Colorado COAGULATION
LabCorp Specialty Testing Group

Laboratory Measurement of Extended Half-Life FVIII

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DISCLOSURES

Conflict	Disclosure
Research Support	No conflict of interest to disclose
Director, Officer, Employee	Officer and Employee of Laboratory Corporation of America® Holdings
Shareholder	No conflict of interest to disclose
Honoraria	Novo Nordisk
Advisory Committee	Novo Nordisk, Siemens Healthcare
Consultant	Novo Nordisk

Outline

- Review factor VIII activity assays commonly used in clinical laboratories to monitor factor product replacement therapy
- Provide an overview of the extended half-life recombinant FVIII (EHL rFVIII) replacement products and review available OSA and CSA data
- Review the challenges facing clinical laboratories when monitoring approved EHL rFVIII products
- Provide recommendations to achieve accurate post-infusion monitoring of EHL rFVIII products

EHL FVIII Background

- A number of EHL FVIII products are currently in development or have recently been approved in the U.S. and/or Europe
- Approaches to extend half-life (i.e., reduce the number of infusions) and to decrease immunogenicity include changes to protein expression systems (e.g., use of human cell lines) as well as modifications to the actual therapeutic protein (e.g., PEGylation, Fc-fusion, single-chain or X-TEN modification)

EHL FVIII Background

- Some EHL rFVIII products show discrepant recovery between different aPTT reagents in one-stage assay (OSA)[1]
  - This can have significant impact on post-infusion monitoring
  - Under-recovery may lead to overdosing of factor product, increased thrombosis potential, and higher cost
  - Over-recovery may lead to inadequate dosing of factor product and increased bleeding potential
- Some EHL FVIII products show discrepant recovery between OSA and CSA

1. Dodt J, et al. *Haemophilia.* 2015;21:543-9.

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FVIII Activity Assays Used to Monitor Factor Replacement Products

- 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
- Two-stage clot assay
  - Complex, cannot be automated, no kit available, rarely performed
- Chromogenic substrate assay (CSA)
  - Based on the two-stage clot assay
  - Limited availability in clinical laboratories, considered more expensive and more complex, this is debatable
  - multiple CE-marked and FDA-approved kits available

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Factor Activity Assays: OSA vs CSA
Does assay methodology matter?

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

1. Trossaert M, et al.*Haemophilia.*2014;20:550-8. 2. Parquet-Gernez A, et al. *Thromb Haemost.*1988;59:202-6. 3. Barrowcliffe TW, et al. *Semin Thromb Hemost.*2002;328:247-56. 4. Sommer JM, et al. *Haemophilia.*2014;20:294-300. 5. St Ledger K, et al. *Thromb Haemost. 2018;*16:555-64.

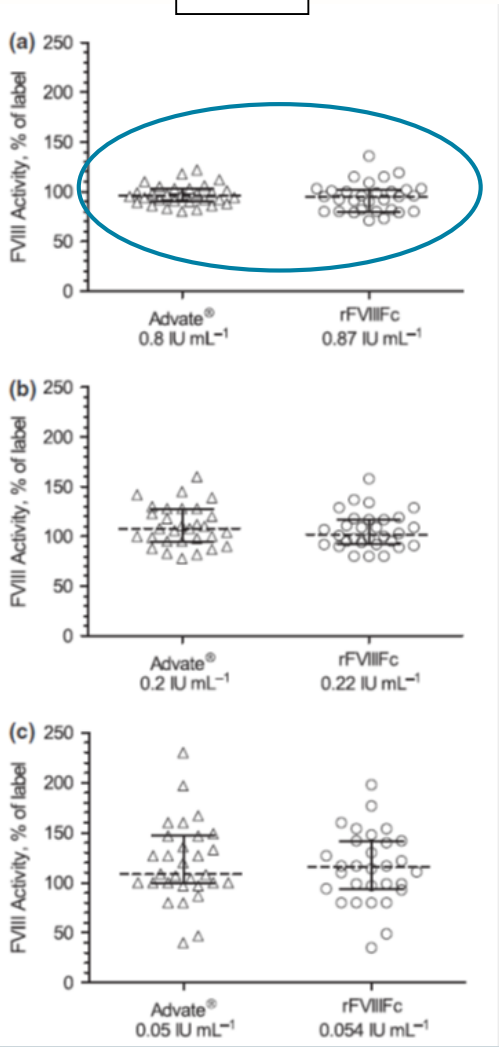
EHL rFVIII Products - Approved

Name	Manufacturer	Modification for Half-Life Extension	Approval Date
rFVIII-Fc (BDD) – ELOCTATE®/ELOCTA®	Bioverativ/SOBI	Fusion to Fc domain of IgG1	FDA Jun 2014 EMA Nov 2015
CSL627 (BDD) – AFSTYLA®	CSL Behring	Single-chain	FDA May 2016 EMA Nov 2015
Bax 855 (FL) – ADYNOVATE/ADYNOVI	Shire	20-kDa branched PEG	FDA Dec 2016 EMA Jan 2018
BAY 94-9027 (BDD) - Jivi®	Bayer	Site-specific 60-kDa PEG	FDA Aug 2018

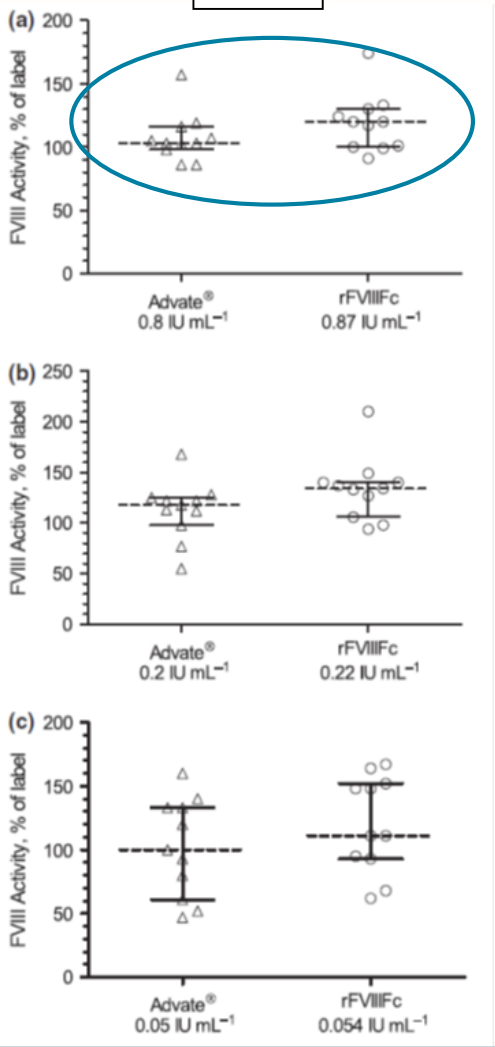
BDD, B-domain-deleted; BDtrunc; FL, full length; PEG, polyethylene glycol.

