Poster 25

Dose Response Curve Validation of a POCT Test to Measure the Effect of Antiplatelet Drugs: An Alternative Approach to Light Transmission Aggregation Validation

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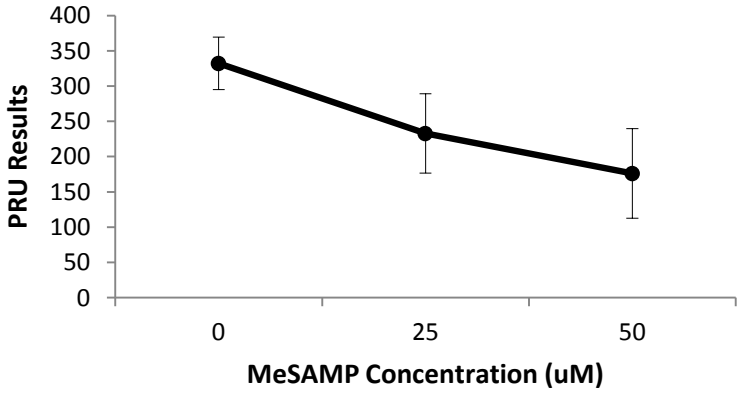
Background: The VerifyNow PRUTest is a point-of-care assay to determine response to anti-platelet medications, such as clopidogrel (Plavix), which target the platelet P2Y12 receptor. The effect of these medications is clinically relevant to patient outcomes, optimal timing of surgical procedures, and utilization of platelet-based blood products. **T**he VerifyNow PRUTest measures the specific activity of the P2Y12 receptor using the platelet agonist ADP. Clopidogrel is a prodrug which must be processed in-vivo in order for the active metabolite to be produced, and therefore cannot be added to blood samples to validate or verify a range of assay response. Moreover, P2Y12 inhibiting drugs are given to higher risk patients following stenting procedures, and it is often difficult to obtain blood samples after dosing from this population for validation activities. For such validation, the manufacturer recommends that accuracy of the PRUTest be determined by running 20 replicates of two levels of quality control samples with assigned values. While light transmission aggregation (LTA) is considered the "gold standard" procedure for comparison, it is not readily available in most clinical situations for routine comparison. Thus we developed an alternative procedure for laboratories to use for whole blood verification of the PRUTest and ascertain the sensitivity of the assay to platelet function

Method: Whole blood samples were obtained from normal volunteers at sites performing validation studies on the VerifyNow PRU Test (formerly P2Y12 test) from January through December 2011. Volunteers were questioned and self-reported any use of an anti-platelet agent within 10 days prior to sample collection, and any volunteer who reported such exposure was excluded from the validation or verification activities. Each sample was collected according to individual institution policies and practices, and no donor identifiers were recorded. Blood used for testing was collected into 3.2 percent sodium citrate using a 21 gauge needle following blood collection into a sodium citrate or no-additive discard tube. Samples were allowed to equilibrate at room temperature for 10 minutes, and all tests were completed within 4 hours. Immediately prior to assay, 20 microliters of 2-methylthio-AMP (2-MeSAMP), a known P2Y12-specific inhibitor, was added in-vitro to a 2 mL aliquot of blood from each donor, resulting in final concentrations of 0, 25 and 50 uM. The samples were then tested with the VerifyNow PRUTest.

Results: Donor results from 21 reagent lots and 21 different instruments were analyzed for an observed dose response. The mean and standard deviation of the PRU results are shown in the figure below.



Conclusion: The 2-MeSAMP procedure is an acceptable approach for a laboratory to perform an optional whole blood validation or verification of dose response for the VerifyNow PRUTest. The dose response characteristic provides the means to assess the effect of therapeutics anti platelet agents.