Point-of-Care Platelet Function Testing with the VerifyNow® and Plateletworks™ Platforms

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VerifyNow
The VerifyNow® System (Accumetrics, San Diego, CA, USA) performs a turbidimetric-based optical detection of induced platelet aggregation in which platelet function (or the level of inhibition of function) is determined from the ability of activated platelets to bind fibrinogen. Whole blood is added to a selected cartridge which contains fibrinogen-coated beads and a specific agonist. The cartridge selected depends on what antiplatelet agent the patient is receiving. During the performance of the assay, the agonist will either activate unblocked platelet receptors or provide a stimulus otherwise inhibited by the antiplatelet agent, resulting in aggregation of functional (non-inhibited) platelets. Clumping of functional platelets will increase the amount of light transmittance through the sample, and from this, the amount of fibrinogen binding by the activated platelets is determined. The level of platelet inhibition is reported based on the amount of apparent fibrinogen binding in the assay.

Three different assays are currently available on this platform to allow assessment of inhibited platelet function due to commonly used anti-platelet agents (aspirin, clopidogrel and IIb/IIIa antagonists). Each type of assay cartridge contains a different agonist which allows assessment of platelet function by a defined pathway.

- Aspirin inhibits the platelet cyclo-oxygenase pathway which generates thromboxane A2. The “aspirin cartridge” thus contains the agonist arachidonic acid, which allows platelet activation and fibrinogen binding via the inhibited thromboxane A2 pathway. Test results are reported in Aspirin Reaction Units (ARU) which indicate the amount of thromboxane A2-mediated activation of GP IIb/IIIa receptors involved in platelet aggregation. ARU is calculated as a function of the rate and extent of platelet aggregation. Expected values are somewhere in the range of 350-700 ARU. 350-549 ARU is the therapeutic range for platelet function (aspirin is effective). 550-700 ARU is the NON-therapeutic range for platelet function (patient is either a non-responder or the effects of aspirin have dissipated). The blood sample must incubate for at least 30 minutes in the agonist tube at room temperature prior to running this assay and the sample should be tested within 4 hours of collection. The aspirin assay itself takes approximately 5 minutes to run. Substances known to interfere with the VerifyNow aspirin assay include: GPIIb/IIIa inhibitors, dipyridamole, clopidogrel, non-steroidal anti-inflammatory drugs (NSAIDS)
which inhibit COX-1 and/or COX-1, COX-2 enzymes (ibuprofen, naproxen, diclofenac, indomethacin, and piroxicam).

- Thienopyridines (e.g., clopidogrel, ticlopidine) inhibit platelet function by binding to the P2Y12 ADP-receptor on the platelet surface. The P2Y12 cartridge (or “clopidogrel cartridge”) thus contains the agonist ADP and also PGE-1 (which suppresses intracellular free calcium levels to reduce the non-specific contribution of the ADP binding to P2Y1 receptors. Concominant signaling from both the P2Y1 and the P2Y12 receptors is required for platelet activation due to ADP in vivo). In a separate chamber, iso-TRAP (a modified thrombin receptor activating peptide) is used as the agonist, and a baseline value (BASE) of platelet function is obtained. Platelet aggregation by iso-TRAP occurs independently of P2Y12 receptors. Results are expressed as P2Y12 Reaction Units (PRU), BASE and percentage of inhibition (% INHIB; which is calculated as [1 – PRU/BASE _ 100). The blood sample must incubate for at least 10 minutes in the agonist tube at room temperature prior to running this test and the sample should be tested within 4 hours of collection. The P2Y12 assay itself takes approximately 3 minutes to run. GP IIb/IIIa antagonists and the drug cilostazol (a phosphodiesterase III inhibitor used to treat intermittent claudication) will interfere with the VerifyNow P2Y12 assay, but the presence of aspirin is not a problem.

- GP IIb/IIIa antagonists (e.g., abciximab, eptifibatide, tirofiban) bind to the IIb/IIIa receptor which is then unable to bind fibrinogen. The “IIb/IIIa cartridge” contains iso-TRAP as the agonist, which activates fibrinogen binding by any unblocked IIb/IIIa receptors. Results are reported in Platelet Aggregation Units (PAU), which are calculated as a function of the rate and extent of aggregation. A percent inhibition for a given patient can also be determined if a baseline PAU value was obtained prior to administration of the GP IIb/IIIa inhibitor. The GP IIb/IIIa assay can be run immediately once the blood sample is introduced into the agonist tube (no incubation period is required) but the sample must be tested within 15 minutes of collection. Each GP IIb/IIIa assay takes approximately 2 minutes to run. The presence of aspirin and/or heparin do not affect the results of the VerifyNow GP IIb/IIIa assay.

