Independent Evaluation of a New Chromogenic Factor VIII Assay Kit

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ABSTRACT

BACKGROUND
Bovine reagent based chromogenic Factor VIII (chFVIII) is based on two-stage assay used for FVIII measurement of selected extended half-life (EHL) FVIII concentrates, inhibitors in hemophilia A patients on emicizumab, and to avoid interferences in the clot-based assay. The available commercial chFVIII kits have the following limitations: 1) lack of an FDA approval on the existing instrumentation, 2) kit components are designed for high volume of testing (~200 tests) over a short stability life, which is undesirable for the laboratories that perform <10 tests in the same time span, 3) reagent compositions (human vs. bovine) vary and can affect results, 4) problematic reagent reconstitution, and 5) long assay time (longer than 30 minutes) for low sample results. To identify a good kit that can address most of the shortcomings of chFVIII assay, we compared a commercial kit with one recently receiving FDA clearance.

METHODS
A chFVIII kit (Precision BioLogic, Dartmouth, NS, CAN) was evaluated for assay characteristics and compared to the chFVIII kit (Chromogenix, Instrumentation Laboratory (IL), Bedford, MA, USA); both assays were performed on the ACL TOP family of instruments (IL, Bedford, MA, USA) and contain bovine reagents. Evaluation studies performed include: method comparisons, linearity, kit stability and analytical sensitivity. Method comparison studies were performed on commercially available factor deficient plasmas, calibrators, proficiency testing samples, purchased rFVIII EHL products (BAX 855, rFVIII-Fc fusion, rFVIII-single chain, rFVIII-HSP 70, and human cl-rhFVIII) diluted and spiked in FVIII deficient plasma (F8DP) and waste de-identified patient samples (including four known emicizumab patients). Linearity was performed on one commercial normal pool sample and one de-identified individual patient sample with elevated chFVIII (in duplicate at a minimum of nine different dilutions with F8DP). Kit stability was evaluated over two days with both fresh and refrozen reagent by performing three levels of quality control (QC) every two hours for eight hours. Analytical sensitivity (limit of detection) was carried out on F8DP and a control material diluted to approximately 1%; each assayed 20 times.

RESULTS
Anticipated reportable range was 1 – 200%. Results of method comparison (Figure 1): All Data (r^2=0.984, slope=1.07, avg % diff=6.0%); Known Assigned and Patient Samples (r^2=0.982, slope=1.06, avg % diff= 8.2%); EHL Spiked Samples (r^2=0.988, slope=1.08, avg % diff=3.1%); emicizumab patients all reported <1%. Linearity average r^2 was 0.997 and average slope was 0.99 for all samples with results ranging from 0.4 – 232.87%. Analytical sensitivity reliably distinguished results down to 1% (coefficient variation [CV] of 4.0%). Kit stability results indicated the reagents were reliable for 8 hours as well as after one additional freeze/thaw cycle.

CONCLUSIONS
The two chFVIII kits are comparable. Ease of use and stability are far superior with the Precision BioLogic kit, however cost effectiveness has not yet been evaluated. Overall, the data demonstrates the Precision BioLogic chFVIII assay can be used to provide reliable FVIII activity estimates for EHL products and emicizumab as well as patient samples with an acceptable CV and lower limit of detection of 1% activity.

KIT BACKGROUND

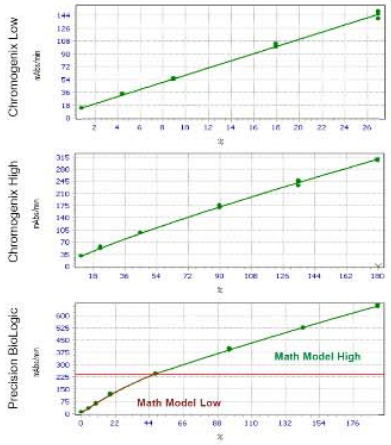
Background Information – Chromogenix

- Combination of lyophilized and liquid reagents.
- Reagent contains bovine factors IXa and X.
- Clinical use requires either batching of samples (~200 tests) or reconstitution of reagents and freezing as aliquots; with an additional reagent dilution prior to testing (~15 tests per aliquot).
- FDA clearance obtained with manual and ACL 9000 chromogenic methodology.
- Calibration (Figure 1) on TOP optimized by completing two individual calibrations with separate sample dilutions and incubation times; 12.5 – 200% (high) and 1 – 30% (low).
- Minimum assay times are 12 and 17 minutes for the high and low calibrations, respectively.

