Platelet PGD*prime*® Test

Rx Only

A. INTENDED USE

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

B. INDICATIONS FOR USE

The Platelet PGD*prime* 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 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 hours of transfusion) of up to six 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 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.

In March 2004, the AABB implemented Standard 5.1.5.1, which required its members to use a method to limit and detect bacterial contamination in platelet components.[2,3] The requirement to control bacterial risk was extended and formalized to apply also to hospital transfusion services by revising regulations governing Good Manufacturing Practices for Blood and Blood Components (21 CFR §606.145) in May 2016.

To satisfy Standard 5.1.5.1, US blood centers and some hospital transfusion services began implementation of 100% Quality Control (QC) testing for bacteria in platelets using growth-based testing systems that were cleared by the FDA for quality control testing of platelet components. Sampling for this QC testing typically occurred 24 hours post collection. 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] The most likely cause for this phenomenon has been identified as sampling error associated with sampling early in unit life, 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].

Another strategy to control bacterial contamination in platelets is testing nearer the time of transfusion. Testing on the day of transfusion has been shown to detect bacterial contamination in platelets after they were distributed to hospitals as negative for bacteria.[10,11] On the day of transfusion, bacteria have likely entered 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.

Like its predecessor, the Platelet PGD® Test, the Platelet PGD*prime* 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 including 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.[12,13] 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.[14,15] By combining the detection of LTA, LPS and other bacterial antigens in a single Test Device, it is possible to detect the bacterial species most frequently implicated in contaminated platelet samples.[16,17]

D. PRINCIPLES OF THE PROCEDURE

The Platelet PGD*prime* Test is a single-use, lateral flow, qualitative immunoassay comprising Reagents, Controls, Disposables and a Test Device containing a test strip specific for the detection of aerobic and anaerobic GP and GN bacteria. Samples from LRAP units suspended in 100% plasma, LRAP suspended in plasma/PAS-C, pre-storage pools of LR whole blood derived platelets, post-storage pools of up to six LR and nLR whole blood derived platelets and individual units of LR and nLR whole blood derived platelets may be tested. Platelet samples are mixed with the sample pretreatment reagent (Reagent 1A) and then brought to the proper testing pH by the addition of the neutralizing detection reagent (Reagent 1B). The processed sample is transferred to Well 1 on the Test Device. As the sample migrates through the test strip, bacteria present in the sample will interact with and bind to GP or GN bacteria-specific biotin-labeled detector antibodies to create biotin-labeled sandwich complexes with capture antibodies immobilized on the nitrocellulose membrane. Upon the addition of buffer reagent (Reagent 2) to Well 2 on the Test Device, streptavidin-coated gold particles will be released and will flow across the nitrocellulose, “chasing” the processed sample. The streptavidin-coated gold will bind to the biotin-labeled sandwich complexes formed in the presence of bacteria in the capture zones, creating a visible red/pink line in the zone(s) where bacteria have been captured. When the sample, detector antibodies and streptavidin gold have all reached the end of the Test Device, an assay procedural control line (C) will become visible in the Procedural Control Window. When the background of the Test Result Window has cleared of pink staining and the control line has formed, the test is valid and can be interpreted by visual examination of the GP and GN Test Result window. Refer to **INTERPRETATION OF RESULTS**.

E. REAGENTS AND MATERIALS

Materials Provided	Quantity	REF
Platelet PGD*prime* Test	100 Tests	PRM100
Platelet PGD*prime* Test	20 Tests	PRM20

Includes the following:

15 °C – 30 °C	20 Test	100 Test	2 °C – 8 °C	20 Test	100 Test
PGD*prime* Test Device	20 each	100 each	Reagent 1A	1 x 10 mL	2 X 10 mL
Sample Pipettes	20 each	100 each	Reagent 1B	1 x 10 mL	2 X 10 mL
Transfer Pipettes	20 each	100 each	Reagent 2	1 x 10 mL	2 X 10 mL
Processing Tubes	20 each	100 each			

Reagents

PGD*prime* Test Device Conjugate Pad: Gold colloid coated with streptavidin. Detector Antibody Pad: biotinylated rabbit polyclonal and mouse monoclonal antibodies and protein (bovine) stabilizer dried in sucrose. Nitrocellulose: mouse monoclonal antibody and rabbit polyclonal antibodies. Preservative: sodium azide

Reagent 1A Water, sodium hydroxide and surfactants.

Reagent 1B HEPES and Tricine buffers, surfactants and protein (bovine, mouse and rabbit) stabilizers, biotinylated rabbit polyclonal and mouse monoclonal antibodies.
Preservatives: ProClin 300® and sodium azide.

