Platelet PGD® Test

Rx Only

A. INTENDED USE

The Platelet PGD Test is a rapid, qualitative immunoassay that detects the presence of bacteria in platelets for transfusion.

B. INDICATIONS FOR USE

The 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) suspended in plasma, LRAP suspended in Platelet Additive Solution C (PAS-C) and plasma, and pre-storage pools of up to six (6) leukocyte reduced whole blood derived platelets suspended in plasma, within 24 hours prior to platelet transfusion as a safety measure following testing with a growth-based quality control test cleared by the FDA for platelet components
- post-storage pools (pooled within four (4) hours of transfusion) of up to six (6) units of leukocyte reduced (LR) and non-leukocyte reduced (nLR) whole blood derived platelets (WBDP) suspended in plasma and
- single units of LR and nLR WBDP suspended in plasma and tested within four (4) hours prior to platelet transfusion as individual platelet units or as components of a post-storage pool.

C. 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.[1] 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.*

Since the issuance of the AABB Standard 5.1.5.1, AABB members are required to employ a method to limit and detect bacterial contamination in all platelet components.[2,3] To meet this standard, US blood centers and some hospital transfusion services have implemented 100% Quality Control testing for bacteria on samples taken at least 24 hours after collection. This testing is typically performed using growth-based testing systems such as BacT/ALERT® (bioMérieux, Durham, NC) or eBDS (Haemonetics Corporation, Braintree, MA), which have been cleared by the FDA for quality control testing of platelet components. Since the implementation of this testing, five studies of both apheresis and whole blood-derived platelets have demonstrated significant rates of contamination in units that tested negative using this testing strategy.[4-8] These studies showed that detection rates using 24 hour post-collection sampling were only 14.9% to 40.0% of detection rates observed when units were sampled and tested on Day 6-7.[5,7] This data indicates that a large number of contaminated platelets escape detection by 24 hour sampling and testing by growth-based methods and are distributed into the US platelet inventory for transfusion in spite of current testing. The most likely cause for this phenomenon has been identified as sampling error associated with sampling at 24 hours, at which time many bacteria are still in lag phase and are therefore at titers too low to be reliably sampled, resulting in False Negative test results.[9]

As a result, other methods to limit and detect bacterial contamination are called for with one strategy being testing on the day of transfusion.[10] This approach offers the opportunity to detect bacteria after they enter logarithmic growth phase and have proliferated to titers that are expected to be higher than at 24 hours post-collection. Sampling and testing on day of transfusion adds a measure of safety by interdicting a proportion of highly contaminated units that pose a serious risk to transfusion recipients.

The Platelet PGD Test is a simple, rapid, day of transfusion test for the detection of bacterial contamination in platelets and is based on Pan Genera Detection® (PGD) technology. It 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.[11,12] 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.[13,14] 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.[15,16]

D. PRINCIPLES OF THE PROCEDURE

The Platelet PGD Test is a single-use, lateral flow, qualitative test comprising 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 and pools of up to six whole blood derived platelets 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 (refer to **INTERPRETATION OF RESULTS**) 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 (refer to **INTERPRETATION OF RESULTS**). Valid test results can be interpreted only after the color change of Procedural Control Windows has occurred.

E. REAGENTS AND MATERIALS

Materials Provided

Platelet PGD Test	REF	P20C	20 Tests
	REF	P100C	100 Tests

Includes the following:

30°C / 15°C	20 Test	100 Test
PGD Test Device	20 each	100 each
Disposable Pipettes	20 each	100 each
Microfuge Tubes	20 each	100 each
8°C / 2°C	20 Test	100 Test
Reagent 1	1 x 6 mL	2 x 12 mL
Reagent 2	1 x 6 mL	2 x 12 mL
Reagent 3	1 x 3 mL	2 x 6 mL
CONTROL -	1 x 1.5 mL	1 x 1.5 mL
CONTROL +	1 x 1.5 mL	1 x 1.5 mL

Reagents

PGD Test Device — Conjugate Pad: Gold colloid coated with rabbit polyclonal and mouse monoclonal antibodies and protein (bovine) stabilzer dried in sucrose.
Nitrocellulose: 0.5 µg mouse monoclonal antibody, 3 µg rabbit polyclonal antibodies, and 2 x 3 µg goat polyclonal antibodies in TRIS buffer and protein (bovine) stabilizer.
Preservative: sodium azide.

Reagent 1 — Water, methanol and surfactants. Preservative: ProClin® 300

Reagent 2 — Water, sodium hydroxide and surfactants. Preservative: sodium azide.

Reagent 3 — Tricine buffer with surfactants, anti-coagulants and protein (bovine, mouse, rabbit) stabilizers. Preservatives: ProClin 300 and sodium azide.

CONTROL - — Phosphate buffered saline, platelet lysates and protein (human, rabbit) stabilizers. Preservatives: ProClin 300 and sodium azide.

CONTROL + — Phosphate buffered saline, Lipoteichoic acid, bacterial antigens, platelet lysates and protein (human, rabbit) stabilizers. Preservatives: ProClin 300 and sodium azide.

See **Reagent Precautions** below.

Materials Available Separately

	Quantity	REF
Platelet PGD Controls	30 Tests	P30C
Platelet PGD Test (without Platelet PGD Controls)	20 Tests	P20
Platelet PGD Test (without Platelet PGD Controls)	100 Tests	P100

Materials Required but not Provided

1. Sterile connecting device or tubing stripper, heat sealer and alcohol pad
2. Sterile secondary sample tubes with caps, minimum volume 1.5 mL
3. Micro-centrifuge 9,000 - 11,000 RCF (relative centrifugal force) capable of holding supplied Microfuge Tubes
   NOTE: Refer to the Micro-centrifuge Operator's Manual for the conversion of revolutions per minute (RPM) to RCF
4. Vortex mixer
5. Pipettes, pipettor or other single use device capable of delivering 500 µL
6. Sterile disposable 500 µL pipette tips
7. Timer
8. Personal protective equipment
9. Bio-hazardous waste equipment

Optional: Reusable transparent covered enclosure

F. WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

Warnings

1. Read the package insert completely before using the product. Follow the instructions carefully. Not doing so may result in inaccurate test results.
2. The Platelet PGD Test has been validated for use with LRAP units (platelets suspended in plasma and platelets suspended in platelet additive solution (PAS-C) and plasma), pre-storage pools of up to 6 LR WBDP suspended in plasma and LR and nLR WBDP suspended in plasma. Except for the Interferring Substances study in which plasma was replaced with 100% PAS-C, the PAS-C studies were conducted using apheresis platelets stored in 65% PAS-C and 35% plasma.
3. The Platelet PGD Test is for use within 24 hours of transfusion of LRAP and pre-storage pools of platelets suspended in plasma as a safety measure following testing using a growth-based quality control test cleared by FDA and for use within 4 hours of transfusion of WBDP suspended in plasma.
4. For optimal sample flow, perform the test at 15 - 30 ˚C and ≥ 20% relative humidity (RH), in a well-lighted area. Ensure that the airflow is minimal across or near the Test Device after addition of sample to the Sample Well. This can be verified by observing no flutter in a tissue paper placed above the laboratory bench surface. Low RH or airflow across the Test Device may result in extended sample flow times and/or invalid assays; invalid assays cannot be interpreted and must be repeated. If laboratory environmental conditions are not optimal, a transparent covered enclosure may be used during Test Device incubation.
5. Each operator performing the test must be able to distinguish between the following colors: Green, Yellow, Blue and Red.
6. Do not use materials after their stated expiration dates.
7. The Positive and Negative Controls contain human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents be handled in accordance with the OSHA Standard on Bloodborne Pathogens using Universal Precautions.[17] Biosafety level 2 or other appropriate bio-safety practices should be used for materials that contain or are suspected of containing infectious agents.
8. The human derived components within the Positive and Negative Controls are non-reactive for hepatitis B surface antigen (HBsAg), human immunodeficiency virus type 1 ribonucleic acid (HIV-1 RNA), antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/HIV-2), antibody to hepatitis C virus (anti-HCV) and HCV RNA, West Nile Virus (WNV) RNA and anti-HTLV-I/II when tested by FDA-licensed assays.

Reagent Precautions

Reagents were classified according to OSHA 29 CFR 1910.1030 and 1910.1200, Globally Harmonized System of Classification and Labeling of Chemicals (GHS) and applicable European Community (EC) Directives. Applicable Classification, Hazard (H) and Precautionary (P) statements are listed below. Safety Data Sheets (SDS) are available upon request. Refer to SDS for complete Precautionary Statements.

Reagent 1 contains methanol and the signal word is Danger.

H 226	Flammable liquid and vapor
H302	Harmful if swallowed
H312	Harmful in contact with skin
H319	Causes serious eye irritation
H370	Causes damage to organs
P210	Keep away from heat/sparks/open flames/hot surfaces. No smoking
P280	Wear protective gloves/protective clothing/eye protection/face protection
P312	Call a Poison Center or doctor if you feel unwell
P501	Dispose of contents/container in accordance with local and national regulations.

Reagent 2 contains sodium hydroxide and the signal word is Warning.

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338	If In EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical attention.
P501	Dispose of contents/container in accordance with local and national regulations.

Reagent 3 contains n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (DDAPS) and the signal word is Warning.

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338	If In EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical attention.
P501	Dispose of contents/container in accordance with local and national regulations.

Reagents 2 and 3, Controls and Test Devices contain sodium azide. Contact with acids liberates very toxic gas.

General Safety Precautions

Follow good laboratory practices and use Universal Precautions when handling all samples and materials.[17,18,19,20] Dispose of all test materials as bio-hazardous waste according to your laboratory procedure and required regulations.

Handling Precautions

Handle and perform test properly:

1. Do not combine leftover volumes of Reagents 1, 2, 3 or Controls.
2. Do not remove dropper tips from bottles.
3. Do not touch exposed dropper tips.
4. Recap bottles immediately after use. Do not interchange bottle caps. The cap color must match the label color.
5. Do not use test components beyond the expiration dates printed on the labels. Always check expiration dates prior to performing test.
6. Do not use Reagents or Controls if they have not been properly stored at 2 - 8 °C. It is not necessary to equilibrate Reagents or Controls to room temperature prior to use.
7. Do not use the PGD Test Device if the pouch has been compromised.
8. Use the PGD Test Device once and dispose of properly after use (see **General Safety Precautions**). Do not re-use Microfuge Tubes, Disposable Pipettes or pipette tips.
9. Use only the Microfuge Tubes and Disposable Pipettes provided with the Platelet PGD Test. Use of other disposables when performing the test may result in inaccurate results.
10. Do not touch the Test Result Windows or Sample Well of the PGD Test Device.
11. Read test results in a well-lighted environment.
12. Disinfect testing area and equipment regularly to avoid accidental contamination.

