Evaluation of the Cy-Quant VASP/P2Y12 ELISA Kit for Sample Stability and Batch Processing

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The platelet P2Y12 receptor is involved in ADP mediated platelet activation. A variety of methods and assays are available to measure P2Y12 function, such as the VerifyNow® (Instrumentation Laboratory), light transmission aggregometry (LTA), and the Cy-Quant VASP/P2Y12 ELISA (Stago). The purpose of this study was to evaluate suitability of batch processing of samples and to determine effects of sample stability for the Cy-Quant VASP/P2Y12 ELISA method. The manufacturer's protocol for Cy-Quant VASP/P2Y12 ELISA requires whole blood samples to be processed within 24 hrs of blood collection which limits batch processing of multiple samples received over time. Therefore, a modification to this protocol was made in which whole blood after stimulation with PGE1 or PGE1+ADP was lysed, and samples were snap frozen for batch analysis. Whole blood was collected from 4 healthy donors in 3.2% sodium citrate anticoagulant (0hr). Stability of blood sample at room temperature was assessed by testing the whole blood according to the manufacturer's instructions at 2hr, 24hr, 32hr, and 48hr time points. A sample of whole blood from each donor was also used to prepare frozen lysates at 0h, and thawed lysate samples were tested at 48h. A sample of the whole blood was treated with ticagrelor (25 µg/mL) for 30 minutes prior to stimulation with PGE1 or PGE1+ADP as an internal control for the assay. Ticagrelor treated samples were tested once per donor, while all other samples were tested in triplicate. The platelet reactivity index (PRI) values obtained at 2h, 24h, 32h, and 48h were 97.4 ± 5.1%, 92.7 ± 4.0%, 89.7 ± 2.1%, and 91.4 ± 4.1%, respectively. Frozen lysates prepared at 0h and assayed 48h later resulted in a PRI of 92.7 ± 2.2% and were not statistically significant ($p>0.05$) compared to PRI values with freshly processed samples. Results from this evaluation study suggest whole blood samples can be collected for VASP analysis and are stable for up to 48h at room temperature when assayed according to the manufacturer's instructions. Alternatively, samples can also be processed for the preparation of lysates that can be frozen for subsequent steps of the ELISA. This alternative method allows for batch processing of samples, providing a logistical means to analyze samples from for a clinical trial. This approach will also minimize inter assay variability when testing patient samples over extended periods of time, and significantly lowers material and labor costs associated with VASP-ELISA testing.