Novel Fluorometric Assay for Pediatric Anti-Factor Xa Testing: Minimizing Bilirubin and Hemolysis Interference in Whole Blood

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BACKGROUND

- High bilirubin levels, common in pediatric patients and those on extracorporeal membrane oxygenation (ECMO), interfere with chromogenic assays for anti-Factor Xa (aFXa). Hemoglobin, platelet activation and platelet factor 4 (PF4) are also known interferents for aFXa assays.
- We are developing a fluorescence assay for aFXa activity on our point-of-care digital microfluidic (DMF) platform using low volume whole blood samples (<50 µL) with integrated plasma separation.
- Frequent monitoring of aFXa activity is essential in acute anticoagulation therapy, but frequent blood draws, particularly in pediatric and neonatal patients, may cause iatrogenic anemia.

METHODS

- The aFXa assay is performed by first separating whole blood into plasma on the DMF cartridge followed by incubation with exogenous FXa and a fluorogenic substrate. The resulting fluorescence is inversely proportional to the concentration of heparin in the sample.
- All required reagents for plasma separation and the aFXa assay are dried within the cartridge, allowing for fully automated performance.
- Interference of the aFXa assay was evaluated by testing whole blood samples (BioIVT, NY) with various concentrations of unconjugated bilirubin (0-40 mg/dL) as well as hemolysate (0-1,000 mg/dL). All experiments were performed with 6 replicates of each concentration.
- For PF4, immunoassays were performed on platelet poor plasma (BioIVT, NY), plasma obtained through centrifugation, and agglutination-separated plasma generated directly on the DMF cartridge.
- For platelet counting, imaging was performed on a microscope with a fluorescent dye (calcein) on whole blood, centrifuged plasma, and plasma generated on cartridge, and the data are reported as absolute counts.

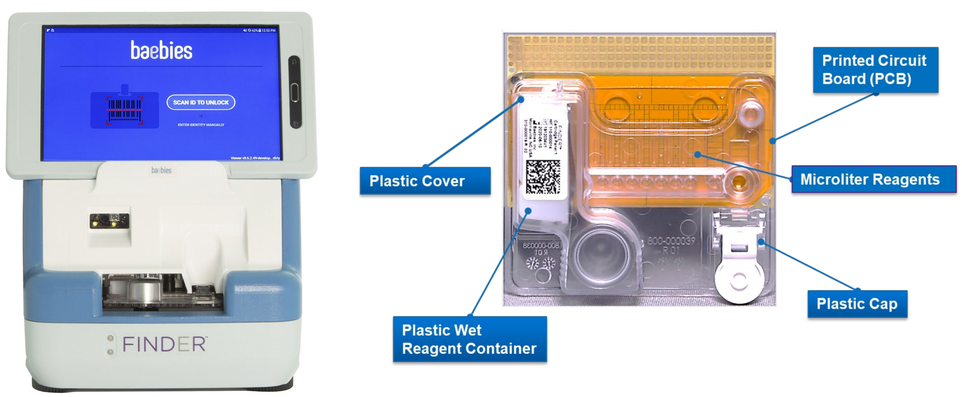

Figure 1. Baebies' point-of-care platform for aFXa testing.

RESULTS

- Bias in the mean aFXa activity was measured to be < 0.05 IU/mL with bilirubin up to 26.67 mg/dL and for hemolysate up to 1,000 mg/dL compared to their respective untreated control values (Table 1).

Table 1: aFXa activity measured over different concentrations of bilirubin and hemolysate

Unconjugated Bilirubin (mg/dL)	Unfractionated Heparin (IU/mL)	Hemolysate (mg/dL)	Unfractionated Heparin (IU/mL)
0	0.38	0	0.64
13.33	0.35	200	0.59
26.67	0.36	500	0.59
40	0.27	1,000	0.64

- The concentration of PF4 in plasma generated on the cartridge is less than that of platelet poor plasma and centrifuged plasma, indicating that the method of generating plasma on the cartridge method does not activate platelets (Table 2).
- Plasma stained with calcein showed that platelet or white blood cell counts between centrifuged plasma and on-cartridge generated plasma (Table 3) are similar; whole blood served as a positive control. The values in Table 3 are an average of 3 experiments.

Table 2: PF4 concentration across methods of plasma preparation

Sample	Concentration (mg/mL)
Platelet poor plasma	0.17
Centrifuged plasma	0.21
On-cartridge generated plasma	0.12

Table 3: Platelet and white blood cell count visualized with calcein fluorescence

Sample	Platelet Count	White Blood Cell Count
Whole blood	609	22
Centrifuged plasma	12	4
On-cartridge generated plasma	6	5

CONCLUSIONS

- **Interference results suggest that our aFXa assay is tolerant to high bilirubin and hemolysate levels.**
- **We also demonstrated that separation of plasma on cartridge is equivalent to platelet poor plasma as measured through PF4 levels and platelet counts.**
- **Further clinical studies are underway to establish clinical performance for a point-of-care aFXa assay.**

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This assay is not available at this time for sale or use in any territory