G. STORAGE INSTRUCTIONS

1. Store Platelet PGD Test Devices at 15 - 30 °C. Do not open the PGD Test Device pouch until time of use. Once opened, Test Devices should be used within 30 minutes.
2. Store Microfuge Tubes and Disposable Pipettes at 15 - 30 °C.
3. Store Platelet PGD Reagents and Controls at 2 - 8 °C. Once opened, use prior to the expiration date on the bottle.

H. INDICATIONS OF INSTABILITY

1. Inspect Reagent and Control bottles for precipitate. Do NOT use if precipitate is present.
2. Failure of the Platelet PGD Controls to perform as expected may indicate deterioration of the Reagents or the PGD Test Device.

I. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types and Handling

1. Testing should include a sample obtained from each LRAP component of an apheresis collection or a sample obtained from a WBDP unit or pool.
2. For optimal performance, sample and test LRAP components and pre-storage pools of leukocyte reduced platelets suspended in plasma:
   - As close to the time of transfusion as practical as bacteria may be in logarithmic growth phase and bacterial titers can increase considerably over a short period of time.
   - From 72 hours post-collection through the expiration date.
3. For optimal performance when testing individual units of WBDP suspended in plasma, sample and test from 72 hours post-collection through the expiration date.
4. For optimal performance when testing post-storage pools of up to 6 WBDP (WBDPp) suspended in plasma, sample and test from 72 hours after collection of the freshest unit in the pool through the expiration date of the oldest unit in the pool. In pools that will be leukoreduced using a leukoreduction filter, optimal performance may be achieved if the PGD Test is performed on the pool prior to filtration.
5. Additional samples will be required for retesting in duplicate following initially reactive PGD results and for retesting at least once following invalid PGD results. For further information, refer to Section L. **INTERPRETATION OF RESULTS** and the Testing/Interpretation Guideline.
6. All samples must be collected and placed in labeled, capped, sterile secondary sample tubes. Samples may be kept at 15 - 30 °C for up to two hours prior to testing. Discard secondary sample tube in the biohazard waste after use.
7. When opening secondary tubes, ensure caps are not mixed up in order to avoid cross-contamination.
8. 500 µL of platelet sample is required to perform this test.
9. Do not use refrigerated or frozen samples as inaccurate test results may occur.

Methods for Sample Acquisition

Ensure that platelet unit is well mixed prior to sampling. Collect platelet samples using sterile procedures in order to maintain a closed system.

If sampling with sterile connecting device, refer to the device manufacturer's instructions.

If sampling from a freshly created segment:

- Using a stripping device, force platelets within tubing segment back into the platelet bag. Strip the segment only one time to avoid activation of platelets.
- While tightly holding the tubing stripper, mix the unit thoroughly by gentle agitation.
- Release the tubing stripper and let tubing segment refill with platelets.
- Create a segment 4-6 inches (10 - 15 cm) long, i.e., sufficient length to yield a 500 µL sample, with heat sealer.
- Cut segment from remainder of tubing with clean cutting instrument that has been wiped with an alcohol pad.
- Drain fresh sample into a sterile secondary sample tube by cutting ends of the segment with a clean cutting instrument.

J. TEST PROCEDURE

Pre-testing Preparation and Notes

1. Inspect bottles for precipitate. Do not use if precipitate is observed.
2. Mix each bottle by gentle inversion 2 to 3 times prior to use.
3. Do not allow exposed dropper tips to come in contact with Microfuge Tubes or other surfaces.
4. Process samples and Controls in a continuous fashion once sample processing has started.

Control Processing

NOTE: Reagent 1 is NOT used when testing Controls.

1. Label two Microfuge Tubes to identify Controls.
2. Add 2 drops of Positive or Negative Control to the appropriately labeled Microfuge Tube.
3. Add 8 drops of Reagent 2 to each Microfuge Tube. Control samples must be blue.
4. Add 4 drops of Reagent 3 to each Microfuge Tube. Cap each Microfuge Tube and briefly vortex. Control samples must be yellow. Proceed directly to **Performing the Test**.

Sample Processing

Prior to adding platelet sample to the Platelet PGD Test Device, perform the following steps for each platelet sample to be assayed.

1. Label a Microfuge Tube to identify the sample being tested.
2. Pipette 500 µL platelet sample into the labeled Microfuge Tube. Properly dispose of pipette tip after transfer.
3. Add 8 drops of Reagent 1 to the Microfuge Tube. Recap the Microfuge Tube and mix by inversion 2 to 3 times. Do not vortex or shake Microfuge Tube; process a new sample if the Microfuge Tube was vortexed or shaken at this step. The platelet sample must turn green after addition of Reagent 1 and mixing. The intensity of the color may range from a pale green or bluish green hue to a dark green/bluish green color. If sample shows no evidence of color change, discard the sample and repeat. If the repeated sample reacts in the same manner, contact Technical Support as the sample cannot be run on the Platelet PGD Test. Samples with a pH lower than 5.5 may fail to turn green upon addition of Reagent 1.
4. Centrifuge Microfuge Tube for 5 minutes (± 30 seconds) at 9,000 - 11,000 RCF. After centrifugation, a cell pellet must be visible near the bottom of the Microfuge Tube.
5. Uncap the Microfuge Tube and decant the liquid into an appropriate waste container by first inverting the tube to drain and then shaking it over the container. After decanting, check to confirm the cell pellet is still adhered to the Microfuge Tube.

   NOTE: A fresh sample must be reprocessed if the pellet was not present after centrifugation or was decanted.
6. Add 8 drops of Reagent 2 to the cell pellet. The sample must be blue after adding Reagent 2. Tap the pellet with the tip of a Disposable Pipette to dislodge the pellet from the bottom of the tube. Carefully aspirate and dispense the solution with the Disposable Pipette. The pellet may either immediately dissolve or break into fragments; either outcome is acceptable. Avoid aspirating the solution into the bulb of the pipette. **Do not vortex**. Minimize forming bubbles or foam. Confirm that no fragments are adhered to the exterior or interior of the Disposable Pipette.

   NOTE: A fresh sample must be reprocessed if:
   - the pellet has adhered to the disposable pipette
   - the processed sample is not blue
   - the processed sample is trapped in the pipette bulb

   Proceed directly to the next step without pause.
7. Add 4 drops of Reagent 3 to the Microfuge Tube. The processed sample must turn from blue to a pale yellow or straw color upon addition of Reagent 3. Total volume in the Microfuge Tube will be approximately 300 µL. Recap the Microfuge Tube and vortex until the pellet goes completely into solution, approximately 5-10 seconds. Cell pellet fragments should not be present. Inspect the tube thouroughly by flicking it so that any particulates that may have settled will rise off the bottom of the tube and become visible. If any cell fragments or particulates are present, continue vortexing until everything is dissolved. Proceed directly to **Performing the Test.**

Performing the Test

For each processed sample or Control to be analyzed on the Platelet PGD Test, perform the following steps:

1. Tear open the notched end of the pouch and remove the PGD Test Device. Verify that a desiccant is present in the pouch. If a desiccant is not present, obtain a new PGD Test Device. Inspect Test Result Windows for surface imperfections. The surfaces should be smooth and white. Visually confirm that both Procedural Control Windows are yellow. See Figure 1.

   NOTE: PGD Test Device should be used as soon as possible, but may be used up to 30 minutes after the pouch is opened.
2. Place the PGD Test Device on a flat surface at 15 - 30 ˚C and ≥ 20% relative humidity with minimal airflow. Use a marker to label the PGD Test Device to identify the sample or Control being added.

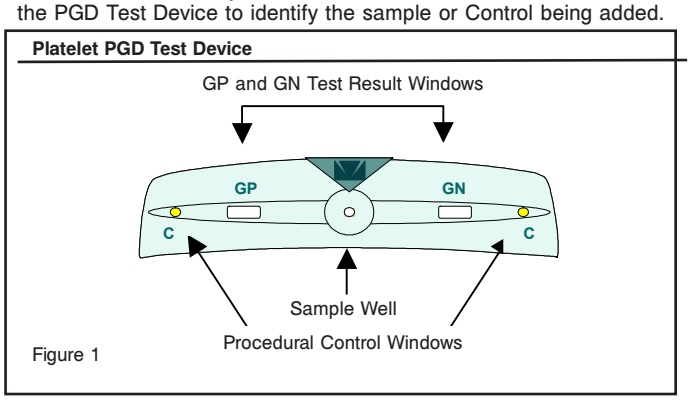


Figure 1

3. Pour the entire processed sample or Control (about 300 µL) in a single action into the Sample Well on the PGD Test Device. See Figure 1. Do not spill or splash sample or reagents on the Test Result or Procedural Control Windows. If this occurs, repeat the test with a fresh sample and PGD Test Device. If laboratory environmental conditions are not optimal, a transparent covered enclosure may be used during Test Device incubation.

   NOTE: The entire Sample Well (300 µL) must be filled for the PGD Test Device to perform properly. Addition of insufficient volume will result in invalid test results.
4. Watch for sample flow, as indicated by red color movement across the Test Result Windows. If sample flow does not proceed on both sides of the device within 5 minutes, press firmly on the sample pad 2 or 3 times with an unused sterile disposable pipette tip to initiate flow. See Figure 2. Start a timer set for 20 minutes once all the Test Devices in the batch have red color movement present on both sides of the device.

   NOTE: This step may be repeated if sample flow does not initiate or the sample flows very slowly.

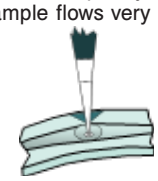


Figure 2

5. After approximately 20 minutes of incubation and approximately every 10 minutes thereafter (up to 60 minutes total), examine both Procedural Control Windows, labeled C on the PGD Test Device, for indications of a yellow to blue/purple color change and clearing of the Test Result Windows. See Figures 3, 4 and 5. When the color change has begun to occur in both Procedural Control Windows and the backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, the test should be read and interpreted. If these criteria are not satisfied within 60 minutes of initiation of sample flow, the test is invalid. Repeat the test with a fresh sample and a new PGD Test Device. Read the results in a well-lighted area within 60 minutes of sample addition.

   NOTE: The entire Procedural Control Window does not have to change to a blue/purple color before the result can be read and interpreted.
6. Refer to **INTERPRETATION OF RESULTS**. Record your results per your laboratory requirements.
7. After interpretation and recording test results, dispose of used PGD Test Device in a bio-hazardous waste container.

K. QUALITY CONTROL

Platelet PGD Controls (Negative and Positive) are for use only with the Platelet PGD Test. The Platelet PGD Controls are used to ensure the User's ability to properly perform and interpret the test. Platelet PGD Controls are also used to verify the performance of the Platelet PGD Test. Test the Platelet PGD Controls under the following circumstances:

- Each new operator, to establish competency prior to testing platelet specimens
- When opening a new lot of Test Devices or Reagents
- Whenever a new shipment of Test Devices or Reagents is received
- At periodic intervals as dictated by the user facility

Each laboratory is responsible for using Platelet PGD Controls to establish an acceptable quality assurance program to monitor the performance of the test under its specific laboratory environment and conditions of use.