rFVIII-Fc (ELOCTATE®/ELOCTA®) – International Comparative Field Study: OSA and CSA Data

OSA



CSA



- N=30 labs using routine OSA (N=30) and CSA (N=11) for FVIII
- rFVIII-Fc and ADVATE spiked at 0.8, 0.2, and 0.05 IU/mL

- Comparable mean recoveries for ADVATE and rFVIII-Fc in both OSA and CSA (slightly higher recovery for rFVIII-Fc in CSA)
- No APTT reagent-dependent recovery observed

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.

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rFVIII-Fc (ELOCTATE®/ELOCTA®) PI

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.

Routine FVIII OSA and CSA can be used to measure rFVIII-Fc in plasma

PI, prescribing Information.

ELOCTATE® Prescribing Information. Biogen Inc; January 2017.

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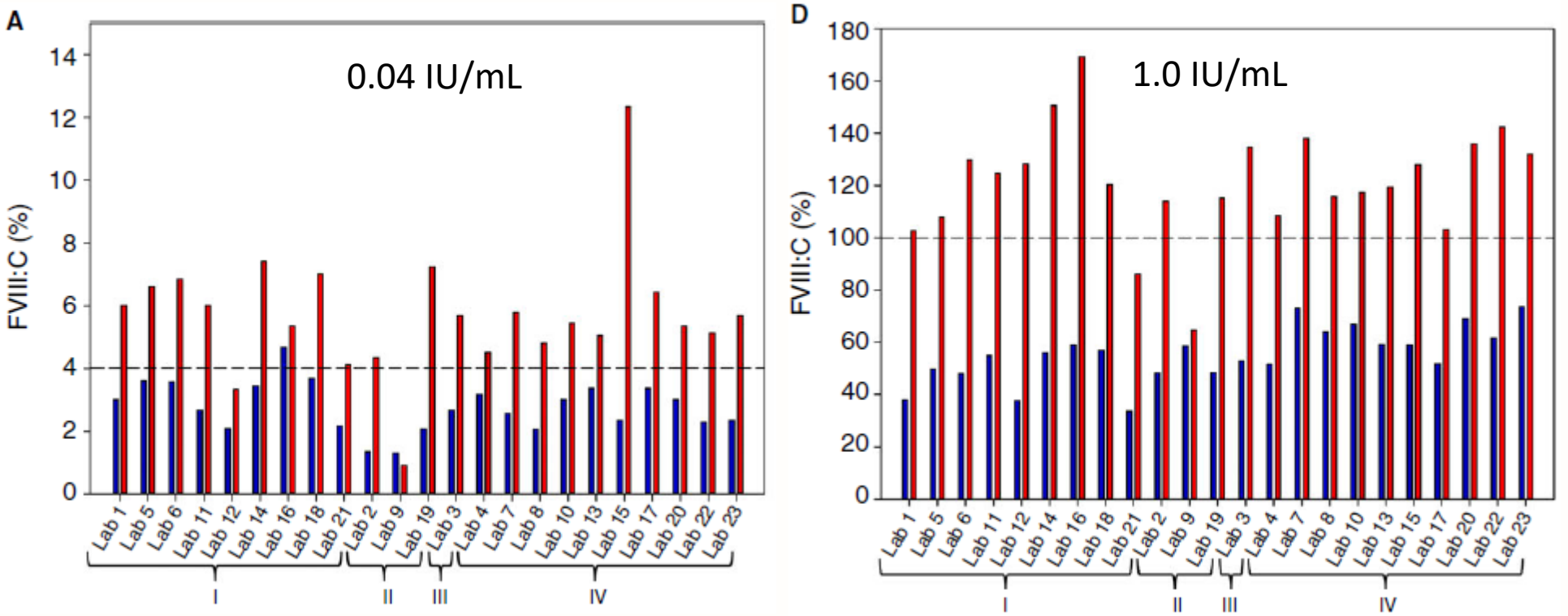
CSL627 (AFSTYLA®) – International Comparative Field Study: OSA Data

- 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



I = silica
II = silicon dioxide particles
III = kaolin
IV = ellagic acid

Adapted from K. ST. Ledger, et al. International comparative field study evaluating the assay performance of AFSTYLA in plasma samples at clinical hemostasis laboratories . J Thromb and Haemost (2018), 16, 555–564

