Colorado COAGULATION
LabCorp Specialty Testing Group

# Hemophilia Treatment and Laboratory Testing

**Stefan Tiefenbacher, PhD**
Technical Director, Vice President
Colorado Coagulation, Laboratory Corporation of America Holdings

LabCorp®

# 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 |

# Laboratory Evaluation of Hemophilia

- Clinical laboratory plays important role in:
  - Diagnosis and classification of disease severity
  - Monitor  treatment (e.g. assessing recovery of replacement factor after infusion)
- With the approval of novel hemophilia treatment therapies, adjustments to the assays commonly used to monitor therapy in the clinical laboratory will be required

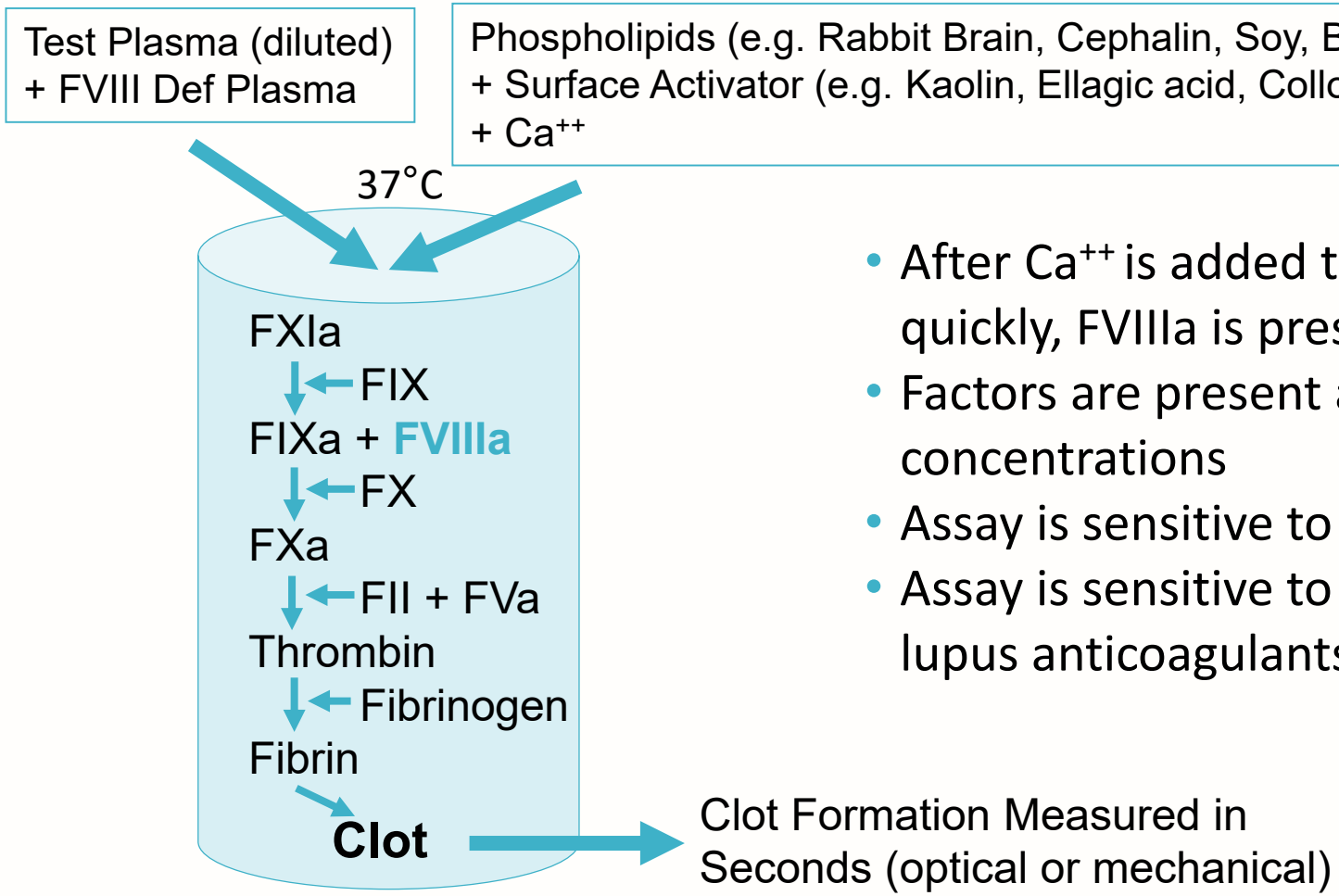
LabCorp

# Laboratory Assays Currently Used to Monitor Hemophilia 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
  - Limited availability in clinical laboratories, considered more expensive and more complex, this is debatable
    - **FVIII:** multiple CE-marked and FDA-approved kits available
    - **FIX:** two CE-marked and no FDA-approved kits available

LabCorp

# One-Stage (aPTT) Factor VIII Activity Assay

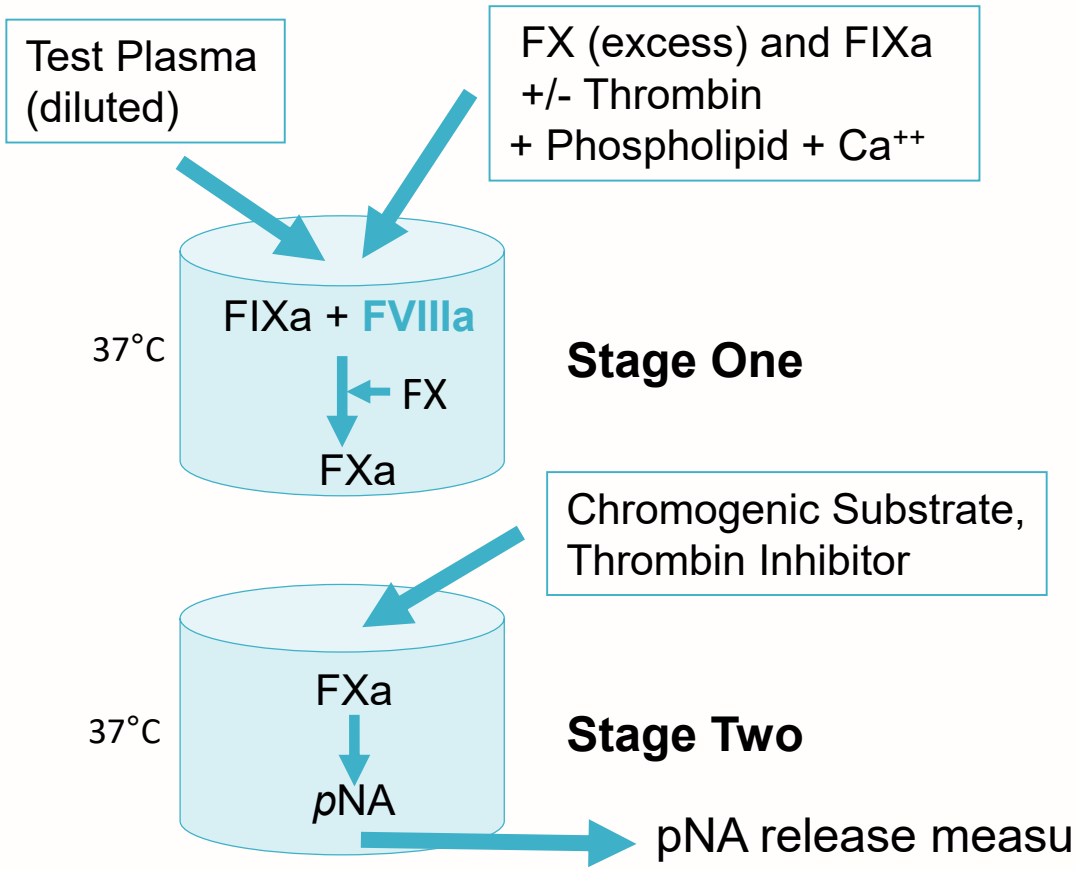



- After $Ca^{++}$ is added the reaction occurs quickly, FVIIIa is present only a short time
- Factors are present at physiologic concentrations
- Assay is sensitive to pre-activation of FVIII
- Assay is sensitive to interference by heparin, lupus anticoagulants and DOAC's

Modified from Peyvandi F, et al. *J Thromb Haemost.* 2016;14:248-61.