L. INTERPRETATION OF RESULTS

The PGD Test Device has built-in Procedural Controls that are used to verify assay validity. Blue/purple color must appear in both Procedural Control Windows for the test to be valid. The color shift of the Procedural Control Windows will occur for both Non-reactive and Reactive samples and Controls. Verify that the Procedural Controls have changed from yellow to a blue/purple color. See Figures 3, 4 and 5.

The backgrounds of both the GP and GN Test Result Windows must be white or have a light pink homogeneous hue, free from streaks or spots that could interfere with interpretation of the test result. Do not confuse extraneous red spots or streaks with Reactive test results. See Figures 3, 4 and 5.

Evaluate the GP and GN Test Result Windows for the presence or absence of GP and GN detection lines. See Figures 3 and 4. Detection lines will be discrete vertical lines that extend from top to bottom of the GP and/or GN result window. The color of the line may range from extremely light pink to a dark purple color. Consider any discrete pink/purple line within either Test Result Window as reactive, no matter how faint the line.

When a detection line is observed, repeat the test twice using a new sample and new Platelet PGD Test Devices. If the 2 retests yield Non-reactive results, the sample is interpreted as Non-reactive. If a detection line(s) is observed in the intial and at least one repeat PGD test, the sample is interpreted as Repeatedly Reactive.

When no detection line(s) is observed, the test result is Non-reactive. No repeat testing is recommended when valid assays yield Non-reactive results. (Refer to Section **M**. **LIMITATIONS** for testing timeframes.)

If either or both Procedural Controls fail(s) to change color or if either or both Test Results Windows fail to clear to white or light pink so as not to interfere with interpretation of results, the test is invalid. An invalid result cannot be interpreted. Repeat the test using a new sample and new Platelet PGD Test Device. Refer to the **Testing/Interpretation Guideline** and Figures 3, 4 and 5.

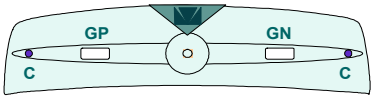
Testing/Interpretation Guideline

If Initial PGD Result is:	Then:	Retest Result(s)	PGD Interpretation
Non-reactive	No additional testing required	Proceed to Interpretation	Non-reactive
Reactive	Retest new sample in duplicate	If 2 retests Non-reactive:	Non-reactive
		If ≥ 1 Retest is reactive:	Repeatedly Reactive
Invalid	Retest new sample	If no valid result after 2 retests:	Invalid - Contact Technical Support

It is recommended that all platelet co-components be retested when a Repeatedly Reactive unit is detected. When Repeatedly Reactive doses are found, notify the platelet provider and determine appropriate follow-up actions such as confirmatory culture and bacterial identification.

Sample Results

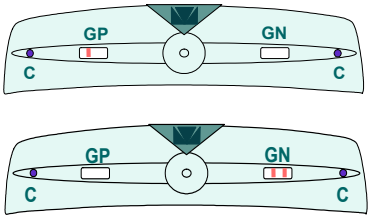
NON-REACTIVE Results



- No detection lines visible in either the GP or the GN Test Result Windows
- The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots that might interfere with interpretation of the results.

Figure 3

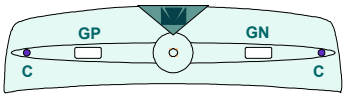
REACTIVE Results



- One or two detection lines visible in either or both of the GP and GN Test Results Windows
- No more than two lines present in either Test Result Window
- When a detection line is observed, repeat the test twice using a new sample and new Platelet PGD Test Devices. If a detection line(s) is observed in the initial and at least one repeat PGD test, the sample is interpreted as Repeatedly Reactive.

Control Results

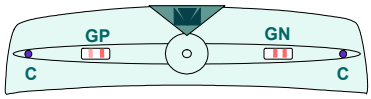
NEGATIVE Control



- No detection lines visible in either the GP or the GN Test Result Windows
- The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots that might interfere with interpretation of the results.

Figure 4

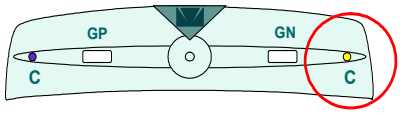
POSITIVE Control



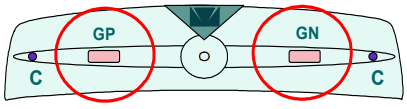
- Two detection lines present in BOTH the GP and GN Test Result Windows (total of 4 lines visible)
- Lines discrete and extend fully from the top to the bottom of the GP and GN Test Result Windows. (Consider any discrete line within either Test Result Window as reactive, no matter how faint the line.)

Invalid Results

INVALID Test Results



- Procedural Control failure where blue/purple failed to appear within 60 minutes for either or both Procedural Controls



- Failure of either or both GP and GN Result Windows to clear to white or light pink so as not to interfere with interpretation of results

An INVALID result cannot be interpreted. When INVALID results occur, repeat the test using a new sample and new Platelet PGD Test Device. Contact Techical Support for assistance if valid results are not obtained after 2 retests.

Figure 5

M. LIMITATIONS

1. The Platelet PGD Test is for use in LRAP and leukocyte reduced pre-storage pools of up to 6 whole blood derived platelets suspended in plasma, within 24 hours prior to transfusion as a safety measure following testing with a growth-based quality control test cleared by FDA for platelet components, for post-storage WBDP pools (pooled within four (4) hours of transfusion) and for single units of WBDP suspended in plasma and tested within four (4) hours prior to platelet transfusion as individual platelet units or as components of a post-storage pool. Performance characteristics for alternate sample types have not been established.
2. The Platelet PGD Test must be performed in accordance with the instructions given in the package insert for an accurate test result.
3. Interpreting the test results before 20 minutes or after 60 minutes of initiation of sample flow may yield inaccurate results.
4. Do NOT use the Platelet PGD Test on clumped or coagulated platelet samples.
5. Processed samples containing fibrin may result in extended sample flow times and/or invalid assays.
6. Do NOT use the Platelet PGD Test on refrigerated or frozen samples as inaccurate test results may occur.
7. Performance characteristics of the product were established using the following anticoagulants: ACD-A (LRAP), CPD and CP2D (WBDP) and in Platelet Additive Solution (PAS-C).
8. For REACTIVE test results, intensity of the test line does not correlate to the titer of bacteria in the sample.
9. A NON-REACTIVE test result does not mean the unit is sterile or bacteria-free. Non-reactive results may occur if:
   a. the samples are not properly obtained or stored
   b. the test procedure was improperly followed
   c. the concentration of bacteria is below the limit of detection of the test
   d. bacterial antigens are present at extremely high concentrations (prozone effect)
   e. the LTA structure is Type IV LTA, which has been identified in a few species of viridans group streptococci (*S. mitis* and *S. oralis*).[12,13] Current PGD antibodies are not targeted against the LTA IV structure found in *S. mitis* and *S. oralis*.
10. For potentially interfering substances and prozone (hook effect) refer to the appropriate section.

N. PERFORMANCE CHARACTERISTICS

Population study of Leukocyte Reduced Apheresis Platelets tested as negative by growth-based methods

Eighteen hospital transfusion services, including academic hospitals, community hospitals and specialty cancer hospitals participated in a bacterial detection study of LRAP units obtained from FDA-licensed and/or registered collection centers. All LRAP units had been distributed by collection centers as culture negative after testing for bacterial contamination using an early storage growth-based system cleared by FDA for quality control testing.

A platelet dose was defined as a unique LRAP available for transfusion to a patient and included in the study, with multiple doses obtained from one apheresis collection unit considered to be different doses. Sites performed PGD testing on platelet doses on the day of transfusion, with 16 sites testing prior to transfusion and 2 sites testing shortly after platelet sampling and subsequent issue for transfusion. Each site integrated the PGD Test into laboratory routine. Testing frequency ranged from testing once per day (at most sites) to once per shift (3 shifts per day) at one site. Platelet age ranged from 2 to 5 days post-collection. Some platelet doses were tested at more than one time-point (e.g. daily as long as they remained in inventory). Of the 27,682 doses tested in this study, 2,461 were tested at a second time-point, 202 were tested at a third time-point and 20 were tested at a fourth time-point.

If no detection lines were observed, the sample was assigned a status of PGD Non-reactive (PGD-NR). In the event that a detection line was observed, PGD testing was repeated on a new sample using new PGD Test Devices to assign the dose a status of either PGD-NR (2 retests PGD Non-reactive following initial Reactive test) or PGD Repeatedly-Reactive (PGD-RR) if lines were detected in $\geq$ 1 retest following the initial Reactive result. Samples having final interpretations of PGD Repeatedly-Reactive (PGD-RR) were cultured for bacteria under aerobic and anaerobic conditions for up to 5 days. Gram stains were also performed on confirmatory culture-positive doses and bacteria that grew were identified using standard microbiological methods.

A total of 27,682 LRAP doses were tested. Of those, 27,620 doses yielded valid PGD results at initial time-point testing and were subsequently included in data analyses. Three of the 18 sites performed plate culture at the time of PGD testing (concurrent culture) regardless of PGD result, with one site performing quantitative culture following a positive cytospin/Gram stain. These 3 sites contributed 10,424 unique LRAP doses that were used to determine True Positives (TP=PGD-RR + concurrent culture positive), True Negatives (TN=PGD-NR + concurrent culture negative), False Positives (FP=PGD-RR + concurrent culture negative), False Negatives (FN=PGD-NR + concurrent culture positive) and Specificity. Note that clinical sensitivity of the Platelet PGD Test was not established by this study. The remaining 15 sites performed confirmatory culture testing on only PGD-RR LRAP doses; TP (PGD-RR + confirmatory culture positive), FP (PGD-RR + confirmatory culture negative) and Non-reactive doses were determined for the 17,196 doses tested at these 15 sites.

Nine (9) bacterially contaminated LRAP doses were detected by the PGD Test and confirmed by culture (TP) at six centers for a detection rate of 1:3069 [1:6,711 - 1:1,617] or 326/million units [149/million - 618/million]. Four of the 9 contaminated doses detected were day 3 platelets, 2 were day 4 and 3 were $\geq$ day 5. All 9 doses were detected by PGD at the first and only time-point when they were tested. Table 1 shows the frequency of contaminated units and platelet age. Table 2 lists the characteristics of the 9 TP identified in the study.