Reagent 2 Phosphate buffer and surfactants.
Preservative: sodium azide.

See **Reagent Precautions** below.

Materials Required and Available Separately	**Quantity**	REF
Platelet PGD*prime* Controls	30 Tests	PRM30C

Materials Required But Not Provided

1. Sterile connecting device or tubing stripper, tube sealer and alcohol pad
2. Clean secondary sample tubes with caps, maximum volume 3.0 mL
3. Vortex mixer for processing non-leukoreduced samples
4. Timer
5. Personal protective equipment
6. Bio-hazard waste equipment

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*prime* 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 six LR WBDP suspended in plasma and LR and nLR WBDP suspended in plasma. Except for the Interfering 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*prime* 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 four hours of transfusion of WBDP suspended in plasma.
4. Perform the test in a well-lighted area.
5. Each operator performing the test must be able to distinguish between the following colors: Yellow, Blue and Red.
6. Do not use materials after their stated expiration dates.

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 1A  Warning

H315 – Causes skin irritation.
H319 – Causes serious eye irritation.
P264 – Wash hands, forearms and exposed areas thourpoughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 – IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 – If skin irritation occurs: Get medical advice/attention.
P337+P313 – If eye irritation persists: Get medical advice/attention.
P501 – Dispose of contents/container according to local, regional, national, territorial, provincial, and international regulations.

Reagent 1B  Warning

H315 – Causes skin irritation.
H319 – Causes serious eye irritation.
P264 – Wash hands, forearms and exposed areas thoroughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 – IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 – If skin irritation occurs: Get medical advice/attention.
P337+P313 – If eye irritation persists: Get medical advice/attention.
P501 – Dispose of contents/container according to local, regional, national, territorial, provincial, and international regulations.

Reagent 2  Warning

H315 – Causes skin irritation.
H319 – Causes serious eye irritation.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 – IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 – If skin irritation occurs: Get medical advice/attention.
P337+P313 – If eye irritation persists: Get medical advice/attention.
P501 – Dispose of contents/container according to local, regional, national, territorial, provincial, and international regulations.

Reagents 1B and 2 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.[18,19,20,21] 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 1A, 1B, 2 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*prime* Test Device if the pouch has been compromised.
8. Use the PGD*prime* Test Device once and dispose of properly after use (see **General Safety Precautions**). Do not re-use Sample Pipettes, Transfer Pipettes or Processing Tubes.
9. Use only the Sample Pipettes, Transfer Pipettes and Processing Tubes provided with the Platelet PGD*prime* Test. Use of other disposables when performing the test may result in inaccurate results.
10. Do not touch Wells 1 or 2 or the GP/GN Test Result Window of the PGD*prime* Test Device.
11. Read test results in a well-lighted area.
12. Disinfect testing area regularly to avoid accidental contamination.

G. STORAGE INSTRUCTIONS

1. Store Platelet PGD*prime* Test Devices at 15 – 30 °C. Do not open the PGD*prime* Test Device pouch until time of use. Use Test Devices within 30 minutes after opening pouch.
2. Store Processing Tubes, Sample Pipettes and Transfer Pipettes at 15 – 30 °C.
3. Store Platelet PGD*prime* 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*prime* Controls to perform as expected may indicate deterioration of the Reagents or the PGD*prime* Test Device.

I. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Sample 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*prime* Test is performed on the pool prior to filtration.
5. Additional samples will be required for retesting in duplicate following initially reactive PGD*prime* results and for retesting at least once following invalid PGD*prime* results. For further information, refer to **INTERPRETATION OF RESULTS** and the Testing/Interpretation Guideline.
6. Place samples in labeled, clean secondary sample tubes. Samples may be held at 15 – 30 °C for up to two hours prior to testing. Cap tubes if samples are not immediately tested. 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. 200 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 the platelet unit is well mixed prior to sampling. Collect platelet samples using sterile procedures in order to maintain a closed system.

<u>If sampling with sterile connecting device</u>, refer to the device manufacturer's instructions.

<u>If sampling from a freshly created segment:</u>

- 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 2 – 3 inches long (~7 cm), i.e., sufficient length to yield a 200 µL sample.
- Cut segment from remainder of tubing with clean cutting instrument that has been wiped with an alcohol pad.
- Drain fresh sample into a clean secondary sample tube by cutting ends of the segment with a clean cutting instrument.