LabCorp

# Chromogenic Substrate Factor VIII Activity Assay




- Stage one is incubated for 2–10 minutes; during this time FVIIIa is generated
- Not sensitive to FVIII pre-activation
- Factors are present at non-physiological concentrations (highly diluted)
- Relatively insensitive to interference from heparin or lupus anticoagulants

Modified from Peyvandi F, et al. *J Thromb Haemost.* 2016;14:248-61.

LabCorp

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

- Conditions where results may not agree:
  - Discrepant hemophilia A[1,2] and hemophilia B[3]
  - In the presence of certain inhibitors
    - Such as lupus anticoagulants
  - Post-infusion monitoring of rFVIII[4], select EHL rFVIII and rFIX replacement therapies[5,6] , FVIII and FIX gene therapies[7,8]
- 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. Kihlberg K, et al. *Haemophilia.* 2016;22(Suppl 4):70 ; 4. Barrowcliffe TW, et al. *Semin Thromb Hemost.*2002;328:247-56.; 5. St Ledger K, et al. *Thromb Haemost. 2018*;16:555-64.; 6. Kitchen S. et al. *Res Pract Thromb Haemost.* 2017;1 (Suppl 1):124-5 (abstract); 7. Rangarajan S, et al. *N Engl J Med. 2017*; 377:2519-2530.; 8. Robinson M, et al. *Blood 2018*; ASH Meeting Dec 2018.

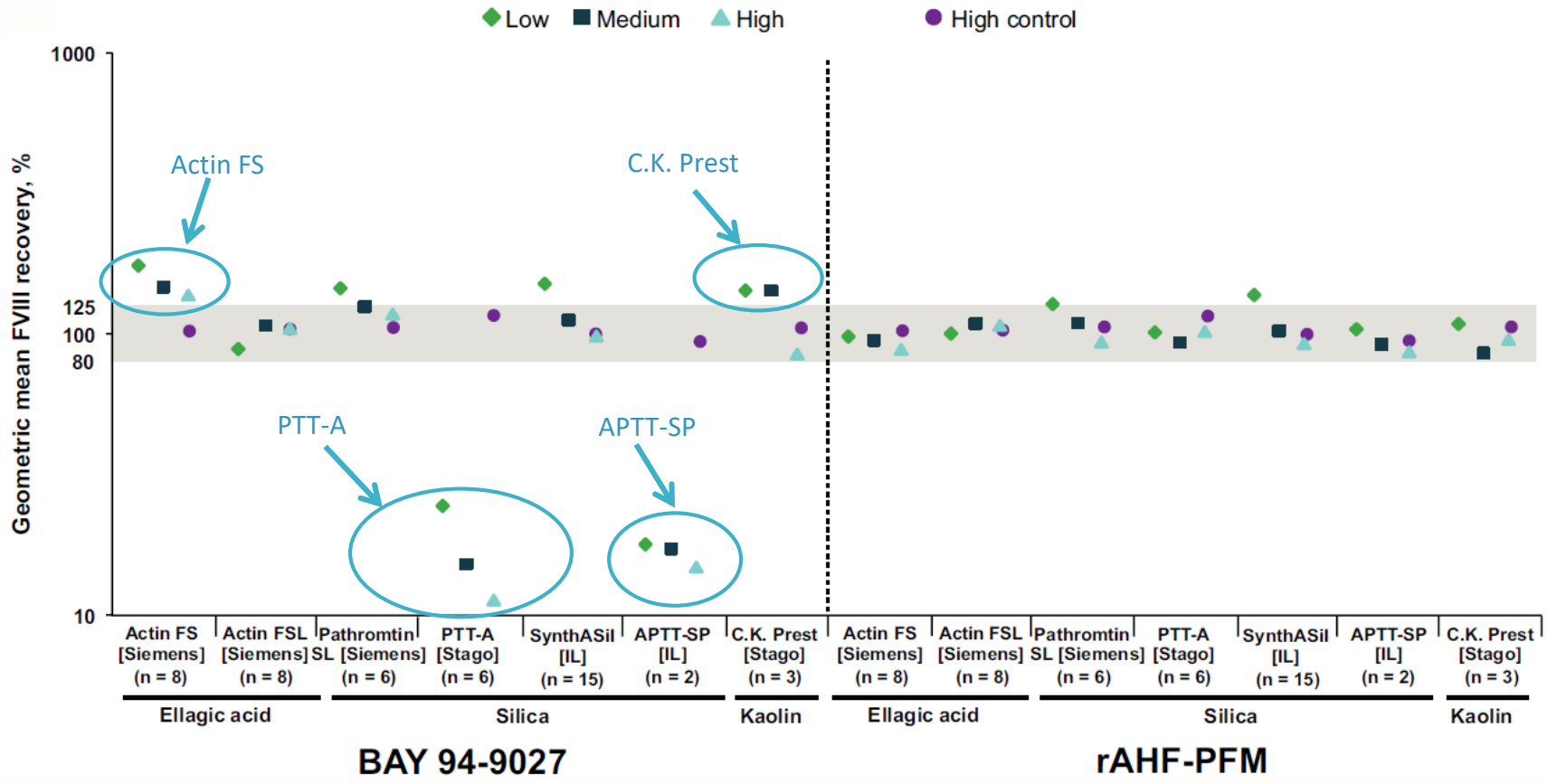
# EHL FVIII Replacement Products

# EHL rFVIII Products

| 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<br>EMA Nov 2015 |
| CSL627 (BDD) – AFSTYLA® | CSL Behring | Single-chain | FDA May 2016<br>EMA Nov 2015 |
| Bax 855 (FL) – ADYNOVATE | Shire | 20-kDa branched PEG | FDA Dec 2016<br>EMA Jan 2018 |
| BAY 94-9027 (BDD) - Jivi® | Bayer | Site-specific 60-kDa PEG | FDA Aug 2018 |
| N8-GP (BDtrunc PEGylated) | Novo Nordisk | 40-kDa glycoPEGylation | BLA filed in US (Feb 2018)<br>MAA filed in EU (Feb 2018) |