Table 1: Frequency of contaminated doses by platelet age

	Platelet Age (Days)				Total
	≤2	3	4	≥5*	
Doses Tested	4,036	8,375	6,660	8,549	27,620
(% Total)**	(15%)	(30%)	(24%)	(31%)	
True Positive	0	4	2	3	9

* Some doses at the 2 sites that tested shortly after platelet issue for transfusion were in-date at time of issue but were several hours past expiration at time of testing.

** Percent of total LRAP doses tested

Table 2: Characteristics of the 9 TP LRAP doses

Case	Site	Bacterial species isolated	Age of dose (days)	Bacterial confirmation method	Bacterial load (CFU/mL)	Transfusion Reaction
1	1	*Bacillus spp.*	3	BC	-	Not Transfused
2*	2	*CoNS*	3	PC; GS	-	Not Transfused
3*		*CoNS*	3	PC; GS	-	Not Transfused
4	3	*Enterococcus faecalis*	3	PC; GS	-	Not Transfused
5	4	*Staphylococcus spp.; Peptostreptococcus spp.*	4	PC; BC; GS	-	Not Transfused
6	5	*CoNS*	4	PC	-	Not Transfused
7	6	*CoNS*	5	PC; GS	1.3×10^6	Transfused, No Reaction
8		*Bacillus spp.*	5+§	PC; GS	1×10^7	Not Transfused
9		*CoNS*	5+§	PC; GS	1.2×10^7	Transfused, Septic shock

* Co-components of same LRAP collection

CoNS, Coagulase-negative staphylococcus

BC; broth culture; PC, plate culture; GS, Gram stain

-, Not done

§ In date at time of platelet issue for transfusion but tested several hours after platelet expiration

In the subset of 10,424 doses tested by concurrent culture, bacteria were detected in 5 doses, 3 of which were detected by PGD testing while 2 were not. The observed false negative (FN) rate among these doses was 2:10,424 = 1:5,212 [1:43,036 - 1:1,443]. A precise FN rate cannot be estimated because of the limited sample size. Four (4) of the 5 doses had sufficiently high bacterial titers based on quantitative culture results to cause septic transfusions.[10] The Platelet PGD Test identified 3 of the 4 doses with high bacterial titers. The 2 doses that were PGD-NR were day 5 doses. Both of these doses were transfused and one of these 2 doses was associated with a septic transfusion reaction; the bacterial species was identified as a viridans group streptococcus (*Streptococcus oralis,* which has a rare lipoteichoic acid class not targeted by current PGD antibodies (LTA Type IV)) at 2 x 10^7 CFU/mL. [21,22] The other dose, which was tested within 4 hours of platelet issue for transfusion, contained a very low level of CoNS (<10^3 CFU/mL) and was not associated with a transfusion reaction.

One other bacterially contaminated dose was found by passive surveillance among the doses tested at the remaining 15 hospitals (1:17,196). This was a day 5 dose tested approximetely 4 hours prior to issue for transfusion that was found following report and subsequent work-up of an allergic transfusion reaction, with the patient showing no febrile or septic reaction. The dose contained *Streptococcus sanguinis*, a member of the viridans Streptococcus group, likely at a titer below the PGD limit of detection. Active surveillance, including concurrent culture, provides a better method for identifying bacterial contaminations than passive surveillance.

Initial invalid results were obtained for 128 of 27,682 (0.46%) doses tested in the study. Forty-eight (48) of the 128 doses with initial invalid results had no follow-up testing. Some study sites had procedures in place regarding retesting samples with invalid results while other sites did not; the valid/invalid status of these 48 doses was not resolved. Eighty (80) of the 128 doses were retested to resolve the initial invalid result. Five (5) of these 80 doses (5/27,682) continued to have invalid results upon retesting. Sixty-six (66) of the 80 doses had valid test results upon retest and nine (9) had valid test results at a subsequent time-point resulting in 62 invalid doses at the first testing time-point. Across all time-points, invalid results were obtained from 149 of 30,991 tests performed in the study for an overall invalid test rate of 0.48%. Across all study time-points 27,545 doses of the total 27,682 doses tested in the study (99.5%) yielded only valid test results while 137 yielded at least one invalid test result sometime during the study. Fifty-four (54) of the 137 doses with invalid results had no follow-up testing. Eighty-three (83) of the 137 doses were retested to resolve the invalid result with only 5 doses continuing to have invalid results and 78 of these 83 doses yielding valid test results. Figure 6 includes a graphic presentation of invalid test rates and information.

Specificity could not be determined for the entire study population of 27,620 doses as concurrent culture results were required to determine the bacterial status of the doses (i.e., culture-negative or culture-positive) and only 3 sites performed concurrent culture. Specificity was calculated using the PGD test results from the 10,424 doses (primarily day 3 - day 5 units) tested at the 3 concurrent culture sites. Observed specificity ranged from 99.2% to 99.5% with an overall specificity of 99.3% [95% CI: 99.1% - 99.4%].

Of the 27,620 doses with valid PGD results at the first testing time point, 259 doses (0.94%) were retested due to initially reactive PGD results. Of those 259 doses, 151 (58.3%) yielded repeatedly reactive PGD results, with 9 of the 151 confirmed as bacterially contaminated. One hundred eight (108) of the 259 (41.7%) were non-reactive upon retesting with the PGD test.

The PGD false positive (FP) rate was determined based on the entire study population of 27,620 doses tested at 18 sites at the first testing time-point. False initial reactive results were observed for 0.91% [0.80 - 1.02%] of doses. The FP rate based on Repeatedly Reactive PGD results ranged from 0.36% to 0.71% with an overall FP rate of 0.51% [0.43-0.61%].

Figure 6 summarizes the study design and results.

Figure 6

PGD Postmarket Surveillance
18 Sites
27,620 Doses

Concurrent Culture Sites
Performing Culture with **Each** PGD Test
3 Sites
10,424 Doses

Sites Performing
Culture **Only** if PGD-RR
15 Sites
17,196 Doses

Final PGD Interpretation	PGD Test Results	Culture Result		Total (N)
		Pos	Neg	
PGD-RR	≥ 2 R	3 (TP)	77(FP)	80
PGD-NR	1R →2NR	0 (FN)	68 (TN)	68
	NR	2 (FN)	10,274(TN)	10,276
Total (N)		5	10,419	10,424

Final PGD Interpretation	PGD Test Results	Culture Result			Total (N)
		Pos	Neg	None	
PGD-RR	≥ 2 R	6 (TP)	65 (FP)		71
PGD-NR	1R →2NR			40	40
	NR	1 (FN)		17,084	17,085
Total (N)		7	65	17,124	17,196

Specificity = 99.3%
(10,274+68)/10,419 = 99.3%

Clinical Sensitivity was not established by the study;
PGD detected 3 of 5 culture positive platelet doses

Precise FN rate was not established by the study;
FN Rate (observed) = 2/10,424 = 1/5,212

Overall Summary

	# Doses	% Total Doses
Total Doses tested at first testing time-point	27,682	
Total Doses with valid results at first testing time-point	27,620	99.78%

	# Doses	% Valid Doses	% Valid Doses retested
Total Doses with valid results at first testing time-point	27,620	100%	
Total Doses not requiring retest (i.e., non-reactive)	27,361	99.06%	
Total Doses retested due to initially reactive results	259	0.94%	100%
Doses with non-reactive results following retest of initially reactive results	108		41.7%
Doses with repeatedly reactive (RR) results following retest of initially reactive results	151		58.3%
Doses with RR results that were not bacterially contaminated (FP)	142	0.51%	54.8%
Doses with RR results that were confirmed as bacterially contaminated (TP)	9	0.03%	3.5%

True Positives (TP)

TP Detection Rate = 0.03% = 1:3,069
where TP = (≥ 2 R + positive culture) =
(3 + 6)/27,620= 9/27,620= 0.03% = 1:3,069

False Positives (FP)

FP Rate = 0.51%
where FP = (≥ 2 R + negative culture) =
(77 + 65)/27,620= 142/27,620= 0.51%

False Initial Reactives (FIR)

FIR Rate = 0.91%
where FIR = (FP + (1R →2 NR)) =
(142 + (68 + 40))/27,620= 250/27,620= 0.91%

Invalid Tests

Invalid Test Results	Invalid Test Results/ Total Tests (%)
Initial Test - First Time-point	128/27,682 (0.46%)
All Tests - All Time-points	149/30,991 (0.48%)

Across All Time-points	# Doses
# Doses in Study	27,682
# Doses with only valid tests	27,545
# Doses with 1 or more invalid tests	137
# Doses retested to resolve invalid results	83
# Doses with valid results upon retest	78
# Doses with only invalid results upon retest	5

Analytical Growth Model Studies for Bacterial Detection in Platelets

LRAP Study 1 Description

The equivalence of the Platelet PGD Test system to BacT/ALERT® for detecting bacterial contamination in LRAP units was evaluated by comparing time to detection of 10 bacterial species. Three sites participated in the study, each using three lots of Platelet PGD Test. All bacterial species were tested with each lot. Platelet units were inoculated with low levels of each bacterial species listed in Table 3. In addition, 28 LRAP units were inoculated with a PBS solution to serve as negative controls for the bacterial inoculation process. The anaerobe *Clostridium perfringens* was only performed at site 3 as the growth model for this species could not be reliably established in LRAP units. Only one cycle of successful inoculations and testing was accomplished using both bags of a single LRAP donation.

For each bacteria species, both bacterially inoculated and negative control units were sampled at 24 hours post inoculation to inject BacT/ALERT BPA and BPN bottles. The units were again sampled at 48hours post inoculation to inject BacT/ALERT BPA and BPN bottles and to perform the Platelet PGD Test. For Platelet PGD testing, 12 blinded samples were prepared (10 or 11 samples from the bacteria-inoculated unit and 1 or 2 samples from the negative control unit). If the Platelet PGD Test detected 100% of the bacteria-inoculated samples at 48 hours, testing was concluded. If any of the bacteria-inoculated samples were not detected by the Platelet PGD Test at 48 hours, the sampling and testing cycle described above was repeated every 24 hours until there was 100% detection by the Platelet PGD Test.

LRAP Study 1 Results

Study results are shown in Table 3. All types of tested bacteria were detectable by the Platelet PGD Test at 48 or 72 hours. For all bacteria except *Staphylococcus epidermidis* (and the *Klebsiella pneumoniae* at Site 1), there was 100% detection by the Platelet PGD Test at 48 hours after inoculation. For specimens spiked with PBS, there were two false positives by BacT/ALERT, both of which were attributable to contamination (bag integrity compromised). One PBS-spiked sample was falsely positive by the Platelet PGD Test. The operator resampled and retested using a new Platelet PGD Test Device and the result was non-reactive.

LRAP Study 2 Description

The objective of Study 2 was to demonstrate that the Platelet PGD Test was able to detect bacteria missed by culture due to sampling error. Sampling error can occur when bacteria are in lag phase at the time of sampling and therefore are not present at sufficient concentration to be consistently captured in the culture sample.[9] This study was performed using three lots of Platelet PGD Test and three bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*).