Background Information – Precision BioLogic

- All liquid reagents in frozen format.
- Reagents contain combination of bovine factor X and human factors IXa and IIa.
- Reagents are ready for use after thawing.
- FDA approved on the ACL TOP Family of instruments.
- Single calibration (Figure 1) on TOP 0 – 200%.
- Minimum assay time is 7 minutes.

FIGURE 1: Example Calibrations



METHODS

The Chromogenix kit was previously assessed and validated within the Mayo Clinic Special Coagulation Laboratory; with discussion in a previous abstract.

The Precision BioLogic kit and protocol was provided by the company.

All method comparison patient samples were de-identified waste samples thawed in a 37˚C waterbath, mixed and tested for both assays.

Linearity samples were comprised of a high patient and a normal pool (NPP) serially diluted with factor VIII deficient plasma.

Analytical Sensitivity was performed by diluting NPP with FVIII deficient plasma down to 1%.

RESULTS

- **Resulting Range** for both assays: 1 – 200%
- **Method Comparison** (Accuracy) (Figure 2)
  - All Data
    - R^2 = 0.984
    - Slope = 1.07
    - Average % Difference = 6.0%
  - Known Assigned and Patient Samples
    - R^2 = 0.982
    - Slope = 1.06
    - Average % Difference = 8.2%
  - Extended Half-Life Spiked Samples
    - R^2 = 0.988
    - Slope = 1.08
    - Average % Difference = 3.1%
  - Emicizumab Patients all reported <1% FVIII activity
- **Linearity** (Figure 3) Precision BioLogic
- **Imprecision Studies** Precision BioLogic and Chromogenix
  - Level 1 ($\bar{x}$ = 61.8 / 95.0): CV% = 4.5% and 3.6%
  - Level 2 ($\bar{x}$ = 18.2 / 28.1): CV% = 5.5% and 3.3%
  - Level 3 ($\bar{x}$ = 5.8 / 8.0): CV% = 8.2% and 10.5%
- **Analytical Sensitivity / Quantitative Limit of Detection**
  - Normal Pool diluted with FVIII deficient plasma to 1%
    - CV% = 14% Chromogenix
    - CV% = 4% Precision BioLogic
- **Kit Stability**
  - Chromogenix: 4 hours on instrument
  - Precision BioLogic: 8 hours on the instrument + one freeze/thaw

FIGURE 2: Method Comparison

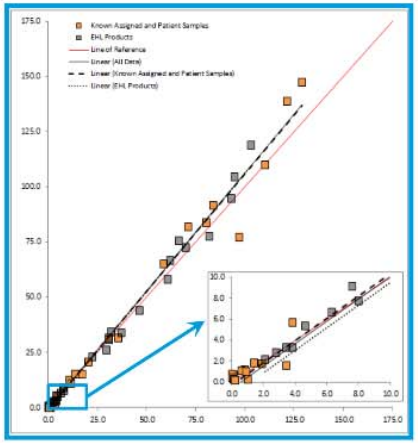
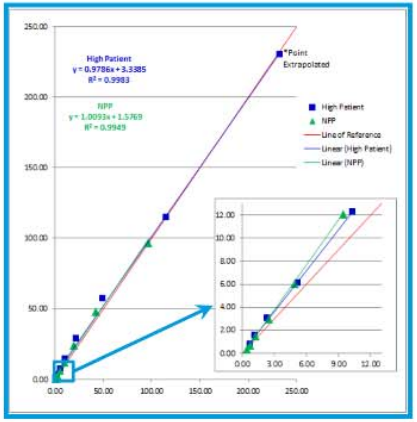


FIGURE 3: Linearity



KIT COMPARISON

Descriptive	Chromogenix	Precision BioLogic
Calibration	2 individual calibrations	1 calibration with two math models
Reagents	Lyophilized & Liquid in large volumes (~200 results) requiring reconstitution , aliquoting and freezing	Frozen liquid in volumes that allow ~15 results
Reagent Stability	4 hours once thawed	8 hours once thawed plus one freeze/thaw
Resulting Range	1 – 200%	1 – 200%
Analytical Time to Result	12 – 17 minutes	7 minutes
Accurate Reporting w/ emicizumab	Yes	Yes
FDA Clearance	Manual and ACL 9000	ACL TOP Family

CONCLUSIONS

- Chromogenic FVIII results are comparable at both the lower (1%) and higher (200%) ranges.
- Precision BioLogic's reagent stability is more amenable to random access/all day testing.
- Precision BioLogic's test setup and analytical time facilitates a short turn around time.

REFERENCES

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