Validation of A New Kit for the Determination of Factor IX Activity

Poster 418

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Background

Factor IX (FIX) activity assay methodology falls into two primary categories: one-stage clot-based assays using an activated partial thromboplastin time (APTT) and chromogenic activity assays.

Unlike APTT-based assays, the chromogenic method offers the advantage of being less prone to interference from lipids or traces of heparin in samples and pre-activation of FVIII during sample collection.

There are a limited number of commercially available chromogenic FIX kits and there is a need for a fully validated FDA-cleared chromogenic FIX assay.

Objective

To develop and validate a new chromogenic assay for the quantitative determination of FIX activity in citrated plasma samples. The kit consists of bovine/human based frozen reagents packaged into a multiple vial set configuration.

Methods

All studies were performed according to applicable CLSI guidelines on IL ACL TOP series instruments.

Precision was determined through a 20-day x 2 run x 2 replicate study measuring six plasma samples with three reagent lots.

The linearity of the assay was evaluated by measuring four replicates of fourteen sample dilutions in the range of 0 to 230% FIX.

The limits of detection and quantification of the assay were determined by quantifying plasma samples from eight donors with severe congenital hemophilia B in a three replicate x five-day study design.

Three hundred and fifty human plasma samples from normal, ostensibly healthy individuals and from patients with congenital or acquired hemophilia B were distributed across three sites and tested for FIX activity using our Chromogenic Factor IX assay and a ROX Factor IX validated laboratory developed test (LDT).

Results

The assay total precision was <10% CV for the four plasma samples with a FIX activity ≥10% and a standard deviation of <1% FIX for the two low plasma samples with a FIX activity of <10% FIX **(Figure 1 and Table 1)**.

The linearity of the assay was 0 to 230% FIX with a 0.5% FIX limit of detection and a 0.5% FIX limit of quantification **(Figure 2)**.

The chromogenic FIX and ROX FIX test results were similar with a correlation (r^2) of >0.98, a slope of 1.1 and an intercept of 0.1 **(Figure 3 and Table 2)**.

Predicted biases were very low (<1%) at medical decision levels for hemophilia B of 1 and 5% FIX activity, <6% at 50% FIX activity and 11% at 100% FIX activity **(Figure 4 and Table 3)**.

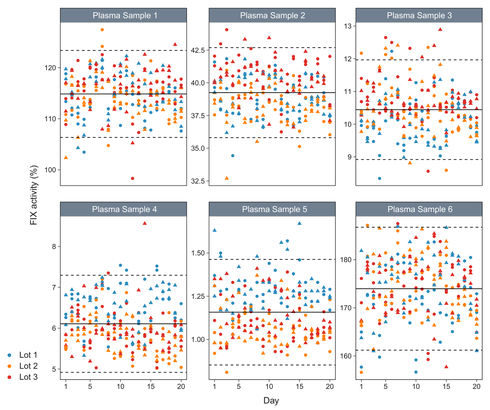
Conclusions

Our findings suggest that our chromogenic FIX assay performs comparably to a clinical LDT chromogenic FIX assay for the quantification of FIX in plasma samples while providing a wide measurement range and low detection and quantification limits, and a frozen format that expedites reagent preparation.

(Figure 1)

Precision Measurements Across Three Lots

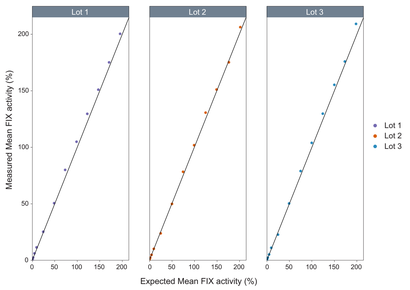
Six plasma samples were measured over 20 days, two runs per day (circle or triangle) and two replicates per run using three reagent lots of Chromogenic Factor IX – Lot 1 (blue), Lot 2 (orange) and Lot 3 (red). The mean measured value (solid black line) and plus/minus two standard deviations (dashed lines) are shown.



(Figure 2)

Linearity Across Three Lots

The mean expected versus measured FIX activity (%) across the range of 0–230% showing regression analysis of the linear fit.



(Table 1)

Overall Precision of cryo*check*™ Chromogenic Factor IX

The mean FIX activity (%), the within-run, between-run, between-day, between-lot, and total within-laboratory precision (SD, %CV) are shown.

Sample	Mean FIX (%)	Within-Run		Between-Run		Between-Day		Between-Lot		Total Within-Lab	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	114.9	3.7	3.2	0.0	0.0	1.5	1.3	1.3	1.1	4.2	3.7
ARP1	39.3	1.3	3.3	0.3	0.8	0.5	1.3	1.1	2.7	1.8	4.5
ARP2	10.4	0.6	6.1	0.2	1.5	0.2	2.0	0.3	3.3	0.8	7.3
PM1	1.2	0.1	NA	0.0	NA	0.0	NA	0.1	NA	0.2	NA
PM2	6.1	0.4	NA	0.1	NA	0.2	NA	0.4	NA	0.6	NA
PM3	174.0	5.4	3.1	3.3	1.9	0.8	0.5	0.4	0.2	6.4	3.7

(Table 2)

Passing-Bablok Regression Analysis

The number of samples (N), the slope, intercept and Pearson correlation coefficient are shown.

N	Slope	Intercept	Pearson Correlation Coefficient
350	1.11	0.10	0.992 (r^2=0.984)

(Table 3)

Bias at Medical Decision Levels

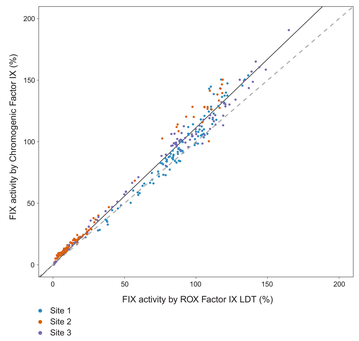
The absolute predicted bias (%) at FIX activity levels of 1, 5, 50, and 100%.

FIX activity (%)	Predicted Absolute Bias (%)
1	0.21
5	0.66
50	5.76
100	11.43

(Figure 3)

Scatter-plot of FIX Activity by Comparator Versus Investigational Methods

Scatter plot of FIX activity (%) by ROX FIX LDT (x axis) versus Chromogenic Factor IX (y axis) measured at three different sites. The solid line indicates the line of best fit by Passing-Bablok regression and the dashed line indicates the line of identity (x=y).



(Figure 4)

Bland-Altman Analysis

Bland-Altman plot of the difference versus average in FIX activity (%) between the two methods. The bolded black line represents the mean bias, the black dashed lines indicate the 95% confidence interval, and the red dashed line indicates the identity line (y=0).