr>
<td>Director, Officer, Employee</td>
<td>Officer and Employee of Laboratory Corporation of America® Holdings</td>
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<td>Shareholder</td>
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<td>Advisory Committee</td>
<td>Novo Nordisk, Siemens Healthcare</td>
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<td>Consultant</td>
<td>Novo Nordisk</td>
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Clinical laboratory plays important role in:

- Diagnosis and classification of disease severity
- Monitor treatment (e.g. assessing recovery of replacement factor after infusion)

With approval of EHL FVIII treatment therapies, adjustments to assays commonly used to monitor therapy in the clinical laboratory will be required
Laboratory Assays Currently Used to Monitor Hemophilia A Therapy

• One-stage clot assay (OSA)
  • Standard factor activity assay used in clinical laboratories
  • Many different instrument/reagent combinations available
  • Simple, rapid, inexpensive and easy to automate

• Chromogenic substrate assay (CSA)
  • Based on the two-stage clot assay
  • Considered more accurate and precise (compared to OSA)
  • Limited availability in clinical laboratories, considered more expensive and more complex (e.g. more difficult to automate)
    • several CE-marked and FDA-approved FVIII CSA kits available
Factor Activity Assays: OSA vs CSA

Does assay methodology matter?

- Conditions where results may not agree:
  - Discrepant hemophilia $A^{1,2}$
  - In the presence of certain inhibitors
    - Such as lupus anticoagulants
  - Post-infusion monitoring of rFVIII$^3$, select EHL rFVIII replacement therapies$^4,5$ and FVIII gene therapies$^6$
- In some instances the OSA result is more accurate, in others the CSA result is more accurate

## Important Differences Between One-Stage Clot and Chromogenic Substrate Assays

<table>
<thead>
<tr>
<th>Features</th>
<th>One-Stage Clot Assay</th>
<th>Chromogenic Substrate Assay</th>
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</thead>
<tbody>
<tr>
<td>Assay Principle</td>
<td>Measures the ability of patient plasma to shorten the prolonged coagulation time of FVIII deficient plasma; endpoint clot formation (optical or mechanical)</td>
<td>Two stage; Stage 1: Measures ability of functional FVIII in the sample to generate FXa; Stage 2: FXa reacts with chromogenic substrate to release pNA (endpoint or kinetic)</td>
</tr>
<tr>
<td>Assay Reagents</td>
<td>Large number of assay reagents and instrument protocols (utilizing different contact activators, phospholipid sources)</td>
<td>Limited number of reagent kits and instrument protocols</td>
</tr>
<tr>
<td>Factor Concentrations</td>
<td>Factors are present at relative physiologic concentrations</td>
<td>Factors are present at non-physiologic concentrations (highly diluted)</td>
</tr>
<tr>
<td>Sensitivity to Interferences</td>
<td>Sensitive to pre-activated FVIII; heparin, lupus anticoagulant and DOAC’s</td>
<td>Not sensitive to pre-activated FVIII; relatively insensitive to heparin or lupus anticoagulant</td>
</tr>
<tr>
<td>Dilutions Tested</td>
<td>3 dilutions, requiring complex result algorithms</td>
<td>1 dilution</td>
</tr>
</tbody>
</table>
Factor Activity Assays: OSA vs CSA

Common Misconceptions

- CSA is more accurate and precise than OSA
  - 67% of HTC physicians in web-based survey believe CSA is more accurate than OSA

OSA and CSA show comparable inter-assay precision in ECAT study\textsuperscript{1}

Factor Activity Assays: OSA vs CSA

Common Misconceptions

OSA and CSA show comparable intra- and inter-laboratory precision in recent field studies\textsuperscript{1,2}


<table>
<thead>
<tr>
<th></th>
<th>OSA</th>
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<th></th>
<th>CSA</th>
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<td></td>
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<td>ADVATE</td>
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<td>0.8</td>
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<td>10.9</td>
<td>7.7</td>
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<td>0.2</td>
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<td>0.05</td>
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<td>16.1</td>
<td>15.1</td>
<td>32.5</td>
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<td>0.8</td>
<td>7.4</td>
<td>14.7</td>
<td>7.6</td>
<td>10.1</td>
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<td>9.5</td>
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<tr>
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<td>8.3</td>
<td>31</td>
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</tbody>
</table>
Factor Activity Assays: OSA vs CSA

Common Misconceptions

- CSA is more expensive than OSA
  - Aliquoting and freezing of reagent, as well as batch testing can reduce cost\(^1\) (must be validated by laboratory)