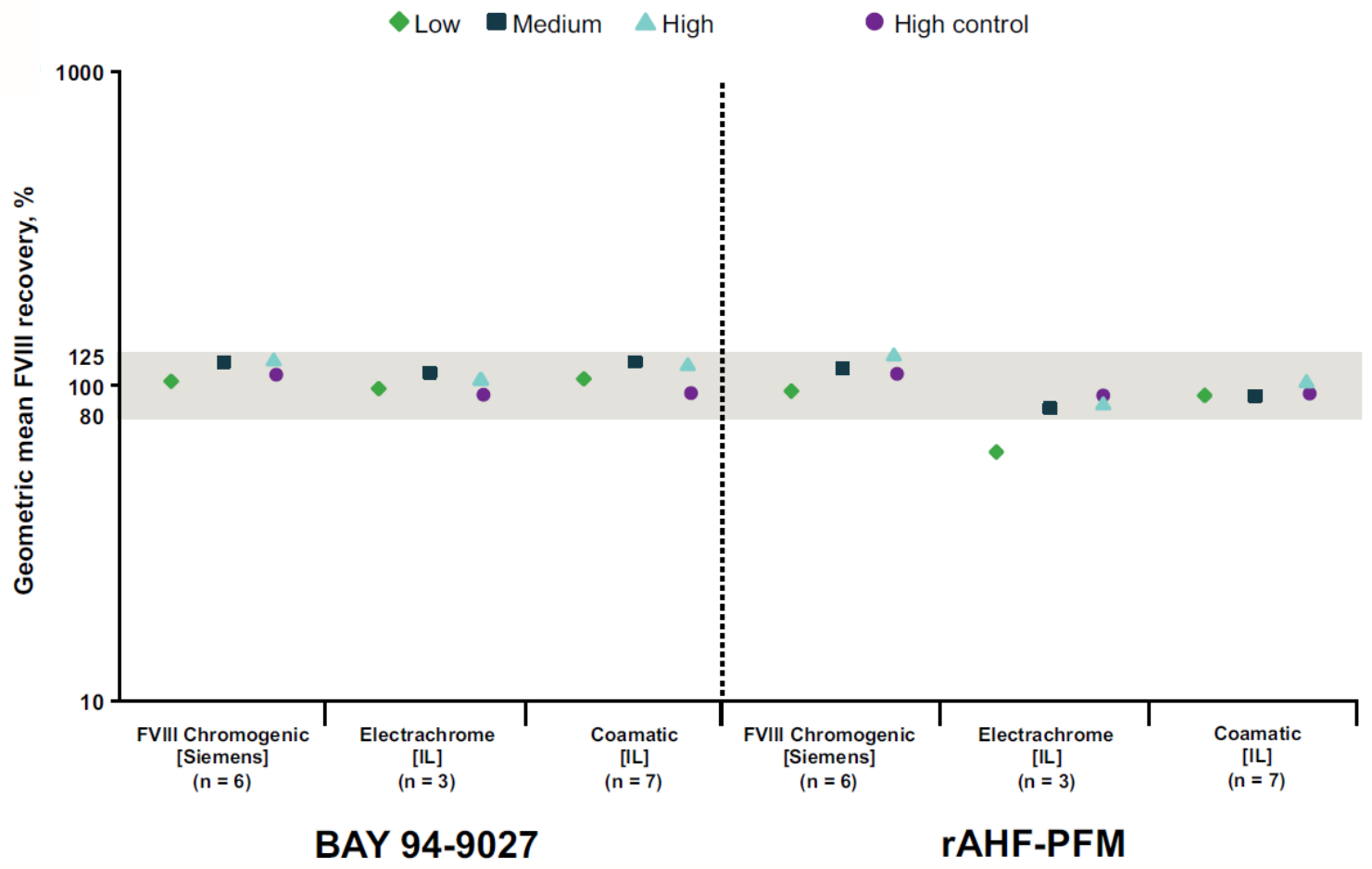
BDD, B-domain-deleted; BDtrunc; FL, full length; PEG, polyethylene glycol.

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




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

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




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

# EHL rFVIII Products – Field Study OSA/CSA Summary

| Name | Manufacturer | One-Stage (aPTT) Assay | Chromogenic Assay |
|---|---|---|---|
| rFVIII-Fc (BDD) – ELOCTATE®/ELOCTA®[1] | Bioverativ/SOBI | √ | √ |
| CSL627 (BDD) – AFSTYLA®[2,3] | CSL Behring | all aPTT reagents ↓ (multiply OSA result x2) | √ |
| Bax 855 (FL) – ADYNOVATE[4] | Shire | √ | √ |
| BAY 94-9027 (BDD) - Jivi®[5] | Bayer | APTT-SP ↓, STA-PTT-A ↓ CK Prest ↑, Actin FS ↑ | √ |
| N8-GP (BDtrunc PEGylated)[6] | Novo Nordisk | APTT-SP ↓, STA-PTT-A ↓ TriniCLOT™ ↓ | √ |

[1] at upper acceptable limit of ± 30% RE ↓indicates under-recovery; ↑ indicates over-recovery; √ indicates acceptable recovery

1. Sommer, et al. Haemophilia (2014), 20, 294–300; 2. ST Ledger et al, Thromb and Haemost (2018), 16, 555–564 (abstract);; 3. AFSTYLA® Prescribing Information. CSL Behring; May 2016 ; 4. Turecek, et al. Haemophilia (2016), 22, 957–965; 5. Church N, *et al. Haemophilia* 2018;00:1–10; 6. Hansen M, *et al.* Poster PB241 presented at ISTH-SSC 2018, Dublin, Ireland.

# Summary EHL FVIII Products

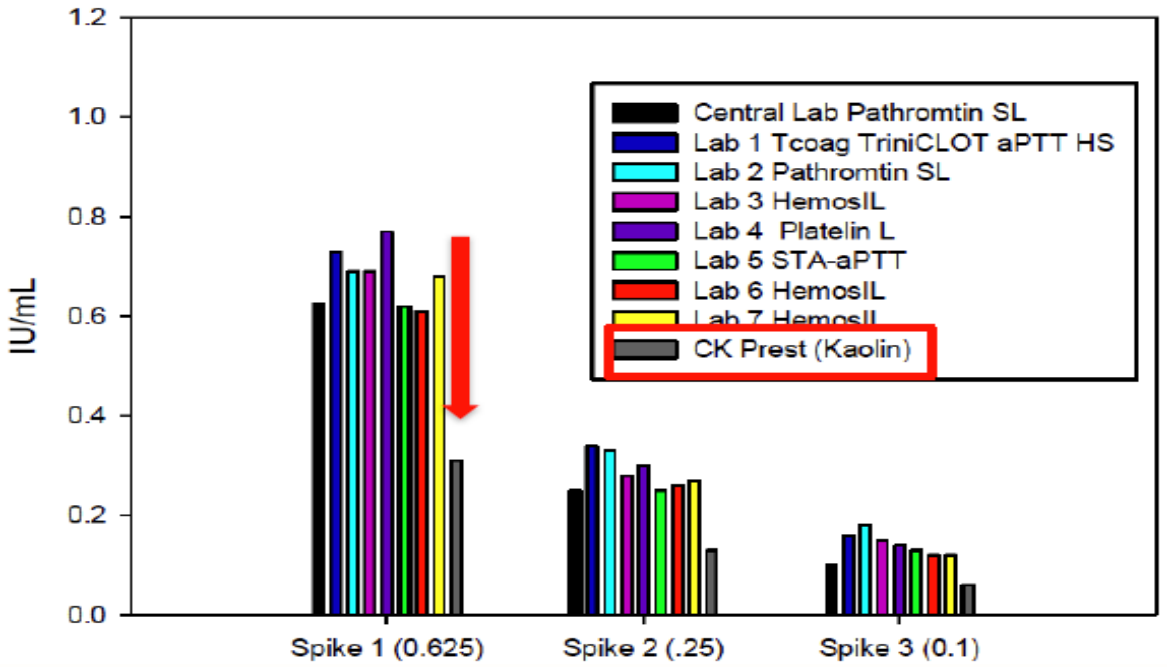
- Field study data available for all of the approved EHL FVIII products
- No single OSA aPTT reagent can measure all of the EHL FVIII products appropriately
- Available results to date suggest that most (if not all) EHL FVIII products (currently approved or in late-stage development) can be monitored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