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

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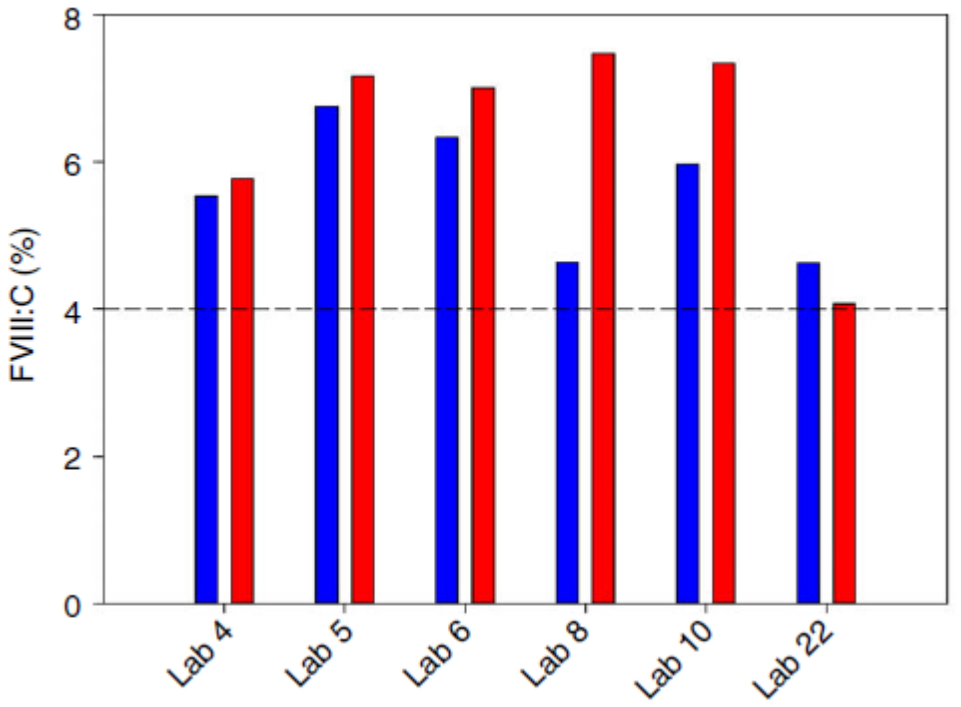
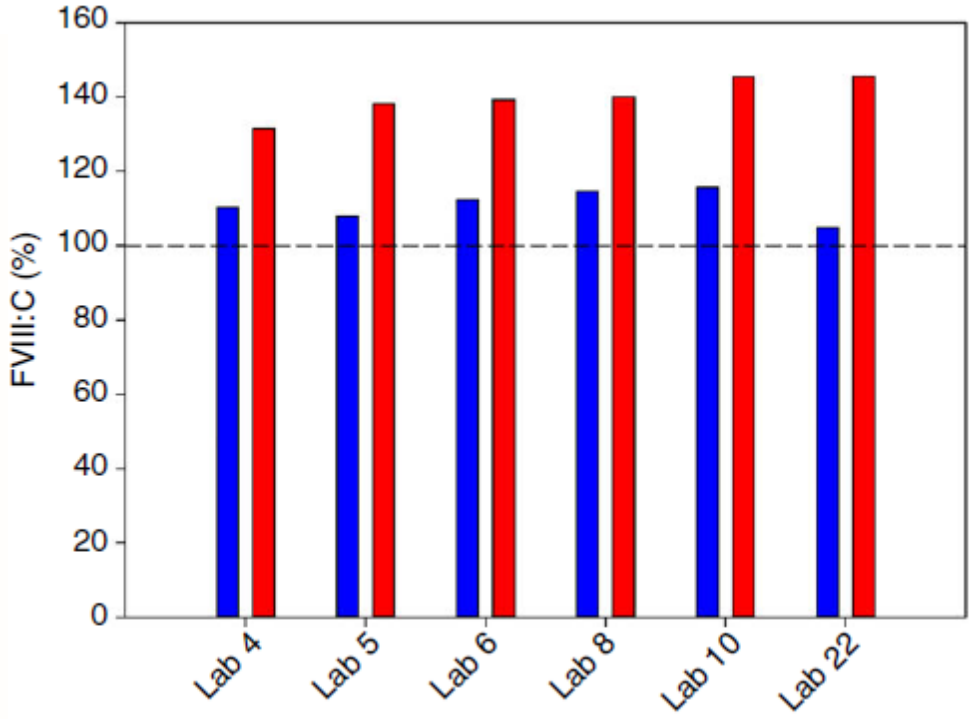
CSL627 (AFSTYLA®) – International Comparative Field Study CSA Data

0.04 IU/mL



1.0 IU/mL



Adapted from K. ST. Ledger, et al. International comparative field study evaluating the assay performance of AFSTYLA in plasma samples at clinical hemostasis laboratories .J Thromb and Haemost (2018), 16, 555–564

AFSTYLA = Blue
ADVATE = Red

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

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CSL627 (AFSTYLA®) PI

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.

FVIII CSA most accurately reflects hemostatic potential of CSL627 in plasma
FVIII OSA underestimates FVIII activity level and a correction factor of 2 must be used

PI, prescribing Information.

AFSTYLA® Prescribing Information. CSL Behring; May 2016.

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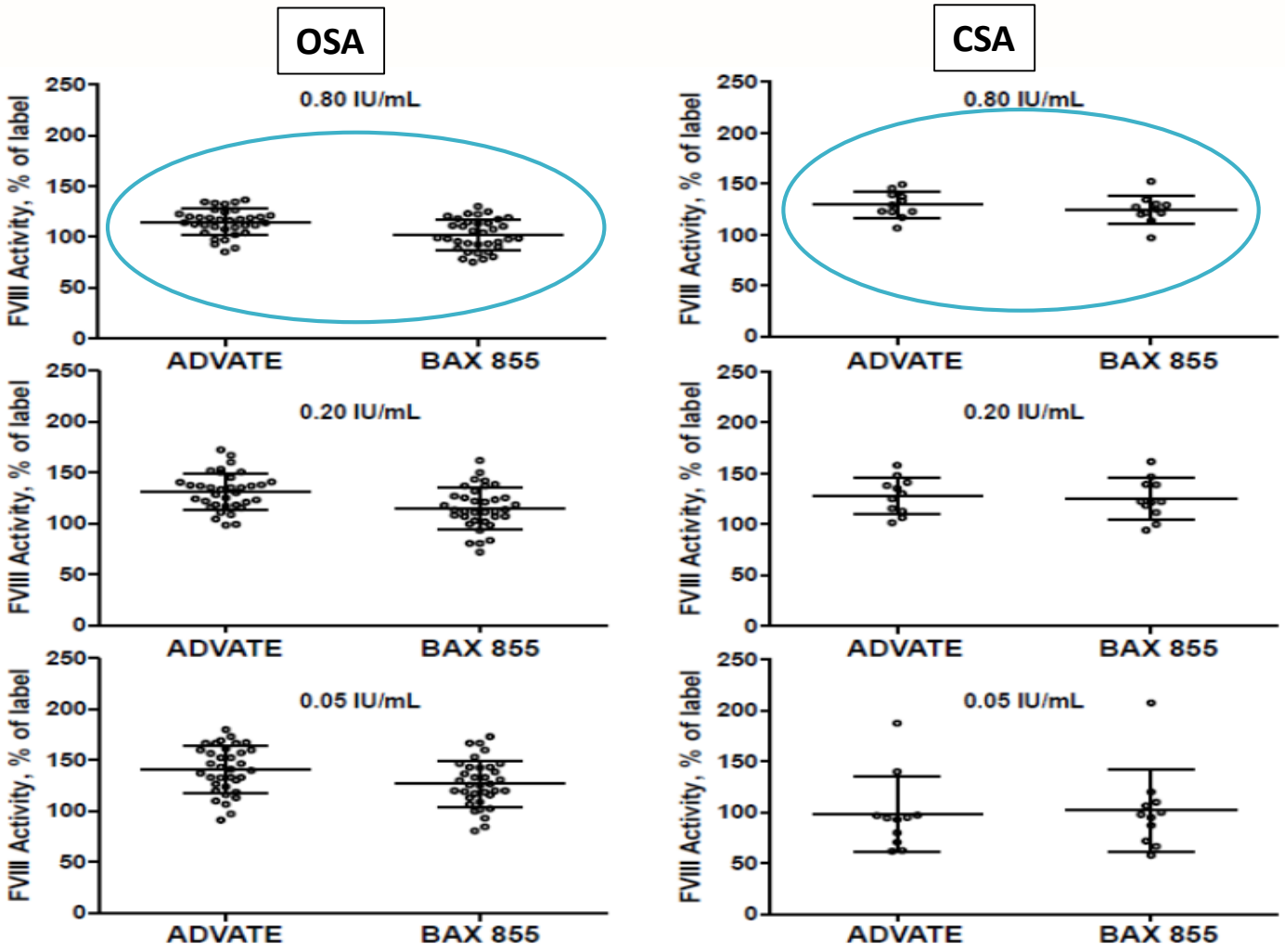
BAX 855 (ADYNOVATE®/ADYNOVI®) – International Comparative Field Study: OSA and CSA Data