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Modification for Half-Life Extension</th>
<th>Approval Date</th>
</tr>
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<tbody>
<tr>
<td>rFVIII-Fc (BDD) – ELOCTATE®/ELOCTA®</td>
<td>Bioverativ/SOBI</td>
<td>Fusion to Fc domain of IgG1</td>
<td>FDA Jun 2014 EMA Nov 2015</td>
</tr>
<tr>
<td>CSL627 (BDD) – AFSTYLA®</td>
<td>CSL Behring</td>
<td>Single-chain</td>
<td>FDA May 2016 EMA Nov 2015</td>
</tr>
<tr>
<td>Bax 855 (FL) – ADYNOVATE</td>
<td>Takeda (Shire)</td>
<td>20-kDa branched PEG</td>
<td>FDA Dec 2016 EMA Jan 2018</td>
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<tr>
<td>BAY 94-9027 (BDD) - Jivi®</td>
<td>Bayer</td>
<td>Site-specific 60-kDa PEG</td>
<td>FDA Aug 2018 EMA Nov 2018</td>
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<tr>
<td>N8-GP (BDtrunc PEGylated) - ESPEROCT®</td>
<td>Novo Nordisk</td>
<td>40-kDa glycoPEGylation</td>
<td>FDA Feb 2019 EMA Apr 2019</td>
</tr>
</tbody>
</table>

BDD, B-domain-deleted; BDtrunc; FL, full length; PEG, polyethylene glycol.
rFVIII-Fc (ELOCTATE®/ELOCTA®) – International Comparative Field Study: OSA and CSA Data

Adapted with permission from J.M. Sommer, et al. Comparative field study evaluating the activity of recombinant factor VIII Fc fusion protein in plasma samples at clinical haemostasis laboratories; Haemophilia (2014), 20, 294–300, © 2013 Haemophilia Published by John Wiley & Sons Ltd.
5.3 Monitoring Laboratory Tests

- Monitor plasma Factor VIII activity by performing a validated test (eg, one stage clotting assay), to confirm that adequate Factor VIII levels have been achieved and maintained. [see Dosage and Administration (2)]

- Monitor for the development of Factor VIII inhibitors. Perform a Bethesda inhibitor assay if expected Factor VIII plasma levels are not attained, or if bleeding is not controlled with the expected dose of ELOCTATE. Use Bethesda Units (BU) to report inhibitor levels.
CSL627 (AFSTYLA®) – International Comparative Field Study: OSA Data

• N=23 labs using routine OSA (N=23) and CSA (N=6) for FVIII
• CSL627 (blue) and ADVATE (red) spiked at 1.0, 0.6, 0.3, and 0.04 IU/mL

- CSL627 demonstrated consistent under-recovery in the OSA at ~45-50% of expected, independent of aPTT reagent used

CSL627 (AFSTYLA®)
International Comparative Field Study CSA Data

- CSL627 at 1.0 IU/mL recovered close to target
- ADVATE recovered slightly higher than expected

5.3 Monitoring Laboratory Tests

- Monitor plasma Factor VIII activity in patients receiving AFSTYLA using either the chromogenic assay or the one-stage clotting assay, which is routinely used in US clinical laboratories. The chromogenic assay result most accurately reflects the clinical hemostatic potential of AFSTYLA and is preferred. The one-stage clotting assay result underestimates the Factor VIII activity level compared to the chromogenic assay result by approximately one-half. If the one-stage clotting assay is used, multiply the result by a conversion factor of 2 to determine the patient’s Factor VIII activity level. Incorrect interpretation of the Factor VIII activity obtained by the one-stage clotting assay could lead to unnecessary additional dosing, higher chronic dosing, or investigations for an inhibitor.
BAX 855 (ADYNOVATE®/ADYNOVI®) –
International Comparative Field Study: OSA and CSA Data

- N=35 labs using routine OSA (N=35) and CSA (N=11) for FVIII
- BAX 855 and ADVATE spiked at 0.8, 0.2 and 0.05 IU/mL
- Comparable mean recoveries for ADVATE and BAX 855 in both OSA and CSA
- No APTT reagent-dependent recovery observed

5.3 Monitoring Laboratory Tests

- Monitor plasma factor VIII activity by performing a **validated one-stage clotting assay** to confirm the adequate factor VIII levels have been achieved and maintained [see Dosage and Administration (2)].

- Monitor for the development of factor VIII inhibitors. Perform the Bethesda inhibitor assay to determine if factor VIII inhibitor is present. If expected factor VIII activity plasma levels are not attained, or if bleeding is not controlled with the expected dose of ADYNOVATE, use Bethesda Units (BU) to determine inhibitor levels.
BAX 855 (ADYNOVATE®/ADYNOVI®) – Swiss Field Study

- 8 labs using routine OSA (N=8) and CSA (N=6) assays for FVIII activity measurements
- BAX 855 spiked at 0.8, 0.5, 0.3, 0.1 and 0.03 IU/mL
- Control-FVIII (standard human plasma spiked into FVIII deficient plasma) at 0.8, 0.5, 0.3, 0.1 and 0.05 IU/mL

Overestimation of BAX 855 (but not control FVIII) using both OSA and CSA when using normal pooled plasma standard