To mimic the low bactericidal properties of LRAP units that support bacterial growth, heat-inactivated plasma (HIP) prepared from LRAP was used as the medium for bacterial inoculation. Following heat treatment, 300 mL of HIP was placed into each of 6 LRAP bags. Bacteria were inoculated at very low titer (< 200 CFU per bag) into each bag, allowed to mix on platelet rockers for 1 to 2 hours and then sampled for initial testing by culture. Ten 8 mL samples were removed from each bag; 4 mL for aerobic culture and 4 mL for anaerobic culture, each of which utilized two 150 mm Mueller-Hinton agar plates. Plates were monitored for growth. An inoculated bag was excluded from further study if colonies were observed on 10 of the 10 samples (indicating no culture sampling error). If colonies were observed on fewer than 10 of the 10 samples (indicating culture sampling error), Platelet PGD testing was performed on that bag.

For Platelet PGD testing, platelet pellets were prepared by centrifugation from in-date LRAP units. Each platelet pellet was then resuspended in 500 µL drawn from an inoculated HIP bag in order to reconstitute a representative platelet sample and tested using the Platelet PGD Test. Samples collected at 24 hours and every 12 hours thereafter were tested with Platelet PGD Test until reactive results were observed using all three Platelet PGD Test lots.

A second culture, including bacterial identification was performed on each bag at the time of the first observed Platelet PGD Test reactive result or at 96 hours if no Platelet PGD Test reactive was observed for a bag. This served to confirm the Platelet PGD Test results and the bacterial growth status of the bag.

Table 3: LRAP Growth Study 1 Results

Bacteria	Site	Bacterial Concentration (CFU/mL) in LRAP at unit inoculation	BacT /ALERT (Hours after inoculation of LRAP unit for a positive result)		Platelet PGD Test (Hours after inoculation of LRAP unit tested and detected)	
			24 hr Sample	48 hr Sample		
Bacillus cereus ATCC 7064	1	2.8	28	52	48	(10/10)
	2	1	28	52	48	(10/10)
	3	1	28	52	48	(10/10)
Clostridium perfringens ATCC 13124	3	0.4	35	69	48	(11/11)*
	3	0.6	36	59	48	(11/11)
Enterobacter aerogenes	1	4	34	54	48	(10/10)
	2	6.4	34	54	48	(10/10)
	3	9.6	32	53	48	(10/10)
Escherichia coli	1	35	28	52	48	(11/11)
	2	89.4	31	55	48	(11/11)
	3	3.4	32	57	48	(11/11)
Klebsiella pneumoniae	1	3.2	33	56	72	(10/10)
	2	7.6	31	54	48	(11/11)
	3	8	Neg	53	48	(11/11)
Pseudomonas aeruginosa	1	3.6	34	53	48	(10/10)
	2	7.8	36	55	48	(10/10)
	3	1.6	33	52	48	(10/10)
Serratia marcescens ATCC 43862	1	4.4	33	52	48	(10/10)
	2	2.4	30	52	48	(10/10)
	3	10	31	52	48	(10/10)
Staphylococcus aureus ATCC 27217	1	4	30	52	48	(10/10)
	2	5	32	52	48	(10/10)
	3	11.2	33	52	48	(10/10)
Staphylococcus epidermidis ATCC 49134	1	32	35	55	72	(10/10)
	2	10.6	34	54	72	(10/10)
	3	10.8	35	55	72	(10/10)
Streptococcus agalactiae ATCC 12927	1	2.8	30	52	48	(11/11)
	2	5	31	52	48	(11/11)
	3	2	32	52	48	(11/11)

*One sample detected as both GP and GN

Unless otherwise noted, bacterial strains were blood culture isolates.

LRAP Study 2 Results

Of 6 bags inoculated, 5 supported bacterial growth (see Table 4). Of 50 initial culture samples taken from these 5 bags, 40 demonstrated sampling error resulting in false negative culture results. Platelet PGD Test results for these 5 bags were reactive by 24 to 72 hours after inoculation of the bag. In the single bag which failed to demonstrate bacterial growth as confirmed by a 96 hour culture sample, 9 of 10 of the initial culture samples were positive, indicating that this bag failed to grow or that it auto-sterilized in spite of initial positive culture results. Platelet PGD Test results were non-reactive 24 to 96 hours after inoculation accurately reflecting the lack of bacterial growth in this bag. Study 2 demonstrated that the Platelet PGD Test, when used following a culture-based test, was able to detect bacterial contamination when an early culture was unable to detect bacteria due to sampling error.

Table 4: LRAP Study 2 Results

Bacteria	Bacterial Concentration CFU/Bag*	Initial Culture Samples Positive	Number of Test Samples Detected at PGD Testing Time-Point (N=3)							Second Culture	
			PGD 24 hr	PGD 36 hr	PGD 48 hr	PGD 60 hr	PGD 72 hr	PGD 84 hr	PGD 96 hr	Sample Time	Result
Bacillus cereus ATCC 7064											
Bag 1	45	5 of 10	0	3	3					36 hrs	Pos
Bag 2	<4.5	0 of 10	0	3	3					36 hrs	Pos
Bag 3	<1	0 of 10	0	3	3					36 hrs	Pos
Klebsiella pneumoniae											
Bag 4	174	9 of 10	0	0	0	0	0	0	0	96 hrs	Neg**
Bag 5	<17.4	0 of 10	3	3						24 hrs	Pos
Staphylococcus epidermidis ATCC 49134											
Bag 6	26	5 of 10	NT	NT	0	0	3	3		72 hrs	Pos

* Concentration of bacteria in bag at time of inoculation

** Culture sample taken at 96 hours was negative indicating no bacterial growth or auto-sterilization of the bag.

Pos = Bacterial growth confirmed; study organism confirmed

NT = Not tested

Unless otherwise noted, bacterial strains were blood culture isolates.

LRAP Study 3 Description

The objective of Study 3 was to demonstrate that the Platelet PGD Test was able to detect bacteria in LRAP suspended in Platelet Additive Solution C (PAS-C) and plasma missed by early storage culture due to sampling error. The study was performed using two lots of Platelet PGD and three bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*). A negative control (platelet unit inoculated with sterile Phosphate Buffered Saline) was prepared and treated in the same way as the units inoculated with bacteria. Inoculated units were blind coded so that the technologist who performed PGD testing was unaware of the expected results.

Bacteria were inoculated into each bag, allowed to mix on platelet rockers for 1 to 2 hours and then sampled for initial testing by culture. Ten 8 mL samples were removed for testing by culture. One mL each sample was added to each of 4 agar plates. As the study species were not anaerobes the culture plates were incubated under aerobic conditions only. Plates were monitored for growth. An inoculated bag was excluded from further study if colonies were observed on 10 of the 10 samples (indicating no culture sampling error). If colonies were observed on fewer than 10 of the 10 samples (indicating culture sampling error), Platelet PGD testing was performed on that bag.

Samples collected at 24 hours and every 12 hours thereafter were tested with Platelet PGD Test until reactive results were observed using both replicates of both PGD Test lots. A second culture was performed on each bag at the time of the first observed Platelet PGD Test reactive result or at 96 hours if no Platelet PGD Test reactive was observed for a bag. This served to confirm the Platelet PGD Test results and the bacterial growth status of the bag.

LRAP Study 3 Results

Of 6 bags inoculated, 5 supported bacterial growth (see Table 5). Of 50 initial culture samples taken from these 5 bags, 35 demonstrated sampling error resulting in false negative culture results. Platelet PGD Test results for these 5 bags were reactive by 36 to 96 hours after inoculation of the bag. In the single bag that failed to demonstrate bacterial growth as confirmed by a 96 hour culture sample none of the initial culture samples were positive, indicating that this bag failed to grow or that it auto-sterilized. Consistent with this assessment, Platelet PGD Test results for this unit were non-reactive 24 to 96 hours after inoculation accurately reflecting the lack of bacterial growth in this bag. Study 3 demonstrated that the Platelet PGD Test, when used following a culture-based test, was able to detect bacterial contamination when an early culture was unable to detect bacteria due to sampling error.

Table 5: LRAP Study 3 Results

Bacteria	Bacterial Concentration CFU/Bag*	Initial Culture Samples Positive	Number of Test Samples Detected at PGD Testing Time-Point (N=4)							Second Culture	
			PGD 24 hr	PGD 36 hr	PGD 48 hr	PGD 60 hr	PGD 72 hr	PGD 84 hr	PGD 96 hr	Sample Time	Result
Bacillus cereus ATCC 7064											
Bag 1	10	1 of 10	0	2	4	4				48 hrs	Pos
Bag 2	100	9 of 10	0	2	4	4				48 hrs	Pos
Klebsiella pneumoniae											
Bag 3	5.75	0 of 10	0	0	0	0	0	0	0	96 hrs	Neg**
Bag 4	57.5	0 of 10	0	4	4					36 hrs	Pos
Bag 5	575	1 of 10	0	0	0	0	0	0	4	96 hrs	Pos
Staphylococcus epidermidis ATCC 49134											
Bag 6	43.5	4 of 10	0	0	0	0	0	4	4	84 hrs	Pos

* Concentration of bacteria in bag at time of inoculation

** Culture sample taken at 96 hours was negative indicating no bacterial growth or auto-sterilization of the bag.

Pos = Bacterial growth confirmed; study organism confirmed

Unless otherwise noted, bacterial strains were blood culture isolates.

WBDPp Suspended in Plasma - Study Description

The objective of the study was to confirm the Platelet PGD Test detected bacteria when a single unit of a 6-member pool contained growing bacteria. The study was performed using 10 bacteria, three lots of Platelet PGD and leukocyte reduced whole blood derived platelets.

Because of the bactericidal properties of fresh platelet rich plasma, Verax used outdated platelet derived plasma (OP) as inoculation media. Plasma was recovered by low speed centrifugation, pooled and placed into empty platelet bags. Following inoculation, the bacterial concentration (CFU/mL) at the time of inoculation was determined. Approximately 18 hours post-inoculation, initial agar plate culture (APC) was performed to confirm growth in the inoculated unit. For the 9 aerobic bacteria, if bacteria were not detected on the 18 hour plates, the cycle ended for that inoculation. Although 18 hour plates for the 20 *Clostridium perfringens* anaerobic test units did not detect viable bacteria, the cycles continued to 96 hours post-inoculation.