- 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

A world-wide survey and field study in clinical haemostasis laboratories to evaluate FVIII:C activity assay variability of ADYNOVATE and OBIZUR in comparison with ADVATE", P.L. Turecek, et al. Haemophilia (2016), 22, 957–965; © 2016 Baxalta Innovations GmbH. Haemophilia Published by John Wiley & Sons Ltd. 2.Turecek PL, et al. J Thromb Haemost. 2016;14(Suppl 1):54 (abstract and poster).

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BAX 855 (ADYNOVATE®/ADYNOVI®) PI

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.

Routine FVIII OSA and CSA can be used to measure BAX 855 in plasma

PI – Prescribing Information

ADYNOVATE® Prescribing Information. Baxalta US Inc; December 2016.

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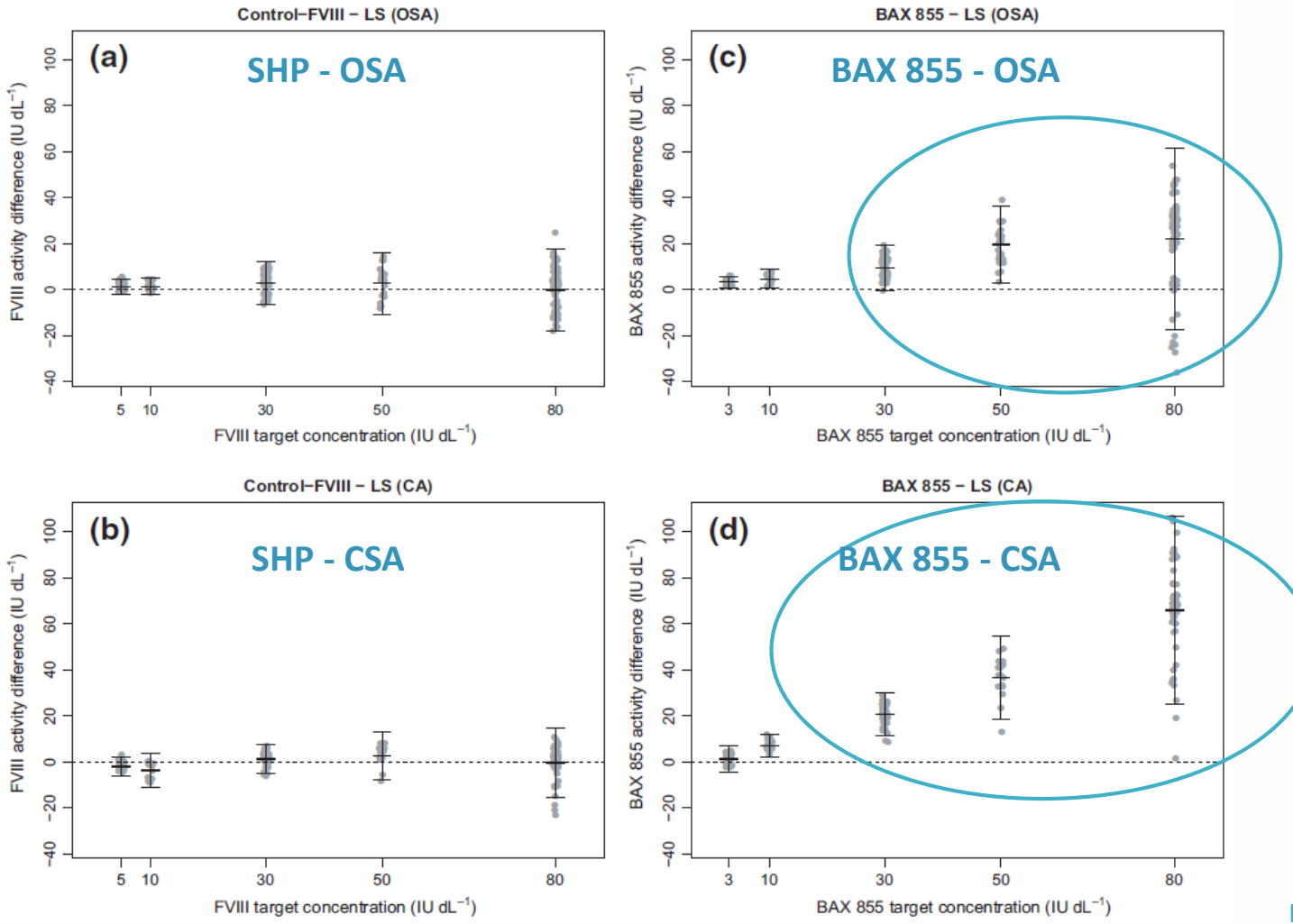
BAX 855 (ADYNOVATE®/ADYNOVI®) – Swiss Field Study



- 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

Bulla O, *et al. Haemophilia* 2017;23(4):e335-339.