J. TEST PROCEDURE

Pretesting 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. Hold Processing Tube in a gloved hand while dispensing Reagents by dropper bottle. Invert the Reagent bottle vertically directly above the Processing Tube while dispensing drops. Hold dropper tip at least ½ inch above tube
3. Do not allow exposed dropper tips to come in contact with Processing Tubes or other surfaces.
4. Process samples and Controls in a continuous fashion once sample processing has started.

Control Processing

1. Label one Processing Tube as the Negative Control. Pick up labeled tube and add 6 drops of Negative Control.

2. Label a second Processing Tube as the Positive Control. Pick up labeled tube and add 6 drops of Positive Control.

3. Pick up one of the Processing Tubes and add 6 drops of Reagent 1A.

Immediately after adding Reagent 1A, cap and invert the Processing Tube 2 to 3 times to mix. The solution will be yellow.

4. Repeat step 3 for the second Processing Tube.
   Wait 2 minutes (but no longer than 5 minutes) and then proceed to Step 5.

5. Uncap and pick up one of the Processing Tubes. Add 6 drops of Reagent 1B; then cap the tube and invert 2 to 3 times. Repeat for the second Processing Tube.

6. Solution will turn orange or red-orange. Proceed directly to **Performing the Test**

Sample Processing

Perform the following steps for each platelet sample to be assayed.

1. Label a Processing Tube to identify the platelet sample to be tested. Use a Sample Pipette (from the clear bag) to add sample to the labeled Processing Tube.

   To use the Sample Pipette, squeeze the upper bulb and immerse the barrel into the sample. Release pressure slowly to fill the barrel completely and overflow sample into the lower bulb. Ensure the barrel is completely full. Slowly squeeze the upper bulb again to dispense the volume in the barrel into the Processing Tube. Note: Only the volume in the barrel will be dispensed.

   Properly dispose of Sample Pipette after transfer.

2. Pick up the Processing Tube**.** Invert the bottle of Reagent 1A vertically directly above the Processing Tube and dispense 6 drops of reagent.

3. Before proceeding to the next sample, cap the Processing Tube and mix by inversion 2 to 3 times. If processing non-leukocyte reduced samples, vortex the sample 2-3 seconds to mix instead of inverting.

   The sample will turn blue to blue-green after adding Reagent 1A and mixing. After 2 minutes but no longer than 5 minutes, proceed to the next step (Step 4).

   NOTE: If the sample does not show 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*prime* Test. Do not proceed to the next step (Step 4) if the solution does not turn the appropriate color.

4. Uncap and pick up the Processing Tube. Invert the bottle of Reagent 1B, hold directly above the Processing Tube and dispense 6 drops of reagent.

5. Before proceeding to the next sample, recap the Processing Tube and mix by inversion 2 to 3 times. The solution will turn red or pink after adding Reagent 1B and mixing.

   NOTE: If processing non-leukocyte reduced samples, vortex the sample 2-3 seconds instead of inverting.

   If the sample does not show 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*prime* Test.

   (a) The intensity of the color may vary ranging from a red to pink color.
   (b) If the solution is purple, do **NOT** proceed. Process a new sample.

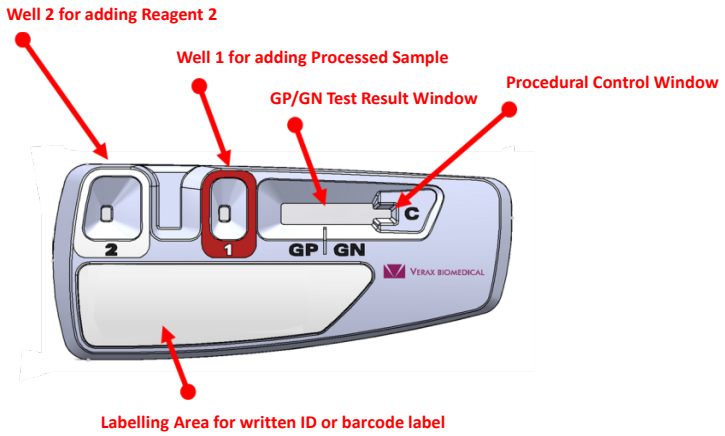
   Proceed directly to **Performing the Test.**

Performing the Test

Perform the following steps for each processed sample or Control to be analyzed on the Platelet PGD*prime* Test.

1. Tear open the notched end of the pouch and remove the PGD*prime* Test Device. Verify that a desiccant is present in the pouch. If a desiccant is not present, obtain a new PGD*prime* Test Device. Inspect GP/GN Test Result Window for surface imperfections. The surface should be smooth and white. See Figure 1.