Approximately 36 hours post-inoculation, units were sampled and samples were pooled at a 1:5 ratio of one part sample from inoculated unit to five parts samples from PGD Non-reactive in-date platelets [equal volumes from 5 different platelet units] to prepare samples for PGD testing. Nine samples including three negative control samples were aliquotted and coded for testing. Each of 3 Platelet PGD lots was used to test 2 replicates of Test samples and 1 replicate of control sample. If all 9 PGD results were correct, the cycle ended. If less than 9 PGD results were correct, sampling and PGD testing was performed approximately every 12 hours until all PGD results were correct or until 96 hours post-inoculation.

All testing cycles ended with performance of plate culture testing either at the time of 100% PGD detection (the 9 aerobic bacteria) or at 96 hours post-inoculation (*Clostridium perfringens*). If bacteria were detected, identification testing was performed to confirm that the inoculated bacterial species was the species detected by both the Platelet PGD Test and APC.

Additional studies demonstrated that bacterial growth was comparable in fresh and outdated plasma.

WBDPp Suspended in Plasma - Study Results

Growth studies were conducted using ten bacteria. Table 6 shows the results for the nine aerobic bacteria evaluated in the WBDPp Growth Model Study. All units inoculated with bacteria that supported bacterial growth are included in the table. The table includes the bacterial concentration at Test Unit inoculation, the initial culture results based on sampling 18 hours after inoculation and the time point when six positive test samples were identified by PGD testing as containing bacteria. The shaded cell corresponds to the final use of the unit in the analysis. For all aerobic bacteria except *Staphylococcus epidermidis*, there was 100% detection by the Platelet PGD Test System within 48 hours after inoculation. Of the two units that grew *Staphylococcus epidermidis*, bacteria were detected by the Platelet PGD Test in one unit at 60 hours and in the other at 72 hours post-inoculation. APC testing and subsequent bacteria identification confirmed the Platelet PGD results agreed with APC results when bacteria grew (the 9 aerobic bacteria).

Although no bacteria were detected by culture at 18 hours post-inoculation, PGD testing was performed for the anaerobe *Clostridium perfringens* starting at 36 hours and continuing until 96 hours post-inoculation. Culture was performed again on all 96 hour samples. Platelet PGD results agreed with culture results; neither the Platelet PGD Test nor APC detected bacteria at 96 hours post-inoculation, thus demonstrating auto-sterilization.

Additional studies demonstrated that *Clostridium perfringens* was detected through a mid-10^5 CFU/mL concentration in 6-member LR WBDP sample pools prepared by spiking a sample from a single unit with bacteria before pooling with equal volumes of five samples from unspiked units and testing with the PGD Test.

Table 6: WBDPp (Plasma) Study Results

Bacteria	Unit	Bacterial Concentration (CFU/mL) at unit inoculation	Initial Culture	Number of Test Samples Detected at PGD Testing Time-Point (N= 6)			
				36 hr	48 hr	60 hr	72 hr
Bacillus cereus ATCC 7064	1	0.6	D	0	6		
Enterobacter aerogenes	2	5.4	D	6			
Escherichia coli	3	2.2	D	6			
	4	3.0	D	6			
Klebsiella pneumoniae	5	4.2	D	6			
Pseudomonas aeruginosa	6	2.0	D	0	6		
	7	3.4	D	6			
Serratia marcescens ATCC 43862	8	2.4	D	0	6		
Staphylococcus aureus ATCC 27217	9	5.0	D	0	6		
Staphylococcus epidermidis ATCC 43194	10	3.6	D	0	0	6	
	11	0.8	D	0	0	0	6
Streptococcus agalactiae ATCC 12927	12	1.4	D	6			

D = Bacteria detected
Unless otherwise noted, bacterial strains were blood culture isolates

Pre-Storage Pools of Platelets Suspended in Plasma Study 1 Description

The objective of this study was to demonstrate that the Platelet PGD Test was able to detect bacteria in pre-storage pools of platelets suspended in plasma missed by culture due to sampling error. This study was performed using two lots of Platelet PGD Test, with 2 PGD tests performed per lot, and three bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*). A negative control (platelet unit inoculated with sterile Phosphate Buffered Saline) was prepared and treated in the same way as the units inoculated with bacteria. Inoculated units were blind coded so that the technologist who performed PGD testing was unaware of the expected results.

In order to have sufficient volume of inoculated platelet for early culture testing, bacteria were inoculated at very low titer (< 200 CFU per bag) into a pool bag comprising 4 – 5 units, allowed to mix on platelet rockers for 1 to 2 hours and then sampled for initial testing by culture. Ten 8 mL samples were removed from each bag. As no anerobic organisms were inoculated 1 mL of each 8 mL sampling was added to each of 4 agar plates and incubated under aerobic conditions only. Plates were monitored for growth. An inoculated bag was excluded from further study if colonies were observed on 10 of the 10 samples (indicating no culture sampling error). If colonies were observed on fewer than 10 of the 10 samples (indicating culture sampling error), the inoculated bag was included in the study. A sterile connection was then made and 60 mL of the inoculated pool was added, 24 hours after the initial inoculation, to an uninoculated pre-storage pool bag comprising 4 - 5 platelet units suspended in plasma of the identical blood type.

Samples collected at 24 hours and every 12 hours thereafter were tested with Platelet PGD Test until reactive results were observed using both replicates of both PGD Test lots. A second culture was performed on each bag at the time of the first observed Platelet PGD Test reactive result or at 96 hours if no Platelet PGD Test reactive was observed for a bag. This served to confirm the Platelet PGD Test results and the bacterial growth status of the bag.

Pre-Storage Pools of Platelets Suspended in Plasma Study 1 Results

Of 15 bags inoculated, six did not support bacterial growth: one each *Bacillus cereus* and *Klebsiella pneumoniae* and four of eight *Staphylococcus epidermidis* inoculations. Of 90 initial culture samples taken from the nine bags that supported growth, 52 demonstrated sampling error resulting in false negative culture results. Platelet PGD Test results for these nine bags were reactive starting at 36 hours after inoculation of the bag. Time to detection was variable, ranging from 36 hours for two *B. cereus* bags to 84 hours for the third *B. cereus* bag. Similarly, *K. pneumoniae* time to detection ranged from 60 to 96 hours depending on the pool involved. Growth and detection of *S. epidermidis* was unexpectedly slow in these in-date pools, ranging from 96 to 108 hours. Exposure of low levels of bacteria to pre-storage platelet pools of 8 – 10 different platelet concentrates suspended in plasma with their variable inhibitory factors may have delayed growth. Table 7 shows the inoculation concentrations and testing results for the 9 bags that supported growth.

Table 7: Pre-Storage Pools of Platelets Suspended in Plasma Study 1 Results

Bacteria	Bacterial Concentration CFU/Bag*	Initial Culture Samples Positive	Number of Test Samples Detected at PGD Testing Time-Point (N=4)								Second Culture	
			PGD 24 hr	PGD 36 hr	PGD 48 hr	PGD 60 hr	PGD 72 hr	PGD 84 hr	PGD 96 hr	PGD 108 hr	Sample Time	Result
Bacillus cereus ATCC 7064												
Bag 1	58	2 of 10	0	0	0	0	0	4	4		84 hrs	Pos
Bag 2	40	6 of 10	0	4	4						36 hrs	Pos
Bag 3	4	2 of 10	0	4	4						36 hrs	Pos
Klebsiella pneumoniae												
Bag 4	170	4 of 10	0	0	0	4	4				60 hrs	Pos
Bag 5	17	1 of 10	0	0	0	0	0	2	4		96 hrs	Pos
Staphylococcus epidermidis ATCC 49134												
Bag 6	50	9 of 10	0	0	0	0	0	0	0		96 hrs	Pos†
Bag 7	99	8 of 10	0	0	0	0	0	0	4		96 hrs	Pos
Bag 8	9	1 of 10	0	0	0	0	0	0	0	4	108 hrs	Pos
Bag 9	29	5 of 10	0	0	0	0	0	0	0	4	108 hrs	Pos

* Concentration of bacteria in bag at time of inoculation
Pos = Bacterial growth confirmed; study organism confirmed
† Bacterial concentration at 96 hours was below PGD LoD
Unless otherwise noted, bacterial strains were blood culture isolates.

Pre-storage Pools of Platelets Suspended in Plasma Study 2 Description

The objective of this study was to confirm that the Platelet PGD was able to detect bacteria growing in pre-storage pools of platelets that are suspended in plasma. This study was performed using two lots of Platelet PGD Test, with at least 2 PGD tests performed per lot, and three bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*). A negative control (platelet unit inoculated with sterile Phosphate Buffered Saline) was prepared and treated in the same way as the units inoculated with bacteria. Inoculated units were blind coded so that the technologist who performed PGD testing was unaware of the expected results.

Bacteria were inoculated into a random donor platelet at approximately 1 – 5 CFU/mL. Approximately 24 hours after inoculation, a random donor platelet with a confirmed concentration between 1 and 10 CFU/mL was pooled with an ABO-identical pre-storage platelet pool (suspended in plasma). After mixing the pool was sampled for culture to determine bacterial viability. PGD testing was initiated 36 hours after inoculation. If at least 1 of the 4 samples was PGD-NR, testing continued to the next time point. If 4 samplings were PGD-R, another 6 samples were tested. If all 10 samples were PGD-R, PGD testing was complete; if at least 1 sample was PGD-NR, testing continued to the next time point until 10/10 samples were PGD-R or to 96 hours. In all cases culture was performed to confirm the PGD results.

Pre-storage Pools of Platelets Suspended in Plasma Study 2 Results

Table 8 shows the results for the three inoculation/pooling events that met study inclusion criteria. There was 100% detection by Platelet PGD of *B. cereus* at 36 hours, *K. pneumoniae* at 48 hours and *S. epidermidis* at 84 hours.

Table 8: Pre-storage Pools of Platelets Suspended in Plasma Study 2 Results

Bacteria	Bacterial Concentration CFU/mL*	Number of Test Samples Detected at PGD Testing Time-Point (N=4 or 10)**					Second Culture	
		PGD 36 hr	PGD 48 hr	PGD 60 hr	PGD 72 hr	PGD 84 hr	Sample Time	Result
Bacillus cereus ATCC 7064								
Bag 1	2.5	10					36 hrs	Pos
Klebsiella pneumoniae								
Bag 2	4.1	0	10				48 hrs	Pos
Staphylococcus epidermidis ATCC 49134								
Bag 3	2.8	0	0	0	0	10	84 hrs	Pos

* Concentration at time of inoculation

** If the results from testing 4 samples were PGD-R, an additional 6 samples were tested for a total of 10 samples tested.

Pos = Bacterial growth confirmed; study organism confirmed

Unless otherwise noted, bacterial strains were blood culture isolates.