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BAY 94-9027 (Jivi®) – International Comparative Field Study

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

Church N, *et al. Haemophilia* 2018;00:1–10.

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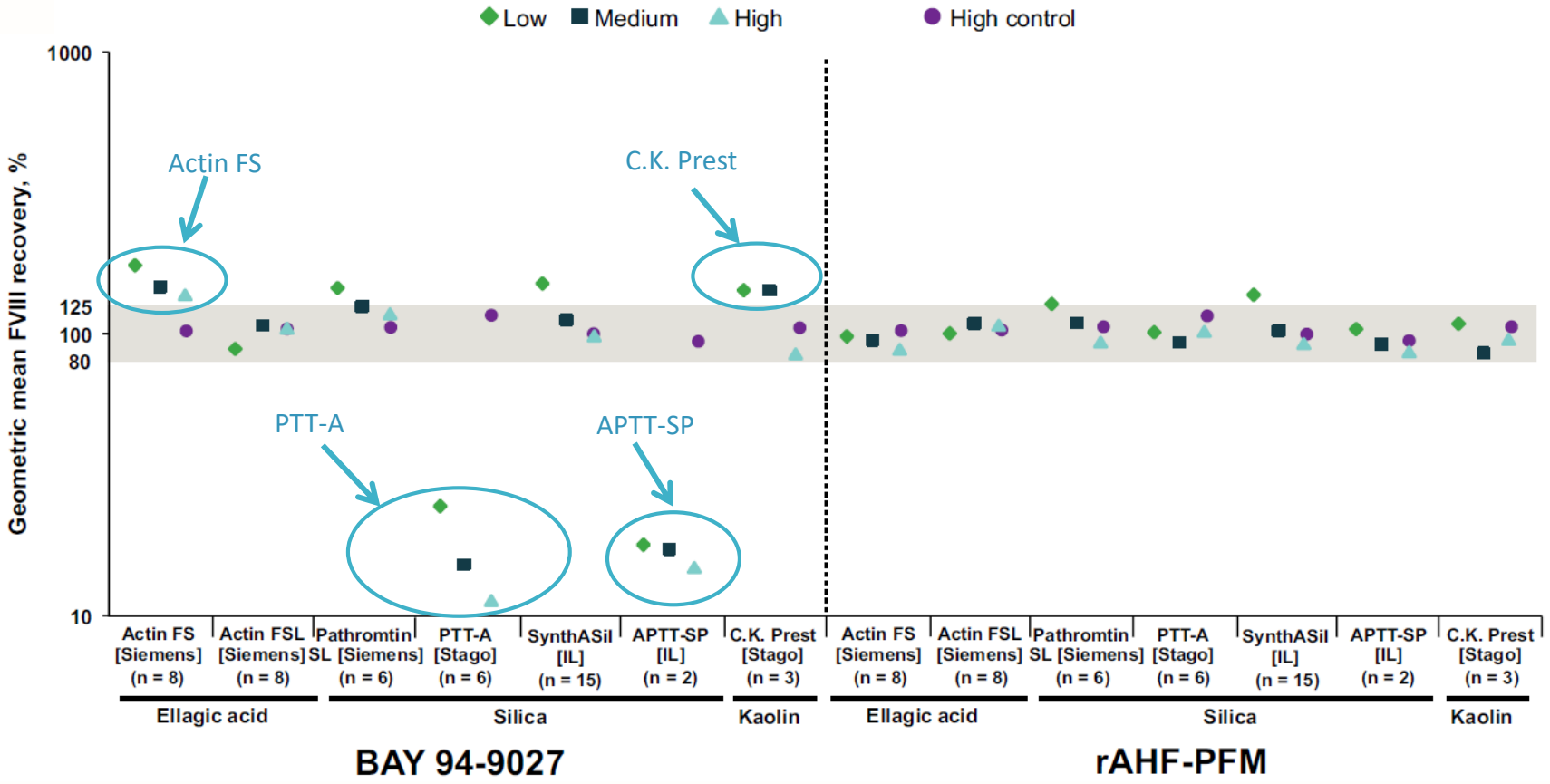
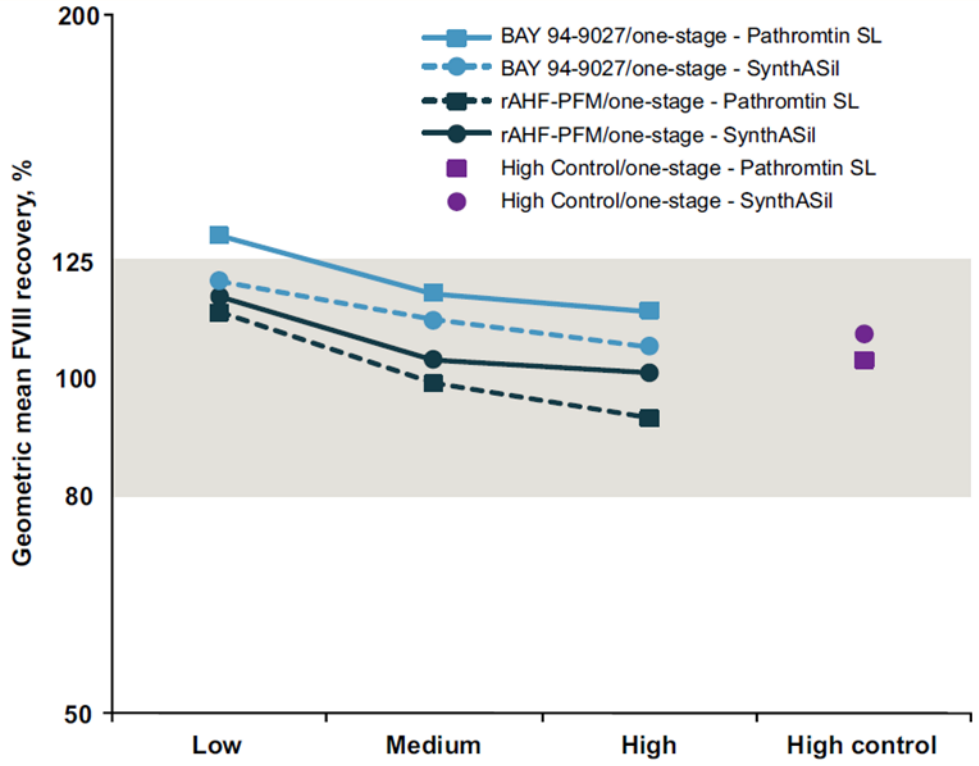
BAY 94-9027 (Jivi®) – International Comparative Field Study: OSA Data (Part I)



