PCR Testing for Epstein Barr Virus in Whole Blood
The Epstein Barr virus (EBV) causes infectious mononucleosis and has infected 90% of the population by early adulthood. After recovery from infection, the virus remains latent in B-lymphocytes. Latency is controlled by cytotoxic T cells. Sporadic low-level lytic infection is also detectable and is roughly inversely proportional to the immunocompetence of the host. The balance, maintained in a healthy individual, can be shifted by immunosuppression, as is the case following transplantation. This results in proliferation of latently-infected B cells, expressed as post-transplant lymphoproliferative disease (PTLPD), and in an increase in lytic expression of the virus.

PTLPD is an important cause of morbidity and mortality in solid organ and stem cell transplant patients. This is particularly true in pediatric patients who are less likely to be EBV positive at the time of transplant and, therefore, to be primarily infected by or after the transplant. Viral load in blood as detected in polymerase chain reactions (PCR) helps diagnose PTLPD. PCR assays developed “in house” are very heterogeneous. Specimens include mononuclear cells, blood, serum, and plasma. Varied nucleic acid extractions, amplification systems, standards, and detection systems have resulted in widely variable results. Cut-offs, indicative of possible disease, which has been suggested, have included 5 copies/ng DNA and 100,000 virions/ml blood plus many others. The net effect has been that while specific viral loads may have some meaning for a given system in a given hospital, there has been little ability to generalize between systems.

We have been doing “homebrew” PCR for EBV for a number of years and have recently changed our method to the Roche LightCycler Real Time PCR instrument, which allows us to do quantitative testing on every specimen. I believe that this system (the first commercially available real time kit-based PCR system) will become the standard. We spent many months perfecting the assay and comparing its performance to our old assay. The overall correlation coefficient between the old and new assays was 0.87. This means the numbers you received from the old assay will vary only slightly with the new assay. The between day coefficient of variation (15%) was also excellent. This means that a number you receive on one day will vary little from the number you receive another day for technical reasons. Nevertheless, it is important not to over interpret small changes in the copy number. An immunocompetent EBV-positive person should be negative in this assay or rarely have a very low copy number. However, an immunosuppressed EBV-positive person will frequently be positive with a low copy number in the absence of EBV disease and indeed may have >100 genomes per ul. Patients with PTLPD will most likely have >1000 genomes per ul but disease may occur at a lower copy number. Each patient is different, and it is important to regularly monitor individual patients. Increasing copy levels, i.e., a doubling or more, of EBV DNA in serial specimens and correlation with clinical symptoms are more meaningful. Regular monitoring also has the advantage of detecting possible disease before it is otherwise clinically evident.
The PFA-100 Platelet Function Analyzer

The PFA-100 is a high shear flow system that measures platelet adhesion and aggregation in citrated whole blood collected within four hours. The PFA-100 induces platelet activation, as blood is made to flow through an aperture cut into a membrane coated with collagen and epinephrine (Col/Epi) or collagen and ADP (Col/ADP). The time taken for a platelet plug to occlude the aperture is measured and is referred to as the closure time (CT). In vitro studies performed to characterize the mechanism of platelet plug formation revealed that von Willebrand Factor (vWF) is the key factor that mediates platelet plug formation in the cartridges. CT above the laboratory-established cut-off could reflect platelet dysfunction or vWF abnormalities.

Pre-analytical variables

There are many pre-analytical variables that could prolong CT. When hematocrit is less than 35%, there is a steady prolongation of CT. Slightly prolonged CT is observed when platelet count is between 100K and 150K and CT is dramatically prolonged when platelet count is less than 100K. Neonates have slightly shorter CT compared to children and adults. CT is 12% shorter when blood samples are collected in 3.2% versus 3.8% buffered sodium citrate. Aspirin prolongs CT in Col/Epi cartridge, but not in Col/ADP cartridge. Heparin and Warfarin do not affect CT unless in high concentrations.

Expected patterns

2. Col/Epi abnormal and Col/ADP normal - this pattern is indicative of drug induced platelet dysfunction, most commonly seen after aspirin ingestion.
3. Col/Epi abnormal and Col/ADP abnormal - this pattern is most commonly seen in patients with von Willebrand disease (vWD) or congenital platelet defects.

Clinical utility of the PFA-100

The PFA-100 is useful mainly as a rapid screening tool. Diagnosis of a specific bleeding disorder requires additional tests.

1. The PFA-100 can replace bleeding time (BT) to rule out platelet dysfunction and vWD. BT was originally designed as an aid in the diagnosis of platelet dysfunction. BT is a difficult test to perform in young children and it is not suitable for serial or repeated testing. A recent study in pediatric patients showed 100% sensitivity and 97% specificity for detection of qualitative platelet abnormality using the PFA-100 compared with 37% and 88%, respectively, with BT. The sensitivity for vWD was 100% compared to 17% for BT. The sensitivities for combined qualitative platelet defects and vWD using the Col/Epi or Col/ADP cartridge were 100% and 87%, respectively, compared with 37% for BT (Cariappa R, et al, J Pediatr Hematol Oncol, 25:474-9, 2003).

2. The PFA-100 can monitor patients with type 1 vWD after therapeutic treatment. The PFA-100 has been proposed for therapeutic monitoring of patients with vWD treated with DDAVP or Factor XIII/vWF concentrate. In one study, 24 patients with type 1 vWD were studied with the PFA-100 before and one hour after the injection of DDAVP. All patients had prolonged CT and decreased vWF: Ag and vWF: RCO levels at baseline. The baseline BT was prolonged in 19 (79.2%) patients. DDAVP injection induced normalization of CT, vWF: Ag and vWF: RCO in all patients. (Franchini M., et al, Haematologica, 87:670, 2002). A lot of studies have also been done on aspirin and other anti-platelet agents to study drug resistance and therapeutic monitoring.

3. The PFA-100 cannot detect fibrinogen or coagulation factor defects.

We are in the process of purchasing the PFA-100. Call the coagulation laboratory (X3232) if you have any questions.