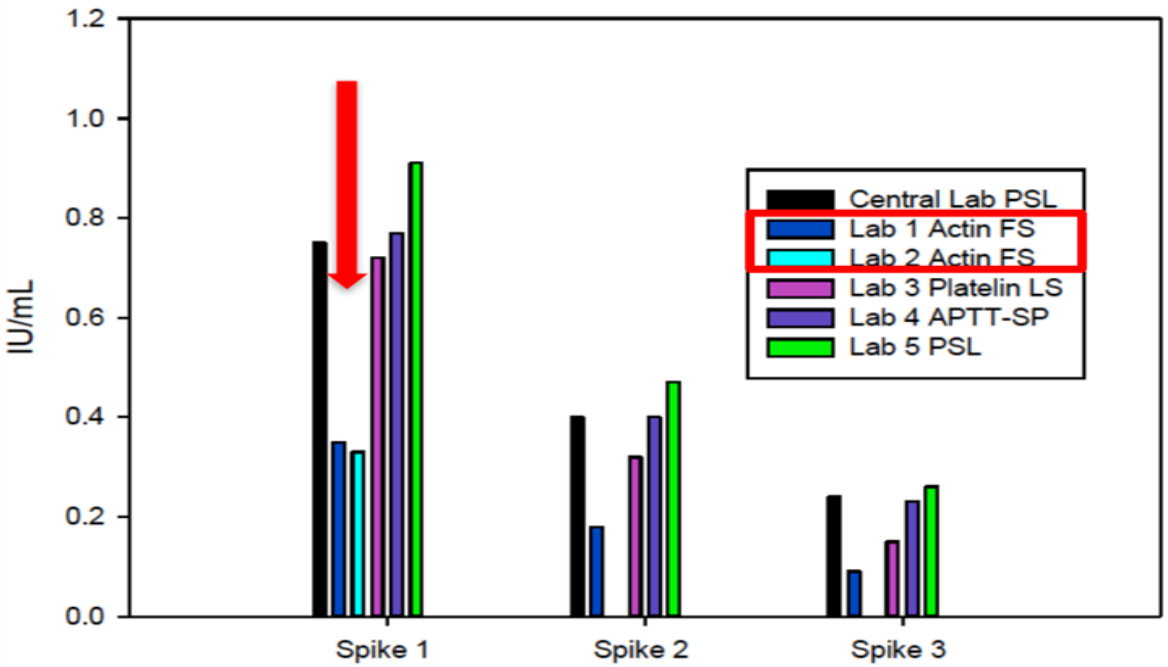
# FF-IL FIX Replacement Products

# EHL rFIX Products

| Name | Manufacturer | Modification for Half-Life Extension | Approval Date |
|---|---|---|---|
| rFIX-Fc – ALPROLIX® | Bioveratif/SOBI | Fusion to Fc domain of IgG1 | FDA Mar 2014<br>EMA May 2016 |
| rIX-FS (CSL654) – IDELVION® | CSL Behring | Fusion to albumin | FDA Mar 2016<br>EMA May 2016 |
| N9-GP – REBINYN®/ REFIXIA® | Novo Nordisk | 40-kDa glycoPEGylation | FDA May 2017<br>EMA Mar 2017 |

# rIX-FP (IDELVION®) – International Multicenter Field Study: OSA Data




St. Ledger K, et al. *Haemophilia.* 2016;22 (Suppl 4):58 (abstract and poster).

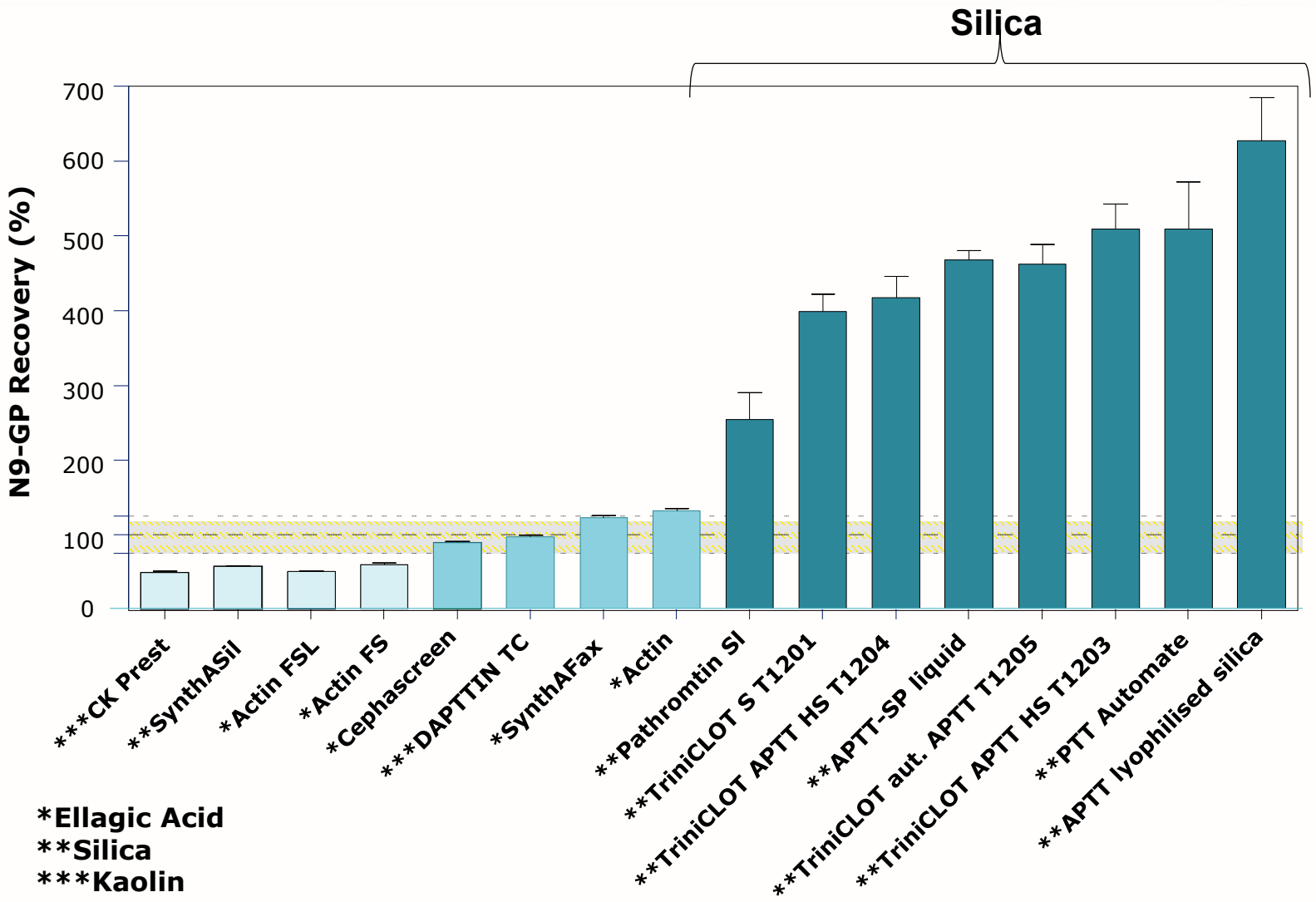
# rIX-FP – OSA and CSA Data[1]

| | | n | Post Replenine | Spiked Replenine | Post Benefix | Spiked Benefix | Spiked Idelvion | Spiked Alprolix |
|---|---|---|---|---|---|---|---|---|
| Calculated FIX in spiked samples | | - | - | 50 IU/dL | - | 70 IU/dL | 60 IU/dL | 60 IU/dL |
| One stage methods Median (IU/dL) | AFS | 19 | 41.8 | 51.5 | 68.8 | 83 | 31.5 | 65.5 |
| | AFSL | 4 | 49 | 51.5 | 81.9 | 89.9 | 50.7 | 75.4 |
| | CK Prest | 5 | 51 | 55 | 76.5 | 86 | 32 | 48 |
| | Pathromtin | 4 | 42.7 | 46.5 | 57.6 | 66.5 | 52.6 | 46.9 |
| | PTT Auto | 3 | 46.3 | 50.7 | 80.5 | 82.6 | 52 | 40 |
| | SynthaSIL | 32 | 45 | 48.1 | 74 | 79.9 | 65.5 | 60.7 |
| Chromogenic Median (IU/dL) | Hyphen | 10 | 44 | 46.7 | 47.9 | 54.4 | 99.4 | 54.0 |
| | Rossix | 13 | 45 | 45 | 55 | 63 | 116 | 66.0 |