- PTT-A and APTT-SP (both silica-based reagents) under-recover BAY 94-9027 by 50% or less
- C.K.Prest and Actin FS over-recover BAY 94-9027
- ADVATE recovers appropriately across all reagents tested

Church N, *et al. Haemophilia* 2018;00:1–10.

BAY 94-9027 (Jivi®) – International Comparative Field Study: OSA Data (Part II)

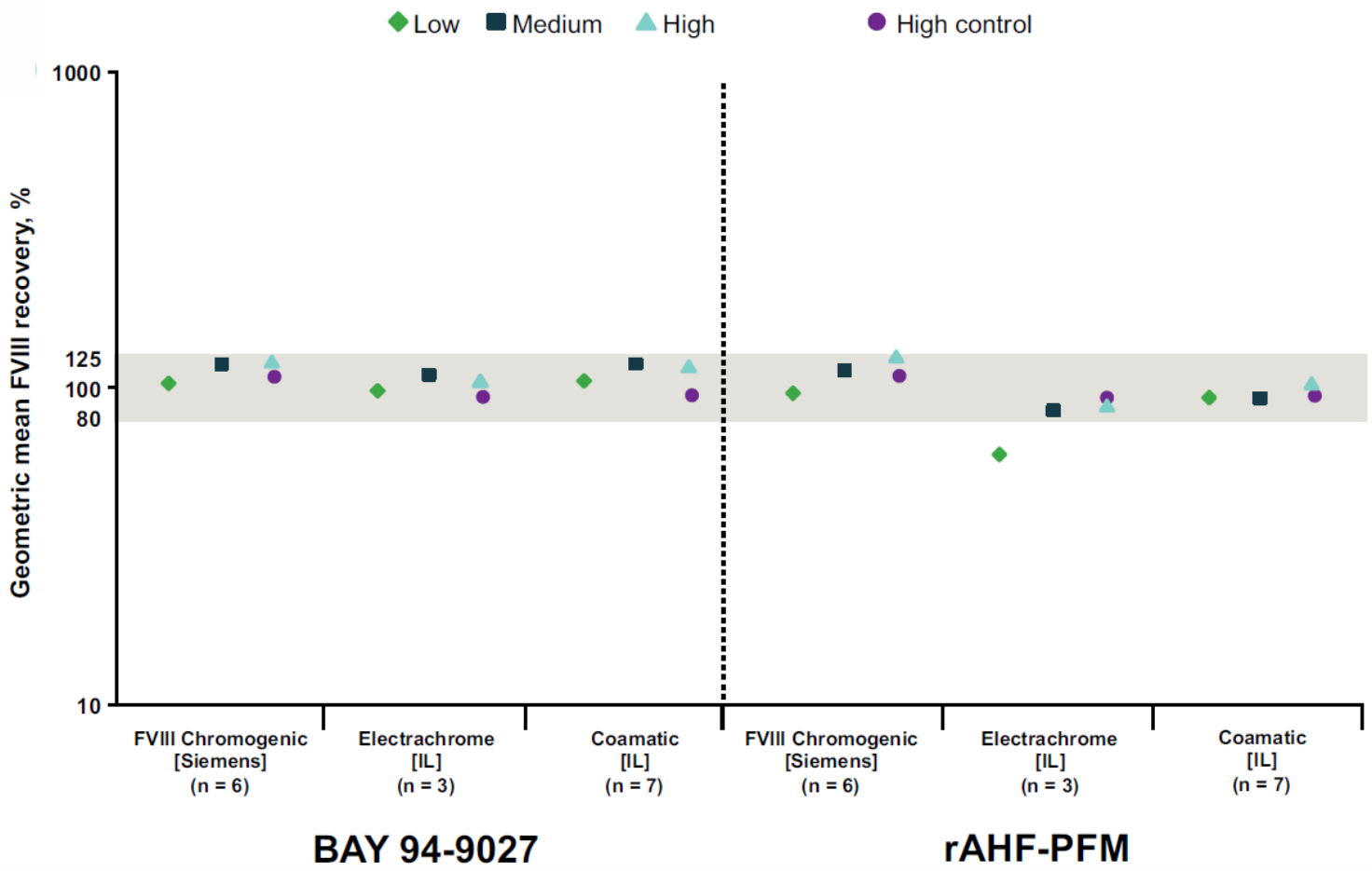


	BAY 94-9027			rAHF-PFM			High control
	Low	Medium	High	Low	Medium	High	
Pathromtin SL, n = 51	129.2 ± 1.8	115.1 ± 1.3	111.0 ± 1.3	111.2 ± 1.8	96.7 ± 1.2	90.0 ± 1.2	101.2 ± 1.2
SynthASil, n = 52	118.0 ± 1.4	109.7 ± 1.2	103.6 ± 1.2	114.6 ± 1.4	100.9 ± 1.2	98.3 ± 1.1	106.3 ± 1.2

- SynthASil® recovers BAY 94-9027 and within ±25% of expected target concentration
- Pathromtin® SL recovers BAY 94-9027 within ±30% of expected target concentration
- SynthASil® and Pathromtin® SL recover ADVATE within ±15% of expected target concentration

Church N, *et al. Haemophilia* 2018;00:1–10.

BAY 94-9027 (Jivi®) – International Comparative Field Study: CSA Data



- Chromogenic assays recover BAY 94-9027 and ADVATE within ±25%

Church N, *et al. Haemophilia* 2018;00:1–10.

BAY 94-9027 (Jivi®) PI

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.