Figure 1: PGD*prime* Test Device Features

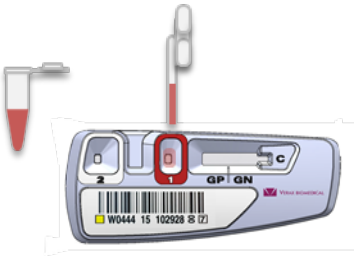


NOTE: PGD*prime* Test Device should be used as soon as possible after the pouch is opened but may be used up to 30 minutes after opening.

2. Place the PGD*prime* Test Device on a flat surface. Use a marker or barcode to label the PGD*prime* Test Device to identify the sample or Control being added. :

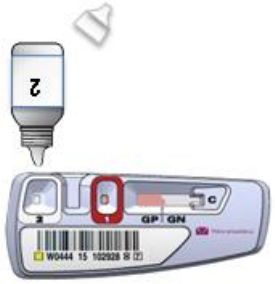
   NOTE: Do not move or pick up the PGD*prime* Test Device after sample is added to Well 1. The PGD*prime* Test Device cannot be moved until the test is completed and valid.

Use a **Transfer Pipette** from the red bag to add the processed sample or Control to Well 1 of the device.



3. To use the **Transfer Pipette**, squeeze the upper bulb and immerse the barrel into the **Processing Tube**. Release pressure slowly to fill the barrel completely and overflow sample into the lower bulb. Make sure the barrel is full. Slowly squeeze the upper bulb again to dispense the volume in the barrel. Note: Only the volume in the barrel will be dispensed.

Watch for sample flow, as indicated by red color movement across the GP/GN Test Result Window. When the front of the red sample liquid has flowed 1/4 to 3/4 of the way across the GP/GN Test Result Window, add 6 drops of Reagent 2 to Well 2.



4. Set a timer for 25 minutes and start it after Reagent 2 has been added to the PGD*prime* Test Device.

5. When the 25 minute timer goes off, examine the device to confirm that:

   - The bottom of Well 1 is red/pink and there is no sample remaining in Well 1.
   - The bottom of Well 2 is white/light gray.

   If these two criterial are not met, the device is invalid. Repeat the test.

   Then confirm that:

   - The Procedural Control line is easily discernible as a strong pink/red line. A faint pink line is not acceptable.
   - The GP/GN Test Result Window is white/light gray.

   If these criteria are not met, recheck device(s) at 5 – 10 minute intervals, until validity criteria are met or a total of 45 minutes have passed.

When these criteria are satisfied, the test is valid and should be read and interpreted. Refer to Section **L. INTERPRETATION OF RESULTS.** Record results according to your laboratory's requirements.

If these criteria are not met within 45 minutes, the test is invalid. Repeat the test with a fresh sample and a new PGD*prime* Test Device.

After interpretation and recording test results, dispose of used PGD*prime* Test Device in a bio-hazardous waste container.

K. QUALITY CONTROL

Platelet PGD*prime* Controls (Negative and Positive) are for use only with the Platelet PGD*prime* Test. The Platelet PGD*prime* Controls are used to ensure the User's ability to properly perform and interpret the test. Platelet PGD*prime* Controls are also used to verify the performance of the Platelet PGD*prime* Test. Test the Platelet PGD*prime* 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*prime* 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*prime* Test Device has a built-in Procedural Control that is used to verify assay validity. This Procedural Control line (C) must appear as a strong pink/red line for the test to be valid. A faint pink line is unacceptable.

The bottom of Well 1 must be red/pink with no liquid sample remaining in the well. The bottom of Well 2 must be white/light gray (not red) for the test to be valid.

The background of the GP/GN Test Result Window must be white/light gray and clear of pink color for the test to be valid.

Evaluate the GP/GN Test Result Window for the presence or absence of red/pink GP and GN detection lines. There are 6 capture zones within the Test Result Window (3 GP and 3 GN). Detection lines will be discrete vertical lines that extend from top to bottom of the window. The color of the line may range from extremely light pink to a dark red color and may not be the same intensity from top to bottom. Consider any discrete red/pink line within the GP/GN Test Result Window as reactive, no matter how faint the line. Do not confuse spots or streaks with Reactive test results. Refer to **Sample Results**, **Control Results**, and **Invalid Results** sections of the **Testing/Interpretation Guideline**, below, and Figures 2, 3 and 4.

When a red/pink detection line is observed in the GP/GN Test Result Window, repeat the test in duplicate using a new sample and new PGD*prime* Test Devices. If the 2 retests yield Non-reactive results, the sample is interpreted as Non-reactive. If one or more red/pink lines are observed in the initial and at least one repeat PGD*prime* 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 required when valid assays yield Non-reactive results.

