Platelet Function Analyzer (PFA)-100

Primary hemostasis involves adhesion of platelets to the sub-endothelial surface of the damaged vessel through von Willebrand factor (vWF). Historically, bleeding time determination was used to evaluate defects in primary hemostasis. Due to the imprecise, impractical, and invasive nature of bleeding time determination, a new method, PFA-100 was introduced as a replacement. This test simulates in vivo platelet adhesion and aggregation under high shear stress. Whole citrated blood is aspirated through a capillary toward a collagen-coated aperture containing either adenosine diphosphate (ADP) or epinephrine (EPI). Time to closure of the collagen aperture, referred to as closure time (CT), and reported in seconds (s) is the indicator of platelet functionality. Values > 300 seconds are reported as non-closure.

Clinically, PFA-100 test may be used in the following conditions:

1. Usefulness for diagnosis and initial work-up of pediatric and adult haemostatic disorders. For vWF and more severe platelet-related disorders, PFA-100 appears to have high negative predictive value (Favalaro, 2004). However, for congenital platelet disorders such as storage pool disease and primary secretion defects, PFA-100 appears to have low sensitivity, similar to bleeding time (Cattaneo et al., 1999b).

2. For monitoring of anti-platelet therapy, especially aspirin and NSAIDS, PFA-100 shows a prolonged CT. In contrast, the test is fairly insensitive to clopidogrel therapy.

3. For monitoring pro-haemostatic therapy such as DDAVP and release of endothelial vWF into blood, the test is mostly sensitive to platelet count, platelet vWF, and high molecular weight vWF. Factors including vWF concentrations and factor VIIa often do not impact PFA-100 CT.

Several pre-analytical factors may influence the PFA-100 closure time:

1. Specimen collection: The amount of anticoagulant (primarily citrate) can significantly increase the closure time. Therefore, the specimen should be collected in tubes with 3.2% citrate. Several studies have shown a more favorable coefficient of variation (CV), if the first volume of draw is excluded.

2. Specimen transport and analysis: Appropriate transportation of the specimen is an important factor to avoid false results. Pneumatic tubes should be not be used for transportation of the specimen. The specimen should be analyzed for CT within 4 hours of collection.

3. Hematocrit and Platelet count: Similar to bleeding time, PFA-100 CT is significantly prolonged with low platelet count or low hematocrit. CT is inversely proportional to platelet numbers in blood samples with <100 x 10^9/L platelets. Similarly, CT is abnormal in specimens with hematocrit <25%.

4. Blood Group: Since vWF levels have an inverse relationship with CT, individuals with blood group O have a slightly prolonged CT, most likely due to naturally low plasma vWF levels.

5. Diet and Drugs: Dietary factors including consumption of flavonoids (e.g. red wine, cocoa, and chocolate) can prolong the CT. Aspirin and non-steroidal anti-inflammatory drugs can prolong CT. A repeat testing 7-10 days after stopping the offending drug is suggested for appropriate evaluation of platelet functionality.

6. Diurnal Variation: Small diurnal variation has been reported in CT with morning blood specimens showing a slightly shorter CT.

7. Age and gender: Age and gender (except neonates) usually do not impact CT.

PFA-100 testing is available at the Saint Luke’s Hospital laboratory Monday – Friday, 0800-2000.
Body Fluid Analysis – New Regulations

For decades, clinical laboratories across the nation have been analyzing nonstandard body fluids (NSBF) (fluids that are not derived from blood or urine) for diagnosis and management of various diseases. NSBF include cerebrospinal, peritoneal, pleural, pericardial, synovial, amniotic fluid, and post-surgical fluid obtained from JP drains. Test requests cover a range of clinical laboratory disciplines: chemistry, hematology, microbiology, flow cytometry, and/or molecular diagnostics. Typically, manufacturers submitting a methodology for FDA approval do not cite NSBF in the “intended use” portion of the package insert. Recently, the laboratory accrediting bodies (i.e. College of American Pathologists, CMS, etc.) began strict enforcement of standards that require extensive validation studies for each NSBF type for each analyte tested.

Validation studies for Saint Luke’s Regional Laboratories are presently underway for a variety of NSBF (peritoneal fluid, pleural fluid, and post-surgical fluid collected from JP drains) for the most common clinically relevant analytes. Meanwhile, non-validated NSBF testing is referred to an external reference laboratory. If urgent testing is required, please contact the Saint Luke’s Hospital Laboratory and ask to speak with a clinical pathologist.

Beware False Positive IgM Serology

Serologic assays for infectious illnesses may be diagnostic for organisms that are not identifiable by culture, including many viruses, rickettsia, Ehrlichia species, and fastidious organisms such as Brucella. Most commonly, serologic testing includes measurement of IgM and IgG antibody titers to the suspected infectious agent. Enzyme immunoassay and immunofluorescence are the usual serologic procedures performed in clinical laboratories. Generally, a measurable IgM antibody response to an infectious organism indicates acute or recent infection, and occurs within a week or two of symptom development. IgM antibodies can persist for several months after an infection. IgG antibody response to infection occurs after the IgM, usually within 2 to 4 weeks of symptoms, and persists lifelong.

False-positive IgM antibody tests have been reported in a variety of scenarios. The two most common causes of false-positivity are cross-reactivity between related microorganisms due to shared antigens, and assay interference from nonspecific antibodies. Examples of non-specific antibodies include rheumatoid factor, anti-nuclear antibodies (ANA), and anti-liver kidney microsomal antibodies (LKM). Of note, rheumatoid factor-like antibodies are found in up to 10% of adults without rheumatoid arthritis, and can be produced during infections including tuberculosis, mononucleosis, influenza, infectious hepatitis, and endocarditis. Examples of cross-reactivity are legion. For instance, false-positive IgM tests for measles have occurred due to parvovirus, rubella, and human herpesvirus-6 infections. All of the human herpesviruses, including herpes simplex (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and varicella virus (VZV) can potentially cross-react on IgM serology assays. False-positive EBV viral capsid antigen IgM and CMV IgM occurs in approximately 3% of HIV-infected patients and up to 30% of patients with hepatitis A. Furthermore, with regard to hepatitis A (HAV), false-positive HAV IgM is likely more frequent than true-positive tests. Current HAV IgM assays may be positive due to any inflammatory or infectious condition that results in a polyclonal B-cell activation and hence a general antibody immune response.

In summary, positive IgM infectious serology results are best interpreted with regard to clinical presentation, compatibility with other diagnostic studies, and epidemiologic likelihood of infection. Confirmation of infection by other means, such as alternate serologic assays or molecular testing, should be considered.