Select silica-based reagents may underestimate and kaolin-based reagents (+Actin FS??) may over-estimate BAY 94-9027 in the FVIII OSA; FVIII CSA assay reagents measure BAY 94-9027 accurately

PI – Prescribing Information

Jivi® Prescribing Information. Bayer Healthcare; August 2018.

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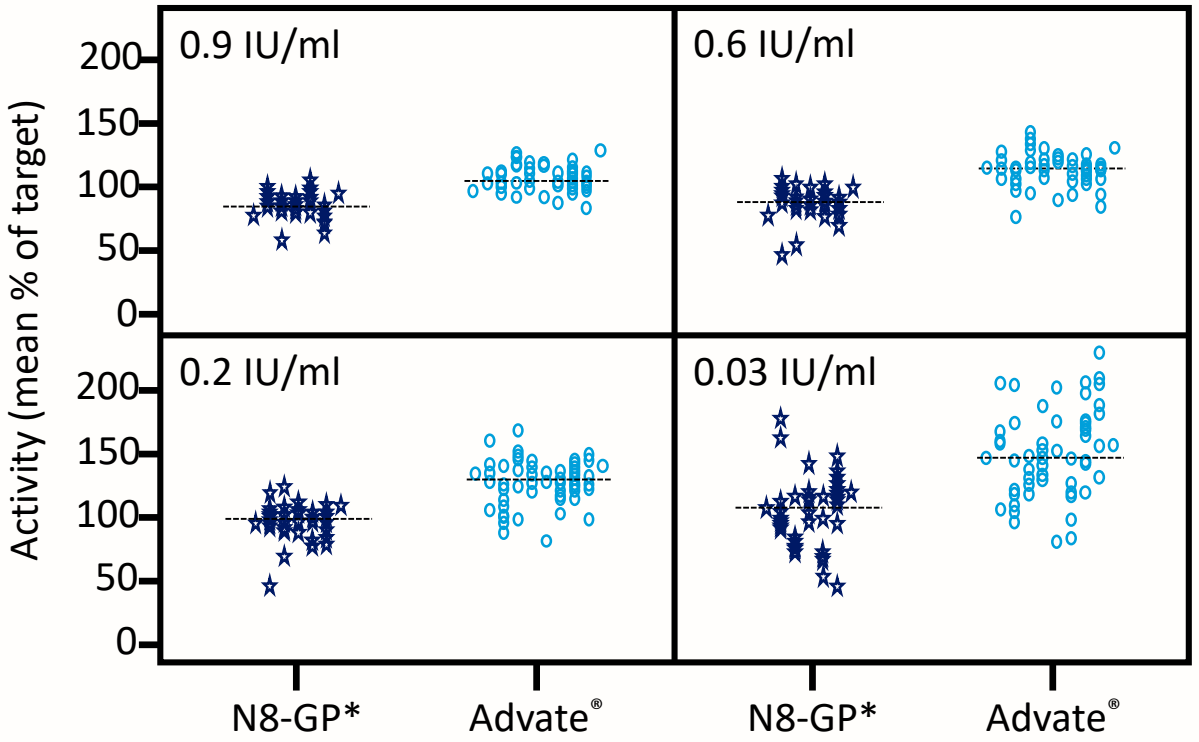
EHL rFVIII Products – In Development

Name	Manufacturer	Modification for Half-Life Extension	Approval Date
N8-GP (BDtrunc PEGylated)	Novo Nordisk	40-kDa glycoPEGylation	BLA filed in US (Feb 2018) MAA filed in EU (Feb 2018)
BIVV001 (BDD) (rFVIIIFc-VWF-XTEN)	Bioverativ	Fusion to Fc domain of IgG1 Fusion to XTEN® protein polymer Fusion to VWF	Phase I/IIa (ongoing)

BDD, B-domain-deleted; BDtrunc, B-domain truncated; PEG, polyethylene glycol; BLA, biologic license application; MAA, market authorization application; VWF, von Willebrand Factor

N8-GP – 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

Hansen M, *et al.* Poster PB241 presented at ISTH-SSC 2018, Dublin, Ireland.

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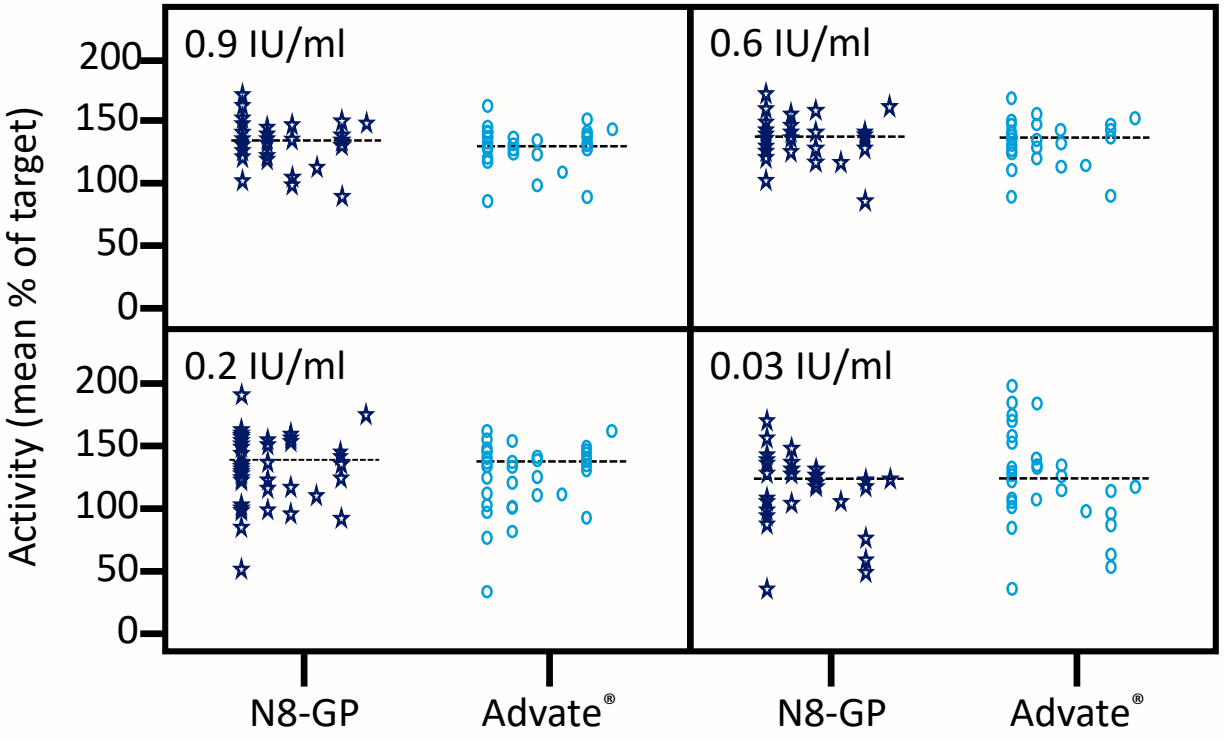
N8-GP – International Comparative Field Study: CSA Data



