Multi-center, post market surveillance study of Verax PGD test

Bacterial contamination of platelets may occur during blood collection. Potential sources of contamination include the donor’s skin surface, the phlebotomist performing the venipuncture and the environment. The skin preparation process results in a reduction of bacterial load but not necessarily a sterile surface. Some contaminations may represent low-level, sub-clinical infections present in the apparently healthy apheresis donor. Since 2004, transfusion services including UCSF have implemented methods to limit and detect bacterial contamination in platelet components. However, the levels of bacterial contamination are extremely low at collection and the organisms may be undetectable by routine culture methods. In recent years, at least 4 UCSF patients have received contaminated platelet products and suffered severe transfusion reactions. In all instances, the contamination was not identified in spite of using standard culture methods on platelet samples obtained soon after collection of the products. The availability of a test that could be performed at a later time, just prior to transfusion, after bacteria enter growth phase, would significantly decrease the risk of infusing bacterially contaminated platelets.

The Platelet PGD Test (PGD Test) is a single-use, qualitative, rapid, immunoassay for the detection of Gram-positive and Gram-negative bacteria in platelets, and has been cleared by the FDA for use as an adjunct QC test for bacterial contamination in leukocyte reduced apheresis platelets (LRAP). A multi-center, post market surveillance study, currently underway, involves testing of a large population of LRAP using the PGD Test to detect contaminated units that may be falsely negative by culture that is performed 24 hour post-collection at the collection center. In the event of a “Reactive” result being observed with the PGD Test, the LRAP in question will be removed from inventory to prevent transfusion of a possibly contaminated component. The LRAP will be further tested following a retest algorithm which will include: 1) repeat testing of a freshly drawn sample from the unit using the PGD Test, 2) culturing the sample under aerobic and anaerobic conditions and 3) identifying any bacteria found on the plates.
Platelet PGD® Test

The Verax Platelet PGD Test is a rapid, qualitative immunoassay for the detection of aerobic and anaerobic Gram-positive and Gram-negative bacteria in leukocyte reduced apheresis platelets (LRAP) as an adjunct quality control test following testing with a bacterial detection device cleared by the FDA for quality control testing of LRAP.

SUMMARY AND EXPLANATION OF THE TEST

Bacterial contamination of platelet units represents the largest infectious disease risk in transfusion medicine with an estimated incidence of 1:2000 to 1:3000 units collected. Bacterial contamination of transfusable blood products is thought to occur by accidental inclusion of skin flora from the site of cannulation or by collection of products from asymptomatic donors with low-level bacteremia. A large number of Gram-positive (GP) and Gram-negative (GN) bacterial species have been implicated in contaminated blood products, including: *Staphylococcus spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Pseudomonas spp.*, *Klebsiella spp.* and *Escherichia spp.* Bacterial concentrations in contaminated platelet units are very low at the time of collection and may not be reliably detectable by available test methods in samples drawn at that time. During component storage this initial small inoculum of bacteria may grow, but by consequence of the diverse interactions of bacteria, donor unit and environmental conditions, the onset and rate of growth is highly unpredictable. QC testing for bacterial contamination at a later phase of component storage may serve to maximize the ability to identify contaminated platelet units compared to testing only at an early phase of storage.

A novel Pan Genera Detection® (PGD) technology has been developed that detects the presence of conserved antigens lipoteichoic acid (LTA) and lipopolysaccharide (LPS) found on aerobic and anaerobic GP and GN bacteria, respectively. LTA and LPS targets are located on the surface of their respective bacteria and are primary constituents of the cell walls. LTA and LPS antigens can be found on rapidly growing as well as stationary phase bacteria and their detection is possible by the use of specific antibodies. By combining the detection of LTA and LPS in a single Test Device, it is possible to detect the bacterial species most frequently implicated in contaminated platelet samples.

PROCEDURE

The Platelet PGD Test is a single-use, lateral flow, qualitative test comprised of reagents, controls, disposables and a Test Device containing two simultaneously run test strips specific for the detection of aerobic and anaerobic GP and GN bacteria. Samples from leukocyte reduced apheresis platelet units may be tested. Samples are mixed with a Reagent and centrifuged, plasma is decanted and platelet pellets are resuspended and solubilized by drop-wise addition of two Reagents with the aid of mixing. The processed sample is transferred to the Test Device. As the sample migrates through the test strips, the sample will interact with GP or GN bacteria-specific binding agents immobilized on colloidal gold and nitrocellulose. When the sample has reached the terminal ends of the Test Device, a dye located beneath the Procedural Control Windows will undergo a yellow to blue/purple color shift and indicate to the user that sufficient volume of processed sample was used and test results can be interpreted. Test results are interpreted from visual inspection of the GP and GN Test Result Windows. Valid test results can be interpreted only after the color change of Procedural Control Windows has occurred.
STUDY PROTOCOL AT UCSF

1. Based on workflow, the blood bank plans to test a limited number of Day 5 platelets only (i.e. on the last day of shelf life). All platelet products routinely undergo bacterial culture testing; only culture-negative products are issued for transfusion. PGD testing is not a requirement to issue platelets.

2. Platelet products that are PGD reactive will not be issued for transfusion. Additional tests including a routine Gram stain and culture will be performed by Microbiology (@ China Basin) on the PGD reactive units.

3. At UCSF, all co-components of PGD reactive units would be quarantined and subjected to additional testing. BCP will be notified about PGD reactive units; quarantine of co-components and recall of shipped co-components will be carried out.

When does the Lab Medicine Resident become involved?

Only in the rare instance that a co-component (e.g. parts B or C of a large apheresis platelet collection) of a PGD reactive unit (tested at UCSF on Day 5) has been transfused at UCSF or Mt. Zion on an earlier date (i.e. Day 3 or Day 4). LMR will be notified by the Blood Bank. A STAT Gram stain will be done on the PGD reactive unit and results communicated to the LMR. If the co-component was transfused at an outside facility, the BCP Medical Director will be notified of the test results.

What should the LMR do if co-components were transfused at UCSF or Mt. Zion?

LMR contacts the clinical team managing the patient that received the co-component and lets them know that the PGD test performed on a co-component on Day 5 was reactive. Remind them that although bacterial culture testing on the transfused product was negative, the point-of-release PGD test may potentially identify contaminated units negative by standard culture methods. LMR also communicates the results of Gram stain as soon as they become available (the BB technologist can pull up the results in MISYS).

What should the clinician do when a co-component from a PGD reactive unit has been transfused?

1. If the Gram stain on the PGD reactive product is positive:
   a. The PGD result is likely to be a true positive. It is very likely that the transfused co-component was also bacterially contaminated. The clinical team should inform ID and consider drawing cultures and initiating antimicrobial therapy.

2. If the Gram stain on the PGD reactive product is negative:
   a. A negative Gram stain could simply represent a false positive PGD test (specificity of the test= 99.7%), i.e. no concern about bacteria in the transfused co-component.
   b. Alternatively, the PGD reactive result could still be a true positive. As the PGD test is more sensitive than the Gram stain (bacterial titers of ~1000 CFU/ml are picked up by the PGD test; several-fold higher titers are required for detection by Gram stain), a negative Gram stain does not rule out bacterial contamination.
   c. Preliminary study data indicate that approximately 1 in 3000-4000 apheresis platelet units falsely negative by the culture method are being identified as being true positive by the PGD test.
   d. Clinical decisions will hence need to be made on a case-by-case basis and not rely solely on results of Gram stain.
   e. Blood Bank will update the clinical teams if cultures subsequently become positive on the index PGD reactive unit.