1. Kitchen S. et al. *Res Pract Thromb Haemost.* 2017;1 (Suppl 1):124-5 (abstract).

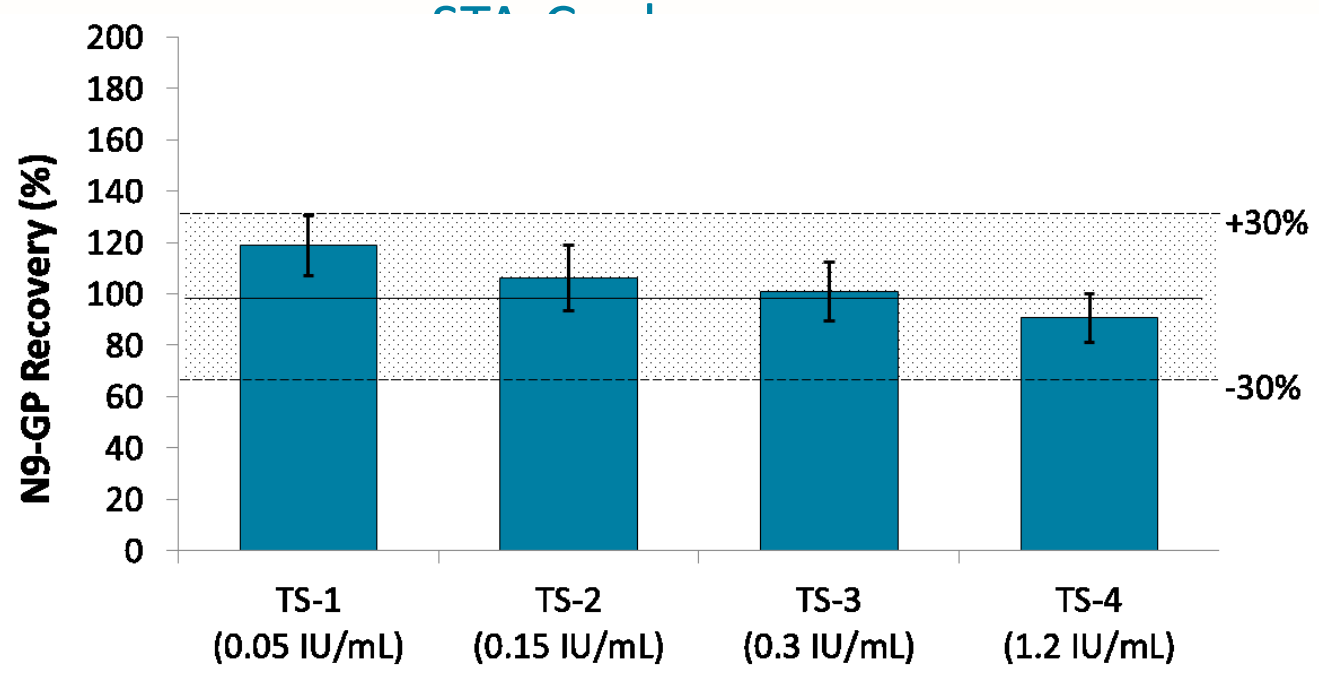
LabCorp

# N9-GP (REBINYN®) – OSA Data



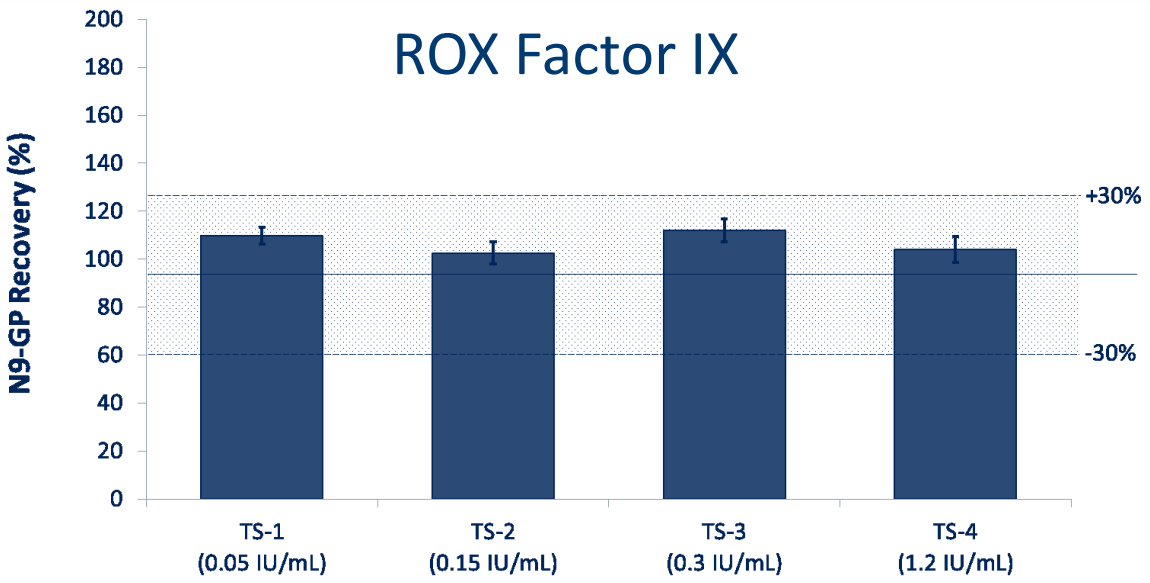

Adapted with permission from P.K. Holm, et al. The activity of GlycoPEGylated recombinant FIX (N9-GP) can be measured in two-stage chromogenic and one-stage clotting assays. J Thromb Haemost. 2013;11(Suppl 2):828 (abstract and poster).

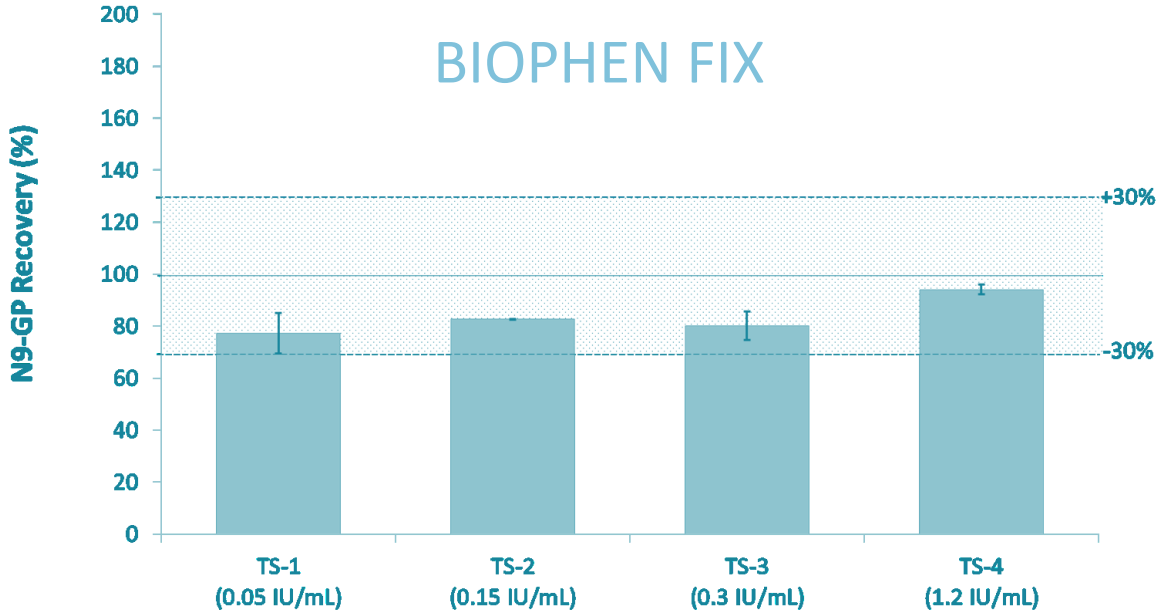
# N9-GP – Multi-Center Qualification Study: OSA Data