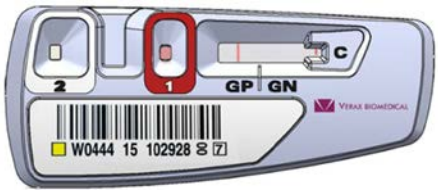
If the Procedural Control line fails to appear as a strong red/pink line, sample remains in Well 1 or Well 2 is not the appropriate color or if the Test Result Window fails to clear to white/light gray, the test is invalid. An invalid result cannot be interpreted. Repeat the test using a new sample and new Platelet PGD*prime* Test Device.

Testing/Interpretation Guideline

If Initial PGD*prime* Result is:	**Then:**	**Retest Result(s)**	**PGD*prime* 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 Verax Technical Support

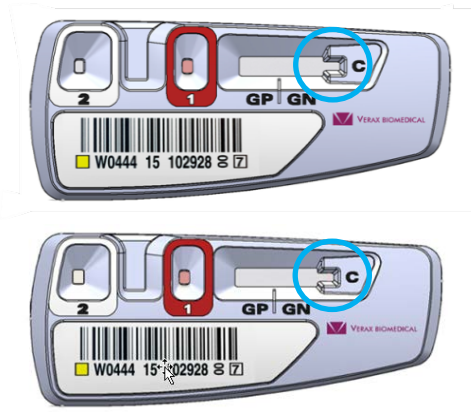
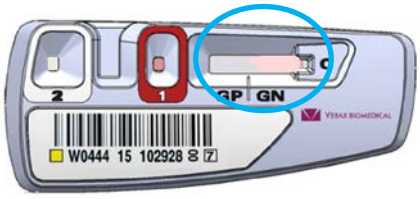
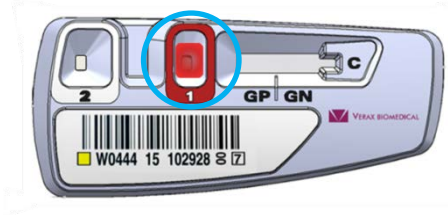
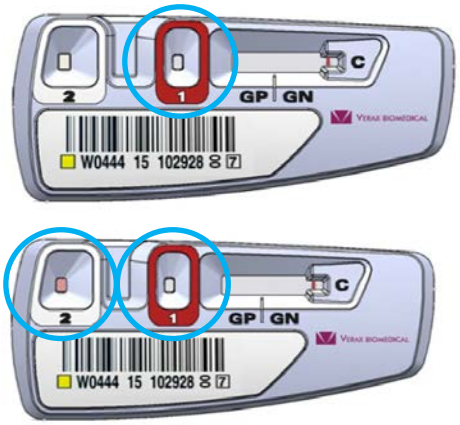
It is recommended that all platelet co-components be retested when a Repeatedly Reactive unit is detected. When Repeatedly Reactive doses are found, notify the platelet provider and determine appropriate follow-up actions such as confirmatory culture and bacterial identification.

Sample Results

NON-REACTIVE Result	REACTIVE Results
	 GP Reactive Result  GN Reactive Result
• No detection lines visible in GP/GN Test Result Window • Background of GP/GN Test Result Window is clear of pink staining • Strong pink/red line in Procedural Control window • Red color in the bottom of Well 1 with no liquid remaining in the well • White/light gray color in the bottom of Well 2 **Figure 2**	• One to six detection lines visible in the GP/GN Test Result Window • Strong pink/red line in Procedural Control window • Red color in the bottom of Well 1 with no liquid remaining in the well • White/light gray color in the bottom of Well 2 • When a detection line is observed, repeat the test in duplicate using a new sample and new Test Devices. If a detection line is observed in the initial and at least one repeat test, the interpretation is PGD*prime* Repeatedly Reactive.

NEGATIVE CONTROL	POSITIVE CONTROL
	
• No detection lines visible in GP/GN Test Result Window • Background of the GP/GN Window is clear of pink staining • Strong pink/red line in Procedural Control Window • Red/orange color in the bottom of Well 1 • White/light gray color in the bottom of Well 2 **Figure 3**	• Six detection lines present in GP/GN Test Result Window • Strong pink/red line in Procedural Control window • Lines are discrete and extend fully from the top to the bottom of the GP/GN Test Result Window • Red/orange color in the bottom of Well 1 • White/light gray color in the bottom of Well 2

Invalid Results

Absent or extremely light Procedural Control line  	Failure of Test Result Window to clear of pink staining to allow interpretation of results 
Liquid sample remains in the sample well (Well 1)  	Bottom of Well 1 or Well 2 is the wrong color 
Figure 4	
An **Invalid** result cannot be interpreted. When an **Invalid** result occurs, repeat the test using a new sample and new PGD*prime* Test Device. Contact Technical Support for assistance if valid results are not obtained after 2 retests.	

