ISTH VIRTUAL CONGRESS 2021 JULY 17–21 ISTH2021.ORG
HOSTED FROM PHILADELPHIA
SCIENCE CONQUERING DISEASE

Laboratory Verification of a Chromogenic Factor IX Assay Kit

J. TANGE, M. SRIDHARAN, N. HEIKAL, D. CHEN, R. PRUTHI
Division of Hematopathology, Department of Laboratory Medicine, Mayo Clinic, Rochester, MN

MAYO CLINIC

BACKGROUND

One-stage clotting FIX assays (OSA) are used for diagnosis of hemophilia B and monitoring clotting factor concentrates (CFC). Selected extended half-life CFC require chromogenic assay (CSA) for monitoring; none of which are US FDA approved. We present our in-house validation of a research use only CSA.

METHOD

REAGENTS/SUPPLIES

- ROX FIX kit – Rossix AB, Sweden
- Factor IX Deficient Plasma – Precision Biologic, Canada
- SynthASil – Instrumentation Laboratory, United States
- ACL TOP 700 – Instrumentation Laboratory, United States

TESTING PERFORMED – per CLSI guidelines

Accuracy (n=68)
- CFC's, ALPROLIX® and IDELVION®, spiked into FIX deficient plasma (n=32)
- Proficiency testing samples procured from ECAT (n=3)
- Waste de-identified patient plasma samples (n=33)

Precision (n=3)
- Intra; 20 replicates performed in one day
- Inter; 2 replicates performed twice daily for 10 days

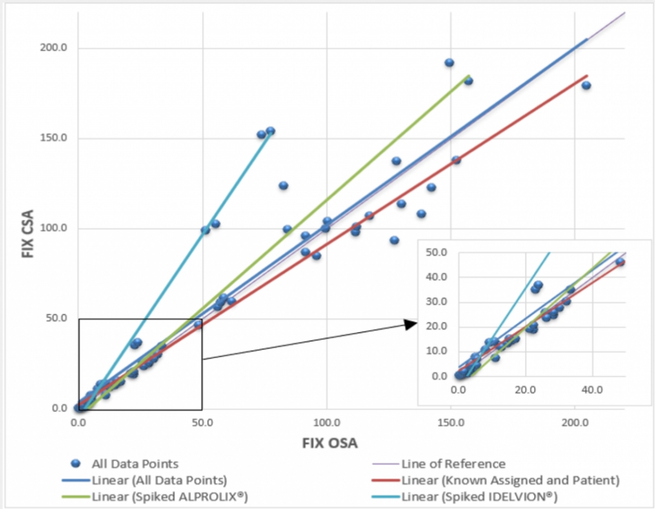
Linearity (n=4)
- One commercial normal pool sample
- 3 de-identified individual patient samples with elevated FIX OSA
- Minimum of 10 different dilutions with diluent buffer
- Tested in triplicate

Reference Range (n=120)
- Verified current one-stage factor IX range

Analytical Sensitivity (n=3)
- Diluent Buffer
- Factor IX Deficient Plasma
- Control Material diluted to ~1%
- 20 replicates per day for 3 days
- Tested on two different lots of ROX FIX kit

RESULTS

ACCURACY



	All Data	Known/Patient	ALPROLIX®	IDELVION®
R-squared (r^2)	0.874	0.958	0.990	0.995
Slope	0.984	0.893	1.205	2.035
Average % Difference	3.5%	-8.1%	-6.6%	46.3%

PRECISION

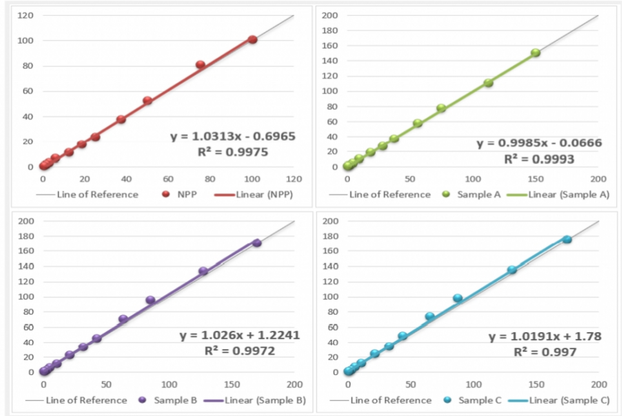
INTRA	Mean (%)	SD	CV
Normal Pooled Plasma	105.4	3.50	3.3%
Low Abnormal	37.5	1.05	2.8%
Very Low Abnormal	10.9	0.35	3.2%

INTER	Mean (%)	SD	CV
Normal Pooled Plasma	107.2	4.19	3.3%
Low Abnormal	37.6	1.76	4.7%
Very Low Abnormal	9.8	0.59	6.0%

REFERENCE RANGE

Mean (%)	SD	Min	Max	Count
104.7	18.1	62.7	153.7	120
Verified Range:		65 – 140%		

LINEARITY / REPORTABLE RANGE 1 – 200%



ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)

Lot #1	Mean (%)	SD	CV
Factor Diluent	<1.0	0.00	0.0%
FIX Deficient Plasma	<1.0	0.00	0.0%
Diluted Control	1.1	0.05	4.3%

Lot #2	Mean (%)	SD	CV
Factor Diluent	<1.0	0.00	0.0%
FIX Deficient Plasma	<1.0	0.00	0.0%
Diluted Control	1.1	0.06	5.4%

CONCLUSIONS

Our CSA kit validation efforts met acceptance criteria for non-spiked patient samples. Data demonstrates the CSA assay can be optimized to provide reliable FIX activity estimates for baseline and ALPROLIX® with an acceptable CV and lower limit of detection of 1% activity. Additional studies are needed to further understand differences between the OSA and CSA when IDELVION® is present.

CONTACT INFORMATION

Tange.Julie@mayo.edu
(507) 284-3138

REFERENCES

CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition*. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.

CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition*. CLSI document EP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* CLSI document EP06-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2003.