Tiefenbacher S, et al. *J. Thromb Haemost*. 2017, Aug 3. doi: 10.1111/jth. 13787

# N9-GP – Multi-Center Qualification Study: CSA Data




Tiefenbacher S, et al. *J. Thromb Haemost*. 2017, Aug 3. doi: 10.1111/jth. 13787

LabCorp

# EHL rFIX Products – Field Study OSA/CSA Summary

| Name | Manufacturer | One-Stage (aPTT) Assay | Chromogenic Assay |
|---|---|---|---|
| rFIX-Fc – ALPROLIX®[1, 2] | Bioveratif/SOBI | CK Prest (kaolin aPTT reagents) ↓ | (√) |
| rIX-FS (CSL654) – IDELVION®[3, 4,5] | CSL Behring | CK Prest (kaolin aPTT reagents) ↓<br>Actin FS ↓<br>aPTT reagents with low lupus responsiveness ↓ | (↑) |
| N9-GP – REBINYN®/ REFIXIA®[6,7] | Novo Nordisk | most aPTT reagents ↑ or ↓<br>Cephascreen √, SynthAFax √ | √ |

↓indicates under-recovery; ↑ indicates over-recovery; √ indicates acceptable recovery; () indicates only limited data are available

1. Sommer et al. Thromb Haemost 2014; 112: 932–40.; 2. ALPROLIX™ Prescribing Information. Biogen Idec; March 2014. ; 3. St. Ledger K, et al. *Haemophilia.* 2016;22 (Suppl 4):58 (abstract and poster).; 4. Kitchen S. et al. *Res Pract Thromb Haemost.* 2017;1 (Suppl 1):124-5 (abstract); 5. IDELVION® Prescribing Information. CSL Behring; March 2016.; 6. Tiefenbacher S, et al. *J. Thromb Haemost*. 2017, Aug 3. doi: 10.1111/jth. 13787; 7. REBINYN® Prescribing Information. Novo Nordisk; June 2017.

# Summary EHL FIX Products

- No single OSA aPTT reagent can measure all EHL FIX products appropriately
- Potential role of CSA assay for monitoring EHL FIX products remains to be demonstrated
  - Limited results available to date suggest that at least two of the three approved EHL FIX products (i.e. rFIX-Fc and N9-GP) can be successfully monitored with CSA
  - FIX CSA not widely used in clinical laboratory
  - Presently only two FIX CSA assay kits available (both CE marked, but neither is approved in the US for diagnostic use)

LabCorp

# FVIII and FIX Gene Therapies

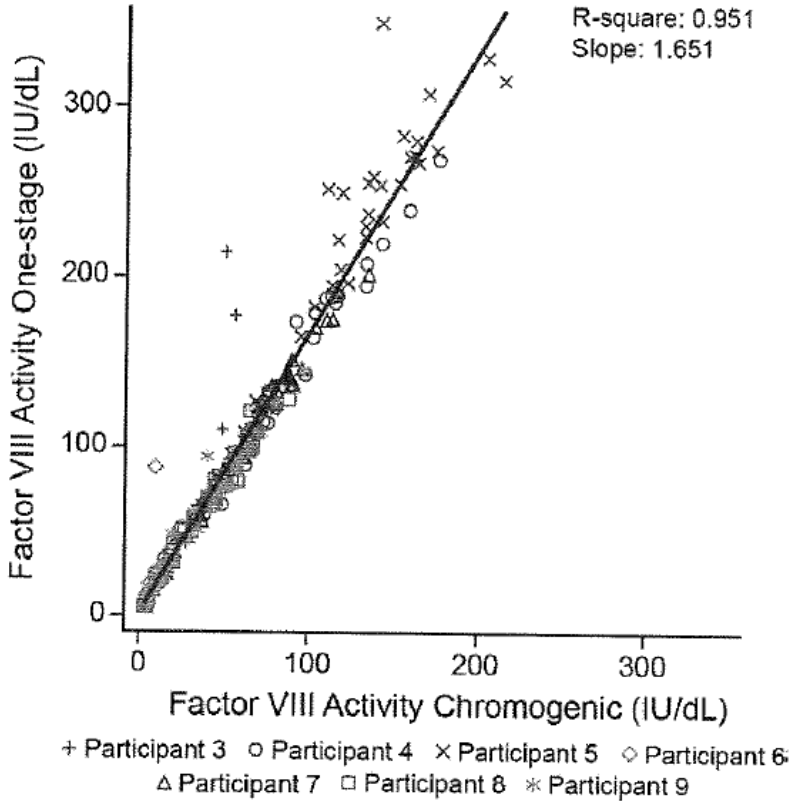
# FVIII Gene Therapies

| Name | Manufacturer | Gene Construct Modification | Clinical Development |
|---|---|---|---|
| BMN-270 | BioMarin | BDD-FVIII | Phase III (Recruiting) |
| SPK-8011 | Spark Therapeutics | BDD-FVIII | Phase I/II |
| SB-525 | Sangamo Therapeutics/Pfizer | BDD-FVIII | Phase I/II |
| SHP654/BAX 888 | Shire | BDD-FVIII | Phase I/II (Recruiting) |

BDD, B-domain-deleted

# FVIII Gene Therapy – OSA and CSA Data

- Adeno-associated virus serotype 5 [AAV5] vector encoding B-domain deleted human FVIII [AAV5- hFVIII-SQ]




- Consistent difference between OSA and CSA FVIII levels are observed
- FVIII OSA levels are ~ 1.65 x higher compared to CSA

Rangarajan S, et al. *N Engl J Med. 2017*; 377:2519-2530

# FIX Gene Therapies

| Name | Manufacturer | Gene Construct Modification | Clinical Development |
|---|---|---|---|
| AMT-061 | Unicure | Padua FIX Variant; AAV5 | Phase III |
| SPK-9001/PF-06838435 | Spark Therapeutics / Pfizer | Padua FIX Variant (FIX-R338L); bio-engineered AAV capsid | Phase III |
| SB-FIX | Sangamo Therapeutics | ZFN genome editing | Phase I/II |

# FIX Gene Therapy: OSA and CSA Data

- Two of the FIX gene therapies currently in development utilize a “Padua variant” (i.e. single R338L gain of function point mutation) which results in ~8 fold higher FIX activity compared to native FIX

- Poster presentation by Robinson M. at ASH Annual Meeting (01 Dec 2018) “Activity of a FIX-Padua Transgene Product in Commonly Used FIX:C One-Stage and Chromogenic Assay Systems following PF-06838435 [SPK-9001] Gene Delivery”

Robinson M, et al. *Blood 2018*; ASH Meeting Dec 2018.