M. LIMITATIONS

1. The Platelet PGD*prime* Test is for use in LRAP and leukocyte reduced pre-storage pools of up to six WBDP units 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 hours of transfusion) and for single units of WBDP suspended in plasma and tested within four 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*prime* 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 25 minutes or more than 45 minutes after initiation of sample flow may yield inaccurate results.
4. Do NOT use the Platelet PGD*prime* Test on clumped or coagulated platelet samples.
5. Samples containing fibrin may result in extended sample flow times and/or invalid assays.
6. 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).

7. For REACTIVE test results, intensity of the test line does not correlate to the titer of bacteria in the sample.
8. 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. contamination is by *Acinetobacter* spp. bacteria since antigens of *Acinetobacter* spp. are not specifically targeted by corresponding antibodies used in the test.
9. For potentially interfering substances and prozone (hook effect) refer to the appropriate section

N. PERFORMANCE CHARACTERISTICS

Evaluation of PGD Technology in a Population study of Leukocyte Reduced Apheresis Platelets tested as negative by growth-based methods

A large study using the original Platelet PGD Test (hereinafter also referred to as the PGD Test) demonstrated the ability of Pan Genera Detection technology to detect bacteria in platelets for transfusion.[11] Results of this study formed the foundation for designation of the PGD Test as a "safety measure." A description and summary of the results of this study follow.

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 Platelet 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 testing 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). If a detection line was observed, PGD testing was repeated on a new sample 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 ≥ 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 most 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 the 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, 95% confidence interval [CI]: 1:6,711 – 1:1,617; or 326/million units, 95% CI 149/million – 618/million]. Four of the 9 contaminated doses detected were day 3 platelets, 2 were day 4 and 3 were ≥ 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 (LTA Type IV), which was not targeted by antibodies used in the original Platelet PGD Test but is targeted by Platelet PGD*prime* antibodies) at 2×10^7 CFU/mL.[22,23] 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 approximately 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.

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 nine 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%, 95% CI [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%, 95% CI [0.43% - 0.61%].

Limit of Detection (Analytical Sensitivity)

Study Description
The limit of detection (LoD) of the Platelet PGD Test was established for LRAP suspended in plasma was determined for each of the 10 organisms listed in Table 3. 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.

The Limit of Detection of the PGD*prime* Test for each of these 10 organisms was established by testing a three level panel that was designed to bracket the LoD established for the PGD Test. Preparation of panel members to achieve specific CFU/mL concentrations is not possible. Each panel member was added to 10 LRAP samples and tested using three lots of PGD*prime* Test and one lot of PGD Test, which served as a control. The PGD*prime* LoD was confirmed as the CFU/mL at which each lot achieved 100% detection, i.e., 10/10 for each lot. The LoD of the PGD Test was confirmed when 10/10 tests gave reactive results. Table 4 shows the organisms and CFU/mL concentrations at which the Platelet PGD*prime* Test and the Platelet PGD Test showed 100% detection.

The LoD for *Streptococcus oralis* was estimated by preparing a bacterial stock, making serial dilutions in LRAP (plasma) and then performing Dilution Plate counts on the dilutions to assign CFU/mL values. PGD*prime* testing was performed on the dilutions to determine the lowest reactive dilution. This dilution was then further tested in 3 replicates in each of 3 lots of PGD*prime*. When all 10 PGD*prime* results were Reactive, the CFU/mL of the tested dilution was established as the LoD. The LoD for *Streptococcus oralis* was 1.95 x 10^6 CFU/mL For further testing, a *Streptococcus oralis* panel member targeted to fall within 0.5 Log of the LoD was prepared. The CFU/mL of the panel member is shown in Table 4.

Additional testing was performed to assess and compare detection of the 11 organisms across platelet types: LRAP in plasma, LRAP in PAS-C/plasma, LR WBDP (pre-storage pools), and nLR WBDP post-storage pools. Each mid-level panel member was added to samples from 10 LR WBDP pools, 7 LRAP in PAS-C/plasma, 6 nLR WBDP pools and 9 LRAP in plasma, which served as control samples. Three lots of PGD*prime* Test were used. Detection was compared across platelet types. There was 100% detection across lots and platelet types.