In general, “low” hematocrit, “low” fibrinogen concentration and “low” platelet count will interfere with the accuracy of the VerifyNow assays. Specific limiting values vary by assay, but are all in ranges that would conventionally be considered “low”. The VerifyNow system is not intended for use in patients with inherited platelet disorders such as von Willebrand Factor Deficiency, Glanzmann Thrombasthenia and Bernard-Soulier Syndrome.

Plateletworks
Plateletworks™ (Helena Laboratories, Beaumont, TX, USA) provides a standard complete blood count (CBC) with platelet count plus information about platelet aggregation (or inhibition, depending on your perspective). Aggregation testing is
based on the performance of a platelet count before and after intentional platelet activation, using either collagen, ADP or arachidonic acid as the agonist. No special sample preparation is required. A 1 ml whole blood sample is added to both a Baseline and an Agonist Reagent Tube. Both tubes are run on a standard impedance cell counter (e.g., the ICHOR or ICHOR II analyzer). CBC and aggregation results are available in approximately 2-3 minutes. Testing can be performed at the point of care with excellent correlation to traditional platelet rich plasma aggregation assays.

In order to determine the percent platelet function (or the percent inhibition of function), a baseline platelet count (BASE) is performed on a 1 ml sample of whole blood in a tube containing EDTA. A second platelet count is then performed in a tube containing a specific agonist (AGONIST). The agonist will stimulate aggregation of functional platelets in the sample. The more functional platelets there are, the more aggregation there will be, and the lower the platelet count will be in the agonist tube. Non-functional (inhibited) platelets will not aggregate in response to agonist. The more non-functional platelets there are, the higher the platelet count will be in the agonist tube.

The percentage of function is given by the formula:

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\frac{\text{[(BASE count - AGONIST count) / BASE count]}}{\times 100}
\]

The percentage of inhibition is given by the formula:

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\frac{\text{[AGONIST count / BASE count]}}{\times 100}
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Significant thrombocytopenia (platelet count < 27,000 x 10^3/L) is a known limitation of the accuracy of this platform, but in this case, platelet function alone is not likely to be the major concern. Additional factors that might artifactually increase or decrease the reported counts should also be considered. According to the manufacturer, platelet counts might be apparently increased by the presence of microcytes, schizocytes, and WBC fragments, by the presence of significant hemolysis, and by the presence of RBC inclusion bodies. As well, platelet counts might be apparently decreased by the presence of agglutinated erythrocytes and giant platelets, following chemotherapy (fragility) or administration of ACD-preserved banked blood (aggregates), and when there has been poor blood collection technique. Pseudothrombocytopenia is a phenomenon that has been observed when aggregation is activated prematurely by EDTA in the sample tube.

**Comparison of the two platforms**

Though the VerifyNow and the PlateletWorks are quite different in their function, the essential advantages of both systems are the same. Both are available for use at the point of care, both are simple to operate, and both provide rapid results from a small volume of whole blood without any special sample preparation. Further, both platforms have been shown to correlate well with standard platelet aggregometry and both have been used to monitor certain
antiplatelet therapies. Perhaps the single most important difference between the two platforms is the actual information each provides, and this has potential implications for clinical arenas/scenarios in which each might be optimally utilized.

VerifyNow is specifically optimized to determine the percentage of inhibition of platelet function through specific pathways or due to the blockade of specific receptors. Thus, it is very useful in the cardiac catheterization laboratory to determine if a therapeutic effect is realized following administration of a specific antiplatelet agent. In the setting of cardiac (or general) surgery, a potential application of the VerifyNow platform might be to detect any residual inhibition of platelet function due to previously administered antiplatelet agents, such as clopidogrel. It must be appreciated, however, that the use of such an assay should not supersede clinical judgment nor recommended practice guidelines (e.g., ASRA guidelines regarding the timing of the performance of a regional anesthetic technique).

PlateletWorks provides a gross overall snapshot of platelet function with the ADP and/or collagen tubes. Inherited or acquired platelet deficiencies, and many of the other caveats listed in the package insert should affect both the pre-activation and the post-activation samples similarly, and thus, this platform can be used in a variety of clinical settings. For example, in the setting of cardiac surgery with hypothermic cardiopulmonary bypass (potentially resulting in an acquired platelet deficiency), this platform is a useful adjunct confirmatory point of care test as part of a transfusion algorithm. This platform can also be used to assess if aspirin is therapeutic with the arachidonic acid tube.

With both platforms, however, one must always be cognizant of manufacturer's recommendations regarding limitations of the accuracy of the assays in specific clinical situations and scenarios.