PART I

• 52 labs using routine OSA (N=49) and CSA (N=16) for FVIII analysis
• BAY 94-9027 and ADVATE® spiked samples were provided at high (50-100 IU/dL), medium (10-50 IU/dL) and low (<10 IU/dL) concentrations

PART II

• 52 labs were provided with SynthASil® and Pathromtin® SL (previously demonstrated to accurately measure BAY 94-9027) for FVIII analysis
• BAY 94-9027 and Advate®-spiked samples were provided at high (50-100 IU/dL), medium (10-50 IU/dL) and low (<10 IU/dL) concentrations

• Chromogenic assays recover BAY 94-9027 and ADVATE within ±25%
5.4 Monitoring Laboratory Tests

- If monitoring of Factor VIII activity is performed, use a validated chromogenic assay or a selected validated one-stage clotting assay [see Dosage and Administration (2.1)].

- Laboratories intending to measure the Factor VIII activity of Jivi should check their procedures for accuracy. For Jivi, select silica-based one-stage assays may underestimate the Factor VIII activity of Jivi in plasma samples; some reagents, e.g., with kaolin-based activators, have the potential for overestimation\(^1\). Therefore, the suitability of the assay must be ascertained. If a validated one-stage clotting or chromogenic assay is not available locally, then use of a reference laboratory is recommended.
N8-GP (ESPEROCT®) – International Comparative Field Study: OSA Data

- **N=67** labs using routine OSA (N=60) and CSA (N=36) for FVIII
- N8-GP and ADVATE spiked at 0.9, 0.6, 0.2, and 0.03 IU/mL


- Three silica based aPTT reagents (APTT-SP, TriniCLOT™, STA® PTT-Automate) underestimated N8-GP recovery by 40-60% and were removed from analysis (data not shown)
- Acceptable overall mean recoveries for N8-GP (92.5% of target*) and ADVATE (123% of target) in the OSA

*APTT-SP, TriniCLOT™ and STA® PTT-Automate removed from statistical analysis*
- Comparable mean recoveries for N8-GP (129%) and ADVATE (127%) in the CSA
- Both N8-GP and ADVATE recovered at upper limit of acceptable range (~130% of target)

5.3 Monitoring Laboratory Tests

- If monitoring of Factor VIII is performed, use a chromogenic or one-stage clotting assay appropriate for use with ESPEROCT [see Dosage and Administration (2)].
- Factor VIII activity levels can be affected by the type of activated partial thromboplastin time (aPTT) reagent used in the assay. Some silica-based aPTT reagents can underestimate the activity of ESPEROCT by up to 60%; other reagents may overestimate the activity by 20%. If an appropriate one-stage clotting or chromogenic assay is not available locally, then use a reference laboratory.
## EHL rFVIII Products – Field Study OSA/CSA Summary

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>One-Stage (aPTT) Assay</th>
<th>Chromogenic Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>rFVIII-Fc (BDD) – ELOCTATE®/ELOCTA®&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Bioverativ/SOBI</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>CSL627 (BDD) – AFSTYLA®&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>CSL Behring</td>
<td>all aPTT reagents ↓ (multiply OSA result x2)</td>
<td>√</td>
</tr>
<tr>
<td>Bax 855 (FL) – ADYNOVATE&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Takeda (Shire)</td>
<td>√</td>
<td>√</td>
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<tr>
<td>BAY 94-9027 (BDD) - Jivi&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Bayer</td>
<td>APTT-SP ↓, STA-PTT-A ↓, CK Prest ↑, Actin FS ↑</td>
<td>√ (?);</td>
</tr>
<tr>
<td>N8-GP (BDtrunc PEGylated) - ESPEROC®&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Novo Nordisk</td>
<td>APTT-SP ↓, STA-PTT-A ↓, TriniCLOT™ ↓</td>
<td>√</td>
</tr>
</tbody>
</table>

<sup>1</sup> at upper acceptable limit of ± 30% RE  
<sup>2</sup> indicates under-recovery; ↑ indicates over-recovery; √ indicates acceptable recovery

• With the recent approval of EHL rFVIII replacement products changes in how clinical laboratories monitor hemophilia replacement therapy are indicated

• Several of the EHL rFVIII replacement therapies demonstrate aPTT reagent dependent recovery
  • No single aPTT reagent recovers all EHL rFVIII replacement products accurately

• Existing data to date suggest that FVIII CSA may be suitable for monitoring currently approved EHL rFVIII products
Proposed Approaches to Achieve Accurate Post-Infusion Factor Product Monitoring

• Laboratory should not solely rely on laboratory test monitoring information provided in respective EHL factor replacement product prescribing information

• Laboratory should verify the recovery of the EHL factor replacement product(s) commonly received at their facility in their existing OS and CS factor activity assay(s)
  - Assess recovery of product-spiked samples across reportable range of assay
  - ECAT/NASCOLA is working on developing EHL factor product evaluation sample sets