- 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)

Hansen M, *et al.* Poster PB241 presented at ISTH-SSC 2018, Dublin, Ireland.

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N8-GP – Preliminary Recommendations

- APTT SP, TriniCLOT™, STA® PTT-A underestimate N8-GP[1,2,3] and should not be used
- Available data to date suggest that chromogenic assays appropriately measure N8-GP (although at upper boundary of acceptable range) [1,2,3]

1. A. Hillarp, et al. Haemophilia (2017), 1-8
2. W. Pickering, et al. J Thromb Haemost. (2016); 14(8), 1579-87
3. Hansen M, *et al.* Poster PB241 presented at ISTH-SSC 2018, Dublin, Ireland.

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Clinical Laboratory Challenges

Challenge 1: Alignment of Clinical Factor Activity Assay with the Potency Assay

- Factor product potency assignment[1]
  - FVIII products: predominantly CSA using WHO IS concentrate
- Factor product monitoring in clinical laboratories usually performed with OSA using pooled normal plasma standard
- Factor activity assays used for post-infusion monitoring should closely align with the assay used by manufacturer to assign potency to factor replacement product or the assay that was used to demonstrate clinical efficacy
  - This can only be achieved if clinical laboratory knows which factor product the patient received

1. Hubbard AR, et al. *J Thromb Haemost.* 2013;11:988-9.

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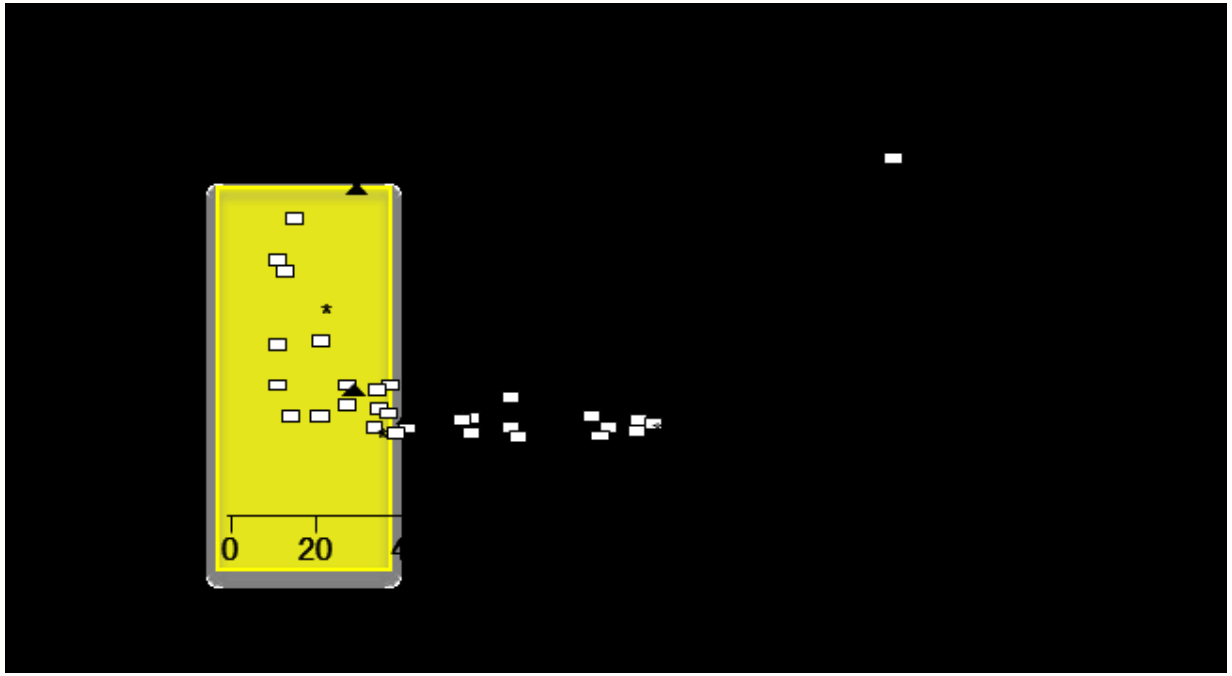
Challenge 2: Variability of FVIII One-Stage Factor Activity Assay



FVIII OSA consistently demonstrate high variability between laboratories in PT surveys (CAP, ECAT, NEQUAS)

- A normal plasma sample in a recent CAP survey (CGE-A 2016) yielded results ranging from 0.72–1.61 IU/mL

Data derived from RCPAQAP Haematology, ECAT, and NEQAS EQA programs (2011-2012).

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Challenge 3: Limited Availability and Use of FVIII CSA Assays in Clinical Labs

- MASAC in 2014 recommends laboratories routinely performing factor assays on patients with hemophilia to add the FVIII chromogenic assay[1]
- To date, only 20-30% of clinical laboratories perform FVIII CSA testing[2]
- Suggested reasons for slow implementation:
  - Considered more expensive than OSA
  - Perceived more complex and difficult to automate
  - Limited availability of approved/validated applications for automated coagulation analyzers
    - In US only 1 of 5 available kits is IVD approved for use on an automated coagulation analyzer

1. MASAC Document #228, 2014
2. ECAT Foundation, Survey 2017-M1

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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

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Summary

- No single OSA aPTT reagent can measure all of the EHL FVIII products appropriately
- Available results to date suggest that most (if not all) EHL FVIII products (currently approved or in late-stage development) can be monitored using the CSA

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THANK YOU