Limit of Detection (Analytical Sensitivity)

Study Description

The Platelet PGD Test's limit of detection (LoD) for LRAP suspended in plasma was determined for each of the 10 organisms listed in Table 9. Testing was performed using 3 lots of Platelet PGD Test with multiple operators and samples withdrawn from multiple LRAP units and tested in replicates of 10. Dilution plate counting was used to assign a CFU/mL concentration. The CFU/mL value of the sample when the Platelet PGD Test achieved 10/10 detection was defined as the assay's LoD. For testing WBDPp suspended in plasma a three level challenge panel (bracketing the LoD) was prepared and tested using three lots of Platelet PGD Test. The panel was tested with LRAP, LR WBDPp and nLR WBDPp. The Platelet PGD Test detected bacteria equally across all three platelet types and all three lots. Table 10 shows the organisms and panel member concentration in CFU/mL at which the Platelet PGD Test detected bacteria in all three platelet types. For testing of pre-storage pools of platelets suspended in plasma and LRAP suspended in PAS-C and plasma, a new three level challenge panel (bracketing the LoD) was prepared and tested using two lots of the Platelet PGD Test. The panel was tested with LRAP suspended in plasma, LRAP suspended in PAS-C and plasma and pre-storage pools of platelets suspended in plasma. The Platelet PGD Test detected bacteria equivalently across all three platelet types and lots. Table 11 shows the organisms and panel member concentration in CFU/mL at which the Platelet PGD Test detected bacteria in all three platelet types.

Note: Preparation of panel members to achieve specific CFU/mL concentrations (e.g. LoD) is not possible.

Results

Study results are shown in Tables 9, 10 and 11.

Table 9: Limit of Detection in LRAP (plasma) (Analytical Sensitivity)

Organism	LoD (CFU/mL)
Bacillus cereus	1.2×10^4
*Clostridium perfringens** ATCC 13124	8.9×10^4
Enterobacter aerogenes	1.0×10^4
Escherichia coli	2.8×10^4
Klebsiella pneumoniae	2.0×10^4
Pseudomonas aeruginosa	8.2×10^3
Serratia marcescens ATCC 8100	8.6×10^5
Staphylococcus aureus	8.2×10^3
Staphylococcus epidermidis	9.2×10^3
Streptococcus agalactiae	5.5×10^4

*Anaerobe
Unless otherwise noted, bacterial strains were blood culture isolates.

Table 10: Confirmation of Detection in LRAP (plasma) and WBDPp (plasma)

Organism	CFU/mL
Bacillus cereus	2.4×10^4
*Clostridium perfringens** ATCC 13124	1.1×10^5
Enterobacter aerogenes	1.9×10^4
Escherichia coli	2.7×10^4
Klebsiella pneumoniae	4.1×10^4
Pseudomonas aeruginosa	4.6×10^4
Serratia marcescens ATCC 43862	8.2×10^5
Staphylococcus aureus	9.3×10^3
Staphylococcus epidermidis	2.1×10^4
Streptococcus agalactiae	6.8×10^4

*Anaerobe
Unless otherwise noted, bacterial strains were blood culture isolates

Table 11: Confirmation of Detection in LRAP Suspended in Plasma and PAS-C and plasma and Pre-storage Pools of Platelets Suspended in Plasma

Organism	CFU/mL
Bacillus cereus	2.6×10^4
*Clostridium perfringens** ATCC 13124	2.1×10^5
Enterobacter aerogenes	1.9×10^4
Escherichia coli	3.1×10^4
Klebsiella pneumoniae	5.0×10^4
Pseudomonas aeruginosa	2.1×10^4
Serratia marcescens ATCC 43862	2.3×10^6
Staphylococcus aureus	2.0×10^4
Staphylococcus epidermidis	3.4×10^4
Streptococcus agalactiae	1.8×10^5

* Anaerobe
Unless otherwise noted, bacterial strains were blood culture isolates.

Reproducibility

Reproducibility of the Platelet PGD Test was assessed using LRAP suspended in plasma and pools of WBDP suspended in plasma

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LRAP Suspended in Plasma - Study Description

The reproducibility of the Platelet PGD Test was evaluated using a 24-member Reproducibility Panel tested over a 12 day period. The panel comprised 4 negative panel members (no bacteria present) and 20 positive panel members, 2 bacterially contaminated panel members for each of the 10 bacteria listed in Table 12. Positive panel members were present in low and mid level concentrations. Five operators at four sites performed the reproducibility study using three Platelet PGD Test lots. Each panel member was tested on 6 different days, using the three Platelet PGD Test lots on each of two days. For each day of reproducibility testing, two Platelet PGD Controls were tested.

LRAP Suspended in Plasma - Results

The Platelet PGD Controls passed on all days. All Test Devices used in the reproducibility study generated valid results. A total of 720 panel members were tested. Of these:

- 600 contained bacteria
- 144 were tested by each operator
- 240 were tested using each lot
- 98.8% were concordant with expected values

Table 12: Reproducibility Panel (LRAP in Plasma)

Bacteria Panel Member	GP or GN	Level	Logs Above LoD	Number Detected (N = 30)	Detection Rate (%)
Bacillus cereus	GP	Low	0.2	30	100
		Mid	0.4	30	100
Clostridium perfringens ATCC 13124	GP	Low	0.1	25	83
		Mid	0.5	30	100
Enterobacter aerogenes	GN	Low	0.4	30	100
		Mid	1.1	30	100
Escherichia coli	GN	Low	0.2	29	97
		Mid	1.0	30	100
Klebsiella pneumoniae	GN	Low	0.4	30	100
		Mid	0.9	30	100
Pseudomonas aeruginosa	GN	Low	0.1	30	100
		Mid	0.7	30	100
Serratia marcescens ATCC 8100	GN	Low	0.6	30	100
		Mid	0.9	30	100
Staphylococcus aureus	GP	Low	0.5	28	93
		Mid	0.6	29	97
Staphylococcus epidermidis	GP	Low	0.4	30	100
		Mid	1.2	30	100
Streptococcus agalactiae	GP	Low	0.0	30	100
		Mid	0.6	30	100

Unless otherwise noted, bacterial strains were blood culture isolates.

For the 120 valid results from negative specimens, 100% gave the expected result of Non-reactive. For the bacteria panel members that were not detected, the bacterial doses in the low panel members for *Escherichia coli, Clostridium perfringens* and *Staphylococcus aureus* were within 0.2, 0.1 and 0.5 log, respectively, above the calculated LoD. The bacterial dose of *Staphylococcus aureus* contained in the mid-range panel member was within 0.6 log of the LoD. On average, the low panel members were less than 0.3 log above the LoDs while the mid-level panel members were less than 0.8 log above the LoDs.

There were no significant differences between operators or Platelet PGD Test lots at either level (p = 1.0 for MID level and p = 0.29 for LOW level) using Fisher's exact test. To determine whether there were any differences in detection rates among the three product lots, Fisher's exact test was performed for each level using the total detected and not detected. The Platelet PGD Test accurately and reliably detected low- and mid-level panel members from a diverse set of GP and GN bacteria and accurately and reproducibly identified negative panel members as non-reactive.

WBDPp Suspended in Plasma - Study Description

Reproducibility was evaluated in 6-unit post-storage pools of LR and nLR WBDP suspended in plasma using a 24-member panel tested over a 6 day period. The panel comprised 4 negative panel members (no bacteria present) and 20 positive panel members, 2 positive panel members for each of the 10 bacteria listed in Table 13. Positive panel members were present in low and mid level concentrations (approximately 0.5 and 1.5 log above LoD, respectively). A total of four operators (2 operators for LR WBDPp and 2 operators for both LR WBDPp and nLR WBDPp) at three sites performed the reproducibility study using three Platelet PGD Test lots. Each panel member was tested in a single platelet type by one operator at each test site on 6 different days using the three Platelet PGD Test lots on each of two days. Each panel member was tested a total of 18 times using LR WBDPp and 18 times using nLR WBDPp. Platelet PGD Controls were assayed on each day of testing.

WBDPp Suspended in Plasma - Study Results

The Platelet PGD Controls passed on all days. 862/864 panel members used in the reproducibility study were tested and generated valid results. Of these:

- 718 valid tests contained bacteria
- 144 panel members were tested by each operator
- 144 panel members tested using each lot and each sample type
- 99.3% concordant with expected values

See Table 13.

For the 144 valid results from negative specimens, 100% gave the expected result of non-reactive.

Fisher's exact test was performed for each level using total numbers of accurate and inaccurate results. There were no significant differences between operators (p=1.0 for both LR and nLR WBDPp) or PGD lots (p=1.0 for LR WBDPp and p=0.33 for nLR WBDPp) for either platelet type.

Table 13: Reproducibility Panel (WBDPp Suspended in Plasma)

Bacteria Panel Member	GP or GN	Level	Number Detected		Detection Rate (%)	
			WBDPp		WBDPp	
			LR (N=18)	nLR (N=18)	LR	nLR
Bacillus cereus	GP	Low	18	18	100	100
		Mid	18	18	100	100
Clostridium perfringens ATCC 13124	GP	Low	18	18	100	100
		Mid	18	18	100	100
Enterobacter aerogenes	GN	Low	18	17	100	94.4
		Mid	18	17	100	94.4
Escherichia coli	GN	Low	18	18	100	100
		Mid	17	18	94.4	100
Klebsiella pneumoniae	GN	Low	18	18	100	100
		Mid	18	17/17	100	100
Pseudomonas aeruginosa	GN	Low	17	18	94.4	100
		Mid	18	18	100	100
Serratia marcescens ATCC 43862	GN	Low	18	17	100	94.4
		Mid	18	18	100	100
Staphylococcus aureus	GP	Low	18	18	100	100
		Mid	18	18	100	100
Staphylococcus epidermidis	GP	Low	18	18	100	100
		Mid	18	17/17	100	100
Streptococcus agalactiae	GP	Low	17	18	94.4	100
		Mid	18	18	100	100
Overall			357/360	355/358	99.2	99.2

Unless otherwise noted, bacterial strains were blood culture isolates

Specificity

Specificity of the Platelet PGD Test was assessed for LRAP suspended in plasma, LRAP suspended in PAS-C and plasma, pools of up to 6 LR and nLR WBDP suspended in plasma and pre-storage pools of up to 6 leukocyte reduced whole blood derived platelets suspended in plasma.

LRAP Suspended in Plasma - Study 1 Description

Specificity was evaluated by testing 610 LRAP samples from volunteer donors using three lots of the Platelet PGD Test. Samples were cultured at the time of Platelet PGD testing and subsequently confirmed negative. Age of the LRAP units sampled and tested ranged from Day 2 through Day 5 post collection.

LRAP Suspended in Plasma - Study 1 Results

Of the 610 samples tested, 608 were non-reactive. Two initially Reactive samples were observed, but upon retest in duplicate only one sample was Repeatedly Reactive. Repeat testing included resampling and retesting by culture to confirm the absence of bacterial contamination in both samples. The specificity of the Platelet PGD Test when testing culture-negative LRAP samples was 99.7% (lower one-sided 95% confidence limit = 99.0%) when initially reactive results were used. The observed specificty on Repeatedly Reactive results was 99.8% (lower one-sided 95% confidence limit = 99.2%).

