CRYO*check*™ Chromogenic Factor VIII: A New Kit for the Determination of FVIII:C Activity

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Background

FVIII clotting activity (FVIII:C) assay methodology falls into two categories: one-stage clot-based (OSC) assays based on activated partial thromboplastin time (aPTT) and chromogenic substrate (CS) assays. Unlike aPTT-based assays, the CS assay offers the advantage of being less prone to impact from common interferents in samples.

Historically, in-market chromogenic FVIII kits have had limitations including a poor limit of detection, undesired measurement range, incomplete interference screening and limited product stability.

Objective

Our objective was to characterize a new chromogenic assay for the quantitative determination of FVIII:C activity in citrated plasma samples. CRYO*check* Chromogenic Factor VIII (Precision BioLogic, Dartmouth, Canada) consists of bovine/human-based frozen reagents ready for use. All studies were performed according to applicable CLSI guidelines on IL ACL TOP® series instruments.

Methods

The linearity of the assay was determined by measuring four replicates of 15 sample dilutions in the range of 0 to 260% FVIII.

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

Interference studies were conducted by measuring replicates (N = 10) of plasma samples representing two levels of FVIII activity spiked with potential interferents, compared with matrix blank controls.

The performance of CRYO*check* Chromogenic Factor VIII was assessed relative to Coatest SP FVIII (Chromogenix / Instrumentation Laboratory, Bedford USA), in a **method comparison** study by testing 300+ human plasma samples from ostensibly healthy individuals (normal) and from patients with congenital or acquired hemophilia A (HA).

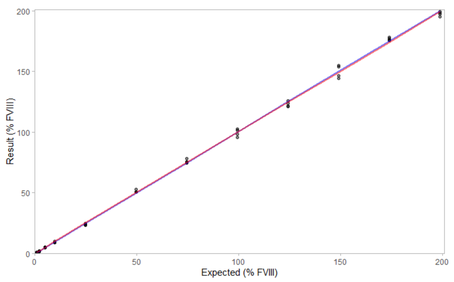
On-board and refrozen stability were assessed by measuring the FVIII activity of six plasma samples when freshly thawed and after refreezing reagents at ≤ -70 °C for up to 2 months.

Conclusions

Our findings suggest that CRYO*check* Chromogenic Factor VIII performs comparably to another in-market chromogenic FVIII assay for the quantification of FVIII:C activity in plasma samples while providing superior characteristics such as a wide measurement range, excellent detection and quantification limits and a robust interference characterization with refreezing capabilities to limit reagent waste.

Results

The linearity of the assay was determined to be 0 to 200% FVIII with a limit of detection and quantification of 0.5% FVIII.

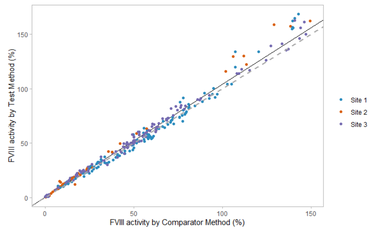


Expected versus resulting FVIII activity (%) of sample dilutions spanning the measurement range. The linear fit from regression analysis is shown in blue and the best fitting polynomial is shown in red.

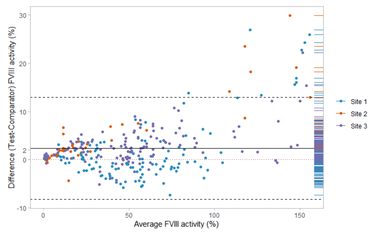
The quantification of FVIII activity using CRYO*check* Chromogenic Factor VIII was not affected by bilirubin (up to 29 mg/dL), hemoglobin (up to 500 mg/dL), intralipid (up to 500 mg/dL), fondaparinux (1.25 mg/L), low molecular weight heparin (up to 2.0 IU/mL), unfractionated heparin (up to 2.0 IU/mL), lupus anticoagulant (up to a 1.8 dRVVT ratio), and vWF (up to 20 µg/mL).

Measurements of Normal FVIII plasma are shown in orange and measurements of FVIII deficient plasma in red. The colored dashed lines represent the limits of acceptable interference.

CRYO*check* Chromogenic Factor VIII and Coatest SP FVIII test results were similar with a correlation (r^2) of > 0.99, a slope of 1.0 and an intercept of 0.4. Predicted biases were very low (≤ 1%) at HA medical decision levels of 1% and 5% FVIII activity and low (< 5%) at ≤ 50% FVIII levels.

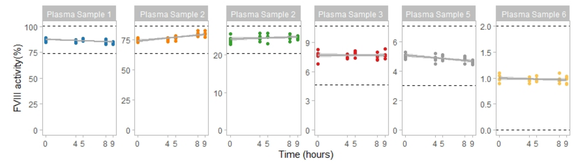


Scatter plot of FVIII activity (%) by comparator method testing (x axis) versus the investigational method (y axis) measured at three different sites. The solid line indicates the line of best fit by Passing-Bablok regression and the dashed line indicates the line of identity (x = y).

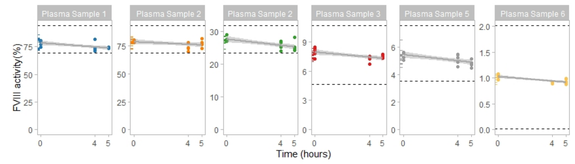


Bland-Altman plot of the difference versus average in FVIII activity (%) between the two methods. The bolded black line represents the mean bias, the black dashed lines indicate the 95% confidence interval and the red dashed line indicates the identity line (y = 0).

CRYO*check* Chromogenic Factor VIII reagents demonstrated 8 hour on-board stability and acceptable re-frozen stability at ≤ -70 °C for up to one month if refrozen within four hours of use.



The on-board stability of reagents was tested by repeated measurement of six plasma samples. The kit produced consistent results up to and beyond eight hours.



The refrozen stability of reagents was tested by repeated measurement of six plasma samples. Reagents were left on board for four hours, then refrozen at ≤ -70 °C for one month. The kit produced consistent results up to and beyond four hours.