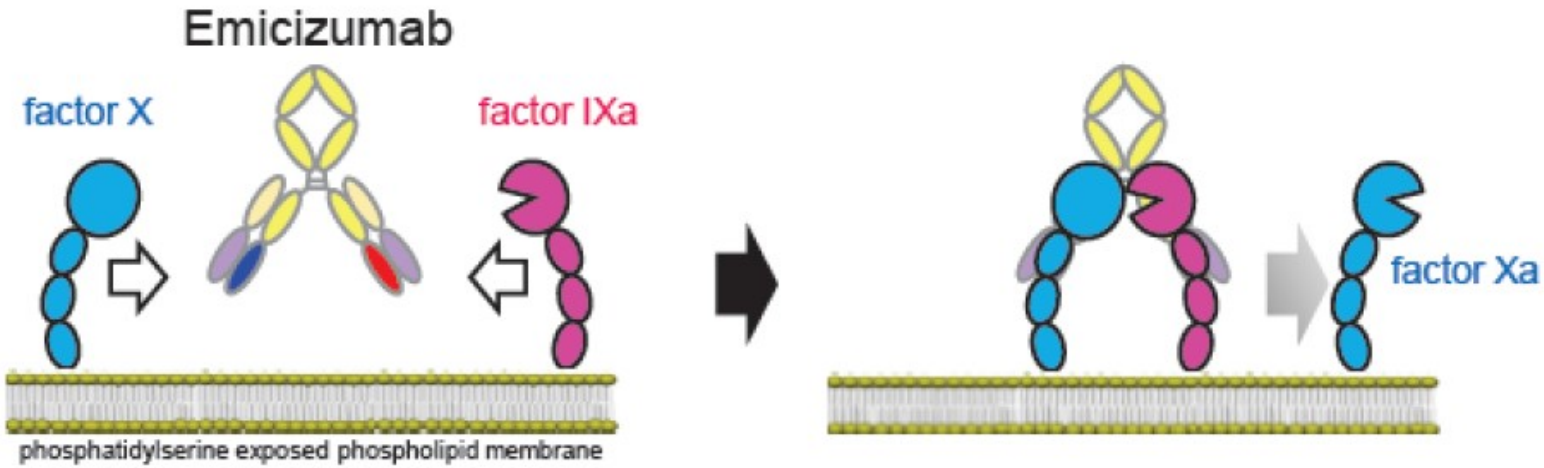
# Non-Factor Replacement Therapies

# None Factor Replacement Therapies

| Name | Manufacturer | Target Mechanism | Approval Date |
|---|---|---|---|
| Emicizumab-kxwh - HEMLIBRA® (Haemophilia A) | Chugai Pharmaceuticals / Hoffman-La Roche | Chimeric bispecific humanised antibody - mimics FVIIIa by bridging FIXa and FX | FDA (Nov 2017)<br>EMA (Feb 2018) |
| Concizumab (Haemophilia A and B) | Novo Nordisk | Anti-TFPI antibody - Inhibition of TFPI | Phase II (ongoing) |
| Fitusiran (Haemophilia A and B) | Alnylam Pharmaceuticals / Sanofi Genzyme | RNA interference therapeutic targeting to lower anti-thrombin (AT) levels | Phase III (ongoing) |

TFPI – Tissue Factor Pathway Inhibitor

# Emicizumab (ACE910)




- Humanized, bispecific, monoclonal antibody that bridges activated FIX and FX, replacing the function of missing activated FVIII
- Although, Emicizumab does not require laboratory monitoring during routine prophylactic dosing[1], situations may arise where laboratory assessment of hemostatic potential in patients is indicated (e.g. surgery or breakthrough bleed)

1. Interim Guidance Medical and Scientific Advisory Committee (MASAC) and National Hemphilia Foundation (NHF) on November 24, 2017
Graphics from Calatzis et al. (2018) poster presented at 62nd Annual Meeting of the Society of Thrombosis and Haemostasis, Vienna Austria

LabCorp

# Important Differences between Emicizumab and FVIII(a)

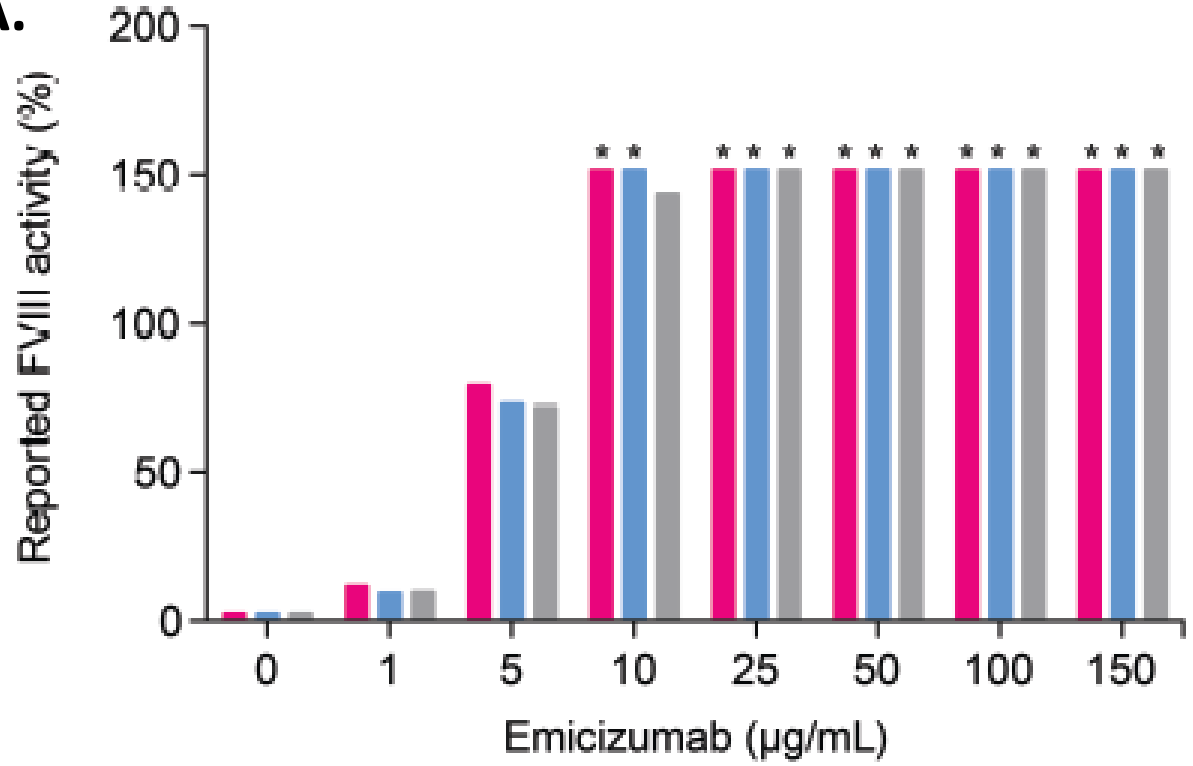
| FVIII(a) | Emicizumab |
|---|---|
| Requires activation by thrombin | Does not require activation |
| Binds to FIXa and FX only | Binds to FIX/FIXa and FX/FXa[1] (human origin only) |
| Promotes PL binding; stabilizes FIXa active site; bridges FIXa to FX | Bridges FIXa to FX |
| Highly regulated (on/off, self-regulation) | Not regulated (no on/off) |
| Half-Life: ~12 h | Half-Life: 27.8 ± 8.1 d (667.2 ± 194.4 h)[2] |
| Neutralized by anti-FVIII Ab | Not affected by anti-FVIII Ab |

[1]Lenting et al. (2017) Blood 130(23): pp 2463-68
[2]HEMLIBRA FDA Prescribing Information, 11/2017