LRAP Suspended in PAS-C and plasma - Study 2 Description

Specificity was evaluated by sampling and testing 634 culture-negative PAS-C and plasma platelet units from volunteer donors using two lots of the Platelet PGD Test.

LRAP Suspended in PAS-C and plasma - Study 2 Results

Of the 634 samples, 632 were PGD non-reactive. Three initially reactive samples were observed but upon retest in duplicate, only two samples were Repeatedly Reactive (PGD-RR). The specificity of the Platelet PGD Test was 99.5% (lower one-sided 95% confidence limit = 98.8%) when initially reactive results were used. The observed specificity on Repeatedly Reactive results was 99.7% (lower one-sided confidence limit = 98.9%).

LR and nLR WBDPp Suspended in Plasma - Study 3 Description

Specificity was evaluated by testing 428 unique pools of WBDP samples (equal volumes of samples from 6 platelet concentrates) or post-storage pools representing 2,569 individual WBDP units. At least three lots of the Platelet PGD Test were used in testing. Samples were cultured at the time of Platelet PGD testing; samples that were culture negative were included in the determination of Platelet PGD Test specificity. Age of the pooled platelets sampled and tested ranged from Day 2 through Day 5 post-collection.

LR and nLR WBDPp Suspended in Plasma - Study 3 Results

Of 428 unique, confirmed culture-negative pools of samples or post-storage pools, 425 were non-reactive in the Platelet PGD Test. Three samples were repeatedly reactive. The specificity of the Platelet PGD Test when testing culture negative pools was 99.3% (lower one-sided 95% confidence limit = 98.2%). Each of the three false reactive results was attributable to a single unit represented in the sample pool tested. Of 2,569 WBDP units included in the study, three units were repeatedly reactive, yielding unit specificity of 99.9% (lower one-sided 95% confidence limit = 99.7%).

Pre-Storage Pools Suspended in Plasma - Study 4 Description

Specificity for pre-storage pooled platelets suspended in plasma was estimated from PGD test results of 1,969 unique platelet products that were culture-negative.

Pre-Storage Pools Suspended in Plasma - Study 4 Results

Of the 1,969 samples, 1,962 were PGD non-reactive. Nine initially reactive samples were observed but upon retest in duplicate only seven were PGD-RR. The specificity of the Platelet PGD Test was 99.5% (lower one sided confidence limit = 99.2%) when initially reactive results were used. The observed specificity on Repeatedly Reactive results was 99.6% (lower one sided confidence limit = 99.3%).

Potentially Interfering Substances

LRAP Study 1 - Description

All testing was performed using LRAP samples, 3 lots of the Platelet PGD Test and five operators. Platelet PGD Test Non-reactive, Gram-positive and Gram-negative samples were tested with all potential interferents listed in Table 14 except WBC concentration.

LRAP Study 2 - Description

In this study, platelet additive solution replaced 100% plasma. Two (2) lots of the Platelet PGD were used and 3 operators performed testing. This study compared results when testing platelets suspended in plasma to those of platelets suspended in 100% PAS-C with and without the presence of Gram-positive and Gram-negative bacteria. See Table 14.

LR and nLR WBDPp Suspended in Plasma - Study Description

Testing was performed using both LR and nLR WBDPp, 2-3 lots of the Platelet PGD Test and five operators. Platelet PGD Test non-reactive, Gram-positive and Gram-negative samples were tested with the potential interferents as noted in Table 14.

Table 14: Potentially Interfering Substances Tested

	Substance Tested	Substance Level
Donor Conditions	Autoimmune antibodies	ds DNA (10 - 252 IU/mL)
		ANA (Positive, qualitative test)
		RF: 13.3 - 773 IU/mL for LRAP 21 - 767.5 IU/mL for WBDP
		Heterophile antibodies: Positive (qualitative test) LRAP and WBDP
		Human anti-mouse antibody (HAMA) 11.4 - 105.0 ng/mL for LRAP 43.7 - 327.1 ng/mL for WBDP
	Hypergammaglobulinemia	IgA (522 - 2470 mg/dL)
		IgG (2030 - 5050 mg/dL)
		IgM (275 - 4550 mg/dL)
	Lipemia	305 - 576 mg/dL
	Hypercholesterolemia	389 - 830 mg/dL
	Hyperproteinemia	> 10 g/dL
	Hypoproteinemia	1.4 - 5.6 g/dL
Sample Conditions	Hemolysis	0 - 350 µg/dL for LRAP 0 - 250 µg/dL for WBDP
	pH	5.5 - 8.5 for LRAP ~5.9 - ~8.2 for WBDP
	Platelet concentration (% normal/native)	50% - 200% average concentration for LRAP and WBDP
	Red blood cells (concentration in %)	0 - 0.35% RBC concentration LRAP 0 - 0.7% RBC concentration WBDP
	White blood cells	~5 x 10^4 - 3 x 10^5 cells/mL for WBDP
	Platelet Additive Solution	0 and 100% for LRAP

Results

With the exception of Rheumatoid Factor (RF) and heterophile antibody samples and one HAMA sample, there were no effects of the substances/conditions tested on performance of the Platelet PGD Test. All Platelet PGD Test Non-reactive samples remained non-reactive in the presence the substances. All reactive samples except for one replicate (1/600) of one *E. coli* sample containing RF remained reactive in the presence of the substances.

For LRAP suspended in plasma seventy-one percent (71%) of the samples tested containing Rheumatoid Factor (RF) as a potential interferent gave expected results. Twenty-nine percent (29%) of the samples tested with a Rheumatoid Factor (RF) ≥16.9 IU/mL resulted in falsely reactive Platelet PGD Test results. One hundred percent (100%) of the samples tested containing heterophile antibodies as a potential interferent gave expected results and one hundred percent (100%) of the samples tested containing human anti-mouse antibody (HAMA) as a potential interferent gave expected results.

Performance when testing LRAP suspended in 100% plasma and 100% platelet additive solution was assessed in culture-negative platelets. All culture-negative samples were PGD-NR in both sample types. All reactive samples were detected in both platelets suspended in plasma and platelets suspended in platelet additive solution.

For WBDP suspended in plasma seventy-six percent (76%) of the samples tested containing Rheumatoid Factor (RF) as a potential interferent gave expected results. Twenty-four percent (24%) of the samples tested with a Rheumatoid Factor (RF) ≥ 33.1 IU/mL resulted in falsely reactive Platelet PGD Test results. Eighty-four percent (84%) of the samples categorized as containing heterophile antibodies as a potential interferent gave expected results. Sixteen percent (16%) of the samples with heterophile antibodies resulted in falsely reactive Platelet PGD Test results. Only 1 of 57 samples tested containing HAMA ≥ 43.7 ng/mL gave a falsely reactive Platelet PGD Test result.

There is no standard or objective method of characterizing samples as containing heterophile antibodies. For example, sample supplier A may categorize a sample with RF > upper limit of normal as an RF sample and sample supplier B may categorize that same sample as a heterophile antibody sample.

Prozone (Hook Effect)

Study Description

High titer, bacteria-inoculated platelet samples were tested in the Platelet PGD Test in order to assess whether excess bacterial antigen would yield false Non-reactive results; see Table 15. Dilution plate counting was used to confirm the sample concentration in CFU/mL. A total of 218 assays were performed using LRAP in plasma and three Platelet PGD Test lots. For WBDP in plasma, 584 assays were performed across three Platelet PGD Test lots. For LRAP in PAS-C and plasma and pre-storage pools of leukocyte reduced whole blood derived platelets suspended in plasma, 202 and 201 assays, respectively, were performed across two Platelet PGD Test lots.

Table 15: Prozone

Bacteria	Maximum Concentration (CFU/mL)			
	LRAP (plasma)	LRAP (PAS-C and plasma)	WBDPp (plasma)	Pre-storage Platelet Pools (plasma)
Bacillus cereus	4.7×10^8	6.2×10^7	1.8×10^8	6.2×10^7
Clostridium perfringens ATCC 13124	1.8×10^9	9.1×10^6*	1.1×10^8	9.1×10^6*
Enterobacter aerogenes	8.9×10^9	2.6×10^9	4.0×10^9	2.6×10^9
Escherichia coli	9.2×10^9	1.4×10^9	1.5×10^9	1.4×10^9
Klebsiella pneumoniae	1.8×10^9	2.4×10^9	2.4×10^9	2.4×10^9
Pseudomonas aeruginosa	1.1×10^9	2.2×10^9	1.7×10^9	2.2×10^9
Serratia marcescens ATCC 43862, ATCC 8100	2.0×10^9	5.3×10^9	1.3×10^9	5.3×10^9
Staphylococcus aureus	2.1×10^9	4.3×10^9	2.1×10^9	4.3×10^9
Staphylococcus epidermidis	8.2×10^9	1.3×10^9	1.1×10^{10}	1.3×10^9
Streptococcus agalactiae	7.7×10^9	7.4×10^{10}	7.0×10^9	7.4×10^9 †

Unless otherwise noted, bacterial strains were blood culture isolates.

* At 9.1×10^7 CFU/mL, 9 of 10 LRAP samples suspended in PAS-C and plasma yielded valid PGD results and were detected; one sample resulted in an invalid test. When 10 samples of pre-storage pools of platelets suspended in plasma were tested, 8 of 10 yielded valid PGD results and were detected; 2 samples resulted in invalid tests.

† At 7.4×10^{10} CFU/mL, 8 of 10 samples of pre-storage pools of platelets suspended in plasma yielded valid PGD results and were detected; 2 samples yielded invalid results.

Results

At concentrations above 6.2×10^7 and up to 7.4×10^{10} (the highest concentrations that yielded consistently valid assays*), the Platelet PGD Test correctly detected the presence of bacteria. There were no false negative results for any of the 10 bacteria tested.

*(During testing, most samples containing > 1.2×10^{10} CFU bacteria/mL and samples of *B. cereus* at 6.2×10^8 and *C. perfringens* at 9.1×10^8 were obviously viscous, cloudy and milky and resulted in invalid assays.)

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Keys to Symbols Used

REF	List Number
IVD	*In vitro* diagnostic medical device
LOT	Lot Number
	Expiration Date
	Temperature limitations
	Do not re-use
	Attention, see instructions for use
	Manufacturer
PGD Test Device	Platelet PGD Test Device
Reagent 1	Reagent 1
Reagent 2	Reagent 2
Reagent 3	Reagent 3
Disposable Pipettes	Disposable Pipettes
Microfuge Tubes	Microfuge Tubes
CONTROL -	Negative Control
CONTROL +	Positive Control
	Consult instructions for use

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