

## BACKGROUND

Diagnosis of hemophilia B and monitoring of coagulation factor concentrates (CFC) is based on the one-stage factor IX (FIX) assays (OSA). Selected extended half-life CFC requires a two-stage based chromogenic FIX assay (CSA). Availability of commercial FIX CSA kits is limited and while they may be CE marked, they have not undergone FDA 510k clearance. In addition, kit components are designed for high volume testing requiring the laboratory to reconstitute, aliquot, and freeze reagents in small batches. Here in we present our experience in evaluation of a CSA in comparison to our current laboratory developed test (LDT).

## METHOD

Instrument	Reference Kit	New Kit
<ul style="list-style-type: none"> <li>Werfen</li> <li>ACL TOP 700</li> <li>Chromogenic</li> <li>Read @ 405 nm</li> </ul>	<ul style="list-style-type: none"> <li>Rossix AB</li> <li>ROX FIX kit</li> <li>RUO – LDT</li> <li>Validated by Special Coag Lab</li> </ul>	<ul style="list-style-type: none"> <li>PrecisionBioLogic</li> <li>FIX CSA kit</li> <li>FDA Clearance Pending. Not yet available for sale in the US.</li> </ul>

### Method Comparison (n=25)

- Factor IX deficient plasmas – commercially available
- Assigned Reference Plasmas: ISTH #5 & Calibration Plasma
- Waste deidentified plasma samples
- Samples provided by PrecisionBioLogic

### Linearity (n=2)

- Pooled normal plasma diluted with Factor Diluent
- Pooled normal plasma diluted with Factor IX deficient plasma
- 7 different dilutions tested

### Stability (n=3)

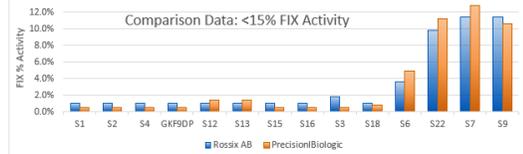
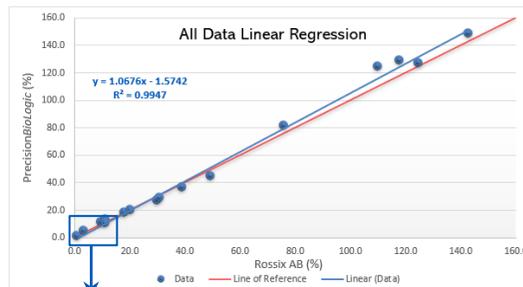
- 3 levels of control material
- Tested hourly for 8 hours (new controls thawed at 6 hours)

### Precision (n=3)

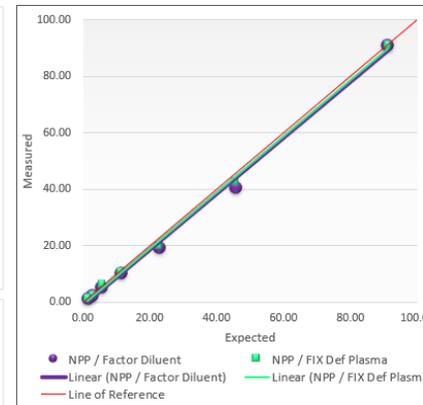
- 3 levels of control material
- Tested daily for 5 days

## RESULTS

### METHOD COMPARISON



### LINEARITY



FIX Activity Range = 1.2 – 91.0%  
 Average r2 = 0.997  
 Average slope = 0.998

### PRECISION

Mean %	SD	CV
11.5	0.83	7.2%
36.1	0.96	2.6%
117.6	2.63	2.2%

Performed over 5 days.

### STABILITY

Mean %	SD	CV
11.5	0.83	7.2%
36.1	0.96	2.6%
117.6	2.63	2.2%

Performed over 8 hours.

## KIT COMPARISON

Features	Rossix AB	PrecisionBioLogic
Calibration	<ul style="list-style-type: none"> <li>One Calibration; one math model</li> <li>AMR 5 – 200%</li> </ul>	<ul style="list-style-type: none"> <li>One Calibration; low and high math models</li> <li>AMR 2 – 200%</li> </ul>
Reagent Characteristics	<ul style="list-style-type: none"> <li>Lyophilized &amp; Liquid</li> <li>Large volumes; ~90 tests per vial set</li> <li>Requires aliquoting and freezing</li> </ul>	<ul style="list-style-type: none"> <li>Frozen liquid</li> <li>Small volumes; ~15 tests per vial set</li> </ul>
Reagent Stability	<ul style="list-style-type: none"> <li>8 hours once thawed</li> </ul>	<ul style="list-style-type: none"> <li>Minimum 8 hours</li> </ul>
Resulting Range	<ul style="list-style-type: none"> <li>1 – 200%</li> </ul>	<ul style="list-style-type: none"> <li>0.5 – 200%</li> </ul>
Analytical Time	<ul style="list-style-type: none"> <li>14 – 17 minutes</li> </ul>	<ul style="list-style-type: none"> <li>11.5 – 13 minutes</li> </ul>
FDA Clearance	<ul style="list-style-type: none"> <li>No; RUO</li> </ul>	<ul style="list-style-type: none"> <li>Pending; Not yet available for sale in the US.</li> </ul>

## CONCLUSIONS

- The two kits obtained comparable FIX activity results.
- Advantageous laboratory workflow with the PrecisionBioLogic kit.
- Shorter turn around time with the PrecisionBioLogic kit.
- Detection at 1% is acceptable; theoretical (due to dilution strategy) detection of 0.5% needs further evaluation.
- Overall, data demonstrates the PrecisionBioLogic kit can be used to provide reliable results with acceptable precision. Complete verification with multiple lot evaluation would need to be performed to fully assess acceptability.

## REFERENCES

- CRYOcheck™ Chromogenic Factor IX package insert. Precision Biologic Inc., Dartmouth, NS, Canada.
- ROX Factor IX package insert. Rossix AB, Mölndal, Sweden.
- Tange J, Sridharan M, Heikal N, Chen D, Pruthi R. Laboratory Verification of a Chromogenic Factor IX Assay Kit [abstract]. *Res Pract Thromb Haemost.* 2021; 5 (Suppl 2). <https://abstracts.isth.org/abstract/laboratory-verification-of-a-chromogenic-factor-ix-assay-kit/>.