Table 3: PGD Limit of Detection in LRAP (plasma) (Analytical Sensitivity)

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

Table 4: Confirmation of PGD*prime* and PGD Detection in LRAP (plasma)

Organism	PGD*prime* CFU/mL	Platelet PGD CFU/mL
Bacillus cereus	2.7 x 10^4	2.7 x 10^4
*Clostridium perfringens** ATCC 13124	2.4 x 10^5	2.4 x 10^5
Escherichia coli	5.6 x 10^4	5.6 x 10^4
Klebsiella aerogenes	3.3 x 10^4	3.3 x 10^4
Klebsiella pneumoniae	6.1 x 10^4	6.1 x 10^4
Pseudomonas aeruginosa	2.6 x 10^3	1.7 x 10^4
Serratia marcescens ATCC 43862	2.5 x 10^6	2.5 x 10^6
Staphylococcus aureus	2.1 x 10^3	1.8 x 10^4
Staphylococcus epidermidis	1.9 x 10^3	2.7 x 10^4
Streptococcus agalactiae	1.6 x 10^5	1.6 x 10^5
Streptococcus oralis	2.5 x 10^6	**

* Anaerobe

** Non-reactive using the Platelet PGD Test

Unless otherwise noted, bacterial strains were isolates from blood cultures or recovered from platelet contamination events.

Reproducibility of Detection

During the Limit of Detection Study, 1356 PGD*prime* test results were generated using 3 lots of PGD*prime* Test, 11 bacterial species and 4 platelet types. The Platelet PGD*prime* Test detected bacteria accurately and reproducibly across lots and platelet types.

Specificity

Study Description

Specificity of the Platelet PGD*prime* Test was assessed for LRAP suspended in plasma (LRAP); LRAP suspended in PAS-C and plasma (PAS); LR-WBD platelet units (LR WBDP) and nLR WBD platelet units (nLR WBDP), some of which were also combined and tested as post-storage pools (LR-WBDPp and nLR WBDPp); and prestorage pools of LR WBDP (PSP). Age of platelets tested in the study ranged from 2 – 6 days post-collection. The Specificity study was performed at multiple sites. Three lots of PGD*prime* were used and lots were evenly distributed across sites.

To be included in the Specificity Study, each platelet sample had to have both a PGD*prime* result and a negative culture result by traditional agar plate culture (APC). Bacterial status of each unit was determined by APC. If no colonies were observed under either aerobic or anaerobic conditions after 3 – 7 days, the unit was deemed to be bacteria-negative.

Repeat testing was performed for samples with initial invalid (INV) or initial reactive (IR) test results. The study protocol specified performing a single repeat test for any sample with an INV result and required two repeat tests for a sample with an IR result (GP or GN or both). Interpretations of PGD*prime* results were made as follows (using only valid tests):

- Repeatedly Reactive (PGD*prime*-RR) if the initial and at least one of the two repeat PGD*prime* tests were reactive.
- Non-reactive (PGD*prime*-NR) if the initial PGD*prime* test was Non-reactive or if the initial PGD*prime* test was reactive but both repeat PGD*prime* tests were Non-reactive.

Specificity was calculated for each platelet type as the percent of culture-negative units that would lead to a PGD*prime*-NR interpretation. One-sided 95% confidence limits were calculated using the Wilson method. Table 5 summarizes the Specificity observed for PGD*prime* with various platelet types. Out of 3,800 unique samples, 5 were initially reactive (IR), 4 were NR upon retest. One sample was classified as indeterminate (IND) as only one repeat test was performed by the test site.

Table 5: Summary of PGD*prime* Results by Platelet Type

Unit Type	Results			All	Initial Specificity		Final Specificity	
	IR	NR	IND		Estimate	Lower 1-Sided 95% CL	Estimate	Lower 1-Sided 95% CL
LRAP	1	1598	0	1599	99.9%	99.7%	100.0%	99.8%
PAS	2	295	0	297	99.3%	98.0%	100.0%	99.1%
LR WBDP	0	611	0	611	100.0%	99.6%	100.0%	99.6%
LR WBDPp	0	75	0	75	100.0%	96.5%	100.0%	96.5%
nLR WBDP*	1	501	1	503	99.6%	98.8%	99.8%	99.1%
				502			100.0%	99.5%
nLR WBDPp	1	64	0	65	98.5%	93.4%	100.0%	96.0%
PSP	0	650	0	650	100.0%	99.6%	100.0%	99.6%
All*	5	3794	1	3800	99.8%	99.7%	100.0%	99.9%
							100.0%	99.9%

* Reported both with (N = 503) and without (N = 502) the sample classified as IND because the IR for that sample was only followed up by a single additional test making it impossible to interpret as either NR or RR.