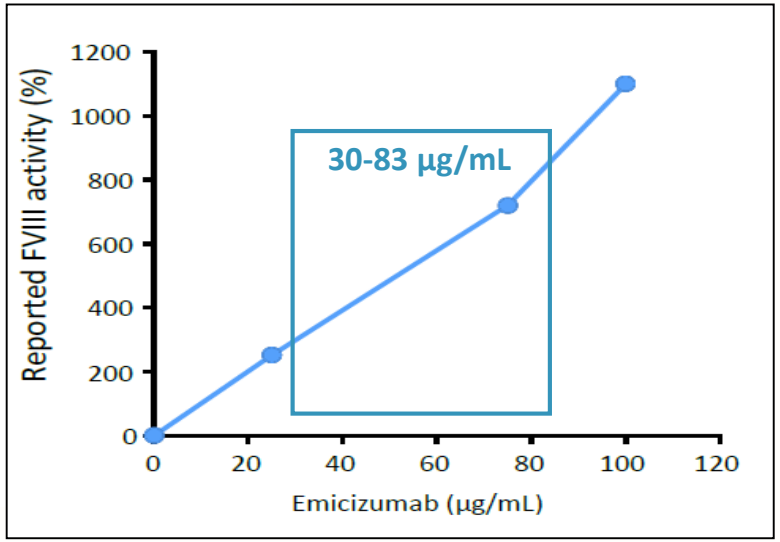
LabCorp

# Effects on FVIII One-Stage Activity Assay

**A.**




**B.**



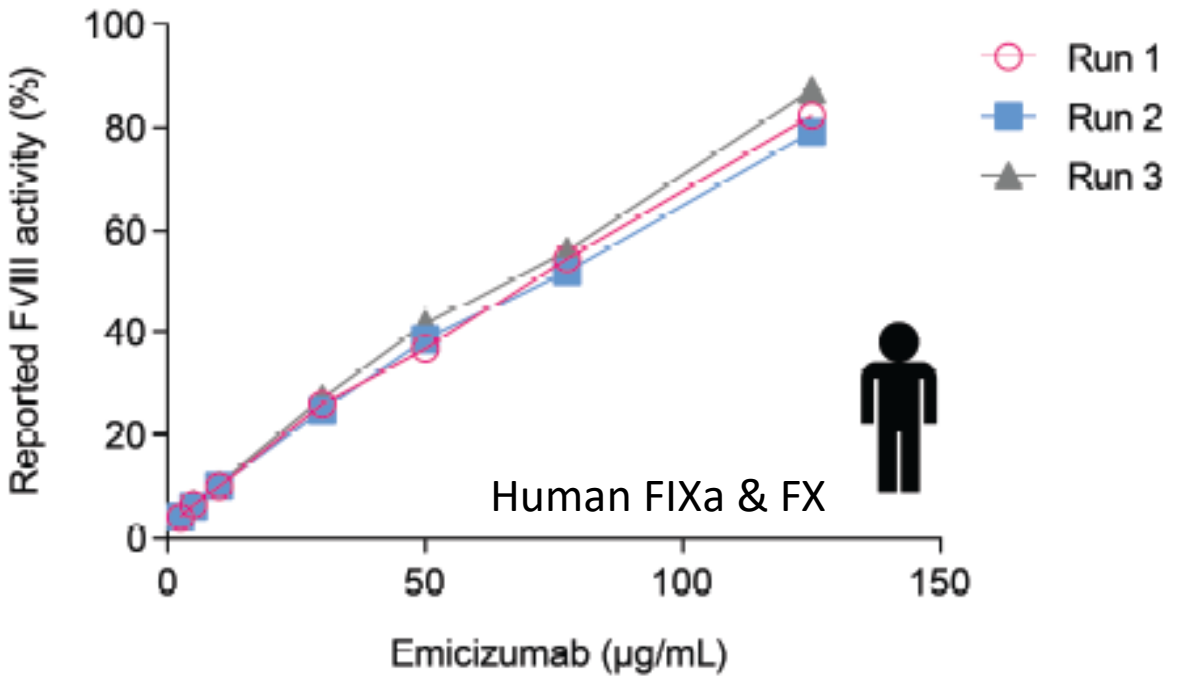

Emicizumab results in overestimation of FVIII activity in the one-stage FVIII activity assay


A. Adamkewicz et al. (2017), Poster presentation at Hemostasis & Thrombosis Research Society, Scottsdale, Arizona
B. Calatzis et al. (2018), Presented at 62nd Annual Meeting of the Society of Thrombosis and Haemostasis, Vienna Austria

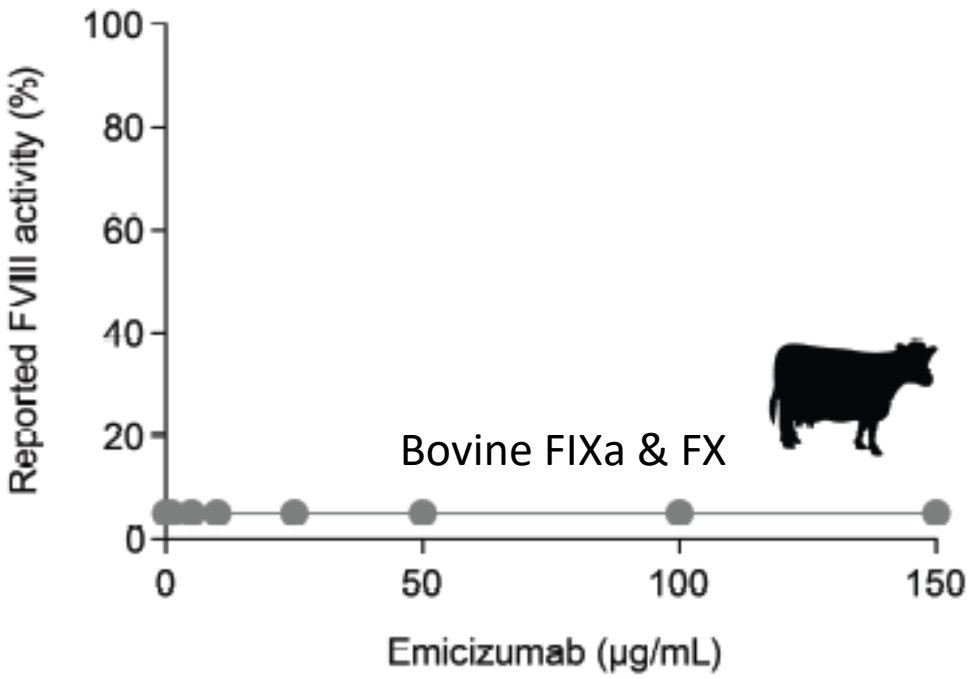

LabCorp

# Effects on FVIII Chromogenic Activity Assay

## Hyphen Biomed




## Siemens Healthcare




Emicizumab co-factor activity is measureable in chromogenic assays containing human FIXa and FX, but is not measurable in assays using bovine origin FIXa and FX

Adamkewicz et al. (2017), Poster presentation at Hemostasis & Thrombosis Research Society, Scottsdale, Arizona

LabCorp

# Summary of Emicizumab Assay Interferences and Recommendations

- Existing data to date suggest that the presence of Emicizumab strongly effect aPTT-based coagulation assays[1,2]
  - One-stage FVIII activity and Bethesda inhibitor assays
- aPTT-based coagulation assays must be avoided when assessing hemostatic potential in patients receiving Emicizumab therapy
- Due to long half-life of Emicizumab, this interference may persist for up to 6 months post treatment[1,2]

[1]HEMLIBRA FDA Prescribing Information, 11/2017; [2]MASAC Interim Guidance on Acute Bleed Management and Use of Laboratory Assays; November 24, 2017

LabCorp

# Summary of Emicizumab Assay Interferences and Recommendations

- Emicizumab does not appear to cause interference in FVIII chromogenic assays that utilize bovine FIXa and FX reagents (i.e. FVIII chromogenic assay, Siemens Healthcare)
- Chromogenic (bovine FIXa and FX) FVIII Bethesda assay should be used when measuring FVIII inhibitors in patients on Emicizumab[1,2,3]
- For measuring Emicizumab a chromogenic (human FIXa and FX) [1,2] or dilute one-stage assay with Emicizumab calibrator and controls can be used[4]

[1]HEMLIBRA FDA Prescribing Information, 11/2017; [2]MASAC Interim Guidance on Acute Bleed Management and Use of Laboratory Assays; November 24, 2017; [3]Collins et al. (2018) Haemophilia 24: pp 344-347; [4]Calatzis et al. (2018) Presented at 62nd Annual Meeting of the Society of Thrombosis and Haemostasis, Vienna Austria

# Summary

- With the recent approval of EHL rFVIII and rFIX replacement products changes in how clinical laboratories monitor hemophilia replacement therapy are indicated
- Several of the EHL rFVIII and rFIX replacement therapies demonstrate aPTT reagent dependent recovery
- Existing data to date suggest that FVIII CSA may be suitable for monitoring EHL rFVIII replacement therapy
- For EHL rFIX additional data are required

LabCorp

# Summary

- FVIII and FIX Gene Therapies
  - Insufficient data exist to determine whether OSA or CSA are more suitable for monitoring therapy
- Emicizumab Therapy
  - Traditional aPTT based FVIII OSA and Bethesda assays are not suitable for measuring hemostatic potential



LabCorp

Thank you