Analytical Growth Model Studies for Bacterial Detection in Platelets

Analytical Growth Study Description

The ability of the Platelet PGD*prime* Test to detect bacteria growing in platelets was evaluated by determining the time to bacterial detection. Three lots of Platelet PGD*prime* were used in the study. All bacterial species and conditions were tested in duplicate with each lot. Platelet units were inoculated at the bacterial levels listed in Table 6. Neither *Clostridium perfringens* nor *Streptococcus oralis* grew reliably in LRAP units, and therefore, were not included in this study.

Platelet units were aliquoted in volumes of at least 50 mL into multiple, smaller platelet bags. At initiation (T=0), each platelet bag was inoculated with an estimated 10 CFU/mL of one bacterial species. One bag was inoculated with PBS only and held as a negative control for growth. After 2 hours, samples were withdrawn from inoculated bags for semi-quantitative culture. Inoculated bags with bacterial concentration between 1 and 30 CFU were blind coded and continued in the study.

At 24 hours post inoculation and every 12 hours thereafter each bag was sampled and tested in duplicate with each of 3 PGD*prime* Test lots until all PGD*prime* results were Reactive for two time points in a row.. A second culture, including bacterial identification, was performed on each bag after reactive results were observed.. The second culture confirmed the Platelet PGD*prime* Test results and the bacterial growth status of the bag. Time to detection was tabulated for each bacterial species. Study results are shown in Table 6. Not all bacterial species grew in one attempt. Two species, required additional growth attempts. Three growth attempts were required for *Klebsiella aerogenes*. S*treptococcus agalactiae* did not grow in four attempts but did grow successfully in a fifth attempt.

Table 6: Analytical Growth Study Results – LRAP Suspended in Plasma

Bacteria	Bacterial Concentration at inoculation (CFU per bag)	Number of Test Samples Detected by *PGDprime* at Testing Time Point (n = 6)								Second Culture Result
		24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr	108 hr	
Bacillus cereus ATCC 7064	18.8	6	6							Pos
Escherichia coli	9.8	0	0	0	0	0	0	6	6	Pos
Klebsiella aerogenes	6.3	0	0	0	6	6				Pos
	5.8	0	0	0	0	6	6			Pos
Klebsiella pneumoniae	6.5	0	6	6						Pos
Pseudomonas aeruginosa	21	0	0	0	0	0	6	6		Pos
Serratia marcescens ATCC 43862	3.8	0	0	6	6					Pos
Staphylococcus aureus ATCC 27217	17.3	0	0	6	6					Pos
Staphylococcus epidermidis ATCC 49134	16.3	0	0	0	0	0	6	6		Pos
Streptococcus agalactiae ATCC 12927	13.8	0	0	0	0	0	0	0	6*	Pos

Unless otherwise noted, bacterial strains were isolates from blood cultures or recovered from platelet contamination events.
* Reactive PGD*prime* results confirmed by subsequent testing at 120 hr.

Ultra Low Inoculum – Study Description
The objective of the study was to demonstrate that the Platelet PGD*prime* Test was able to detect bacteria missed by early culture due to sampling error. This study was performed using three lots of Platelet PGD*prime* Test. Leukocyte reduced platelets (LRAP in plasma, LRAP in PAS-C and plasma, and pools of LR WBDP were inoculated with 3 bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*). A negative control was also prepared by inoculating PBS into the same platelet matrices. Inoculated units were blind coded so that the technologist performing testing was unaware of the expected results.

Bacteria were inoculated at very low titer (targeting < 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. A 1 mL volume from each sampling was added to each of four agar plates and incubated under aerobic conditions only as no anaerobic organisms were inoculated. 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 qualified for study inclusion.

Samples collected at 24 hours post inoculation and every 12 hours thereafter were tested in duplicate using three PGD*prime* Test lots. For LR WBDP, one volume of inoculated platelet was combined with five volumes of uninoculated pooled platelets to prepare a pooled sample at the time of testing. Testing continued until reactive results were observed on all six PGD*prime* Test devices for at least two samplings in a row. A second culture, including bacterial identification, was performed on each bag after reactive results were observed. The second culture confirmed the Platelet PGD*prime* Test results and the bacterial growth status of the bag.

Ultra Low Inoculum Study—LRAP Suspended in Plasma Results
Of 10 bags inoculated, five supported bacterial growth (see Table 7). Of 50 initial culture samples taken from these five bags, 35 demonstrated sampling error resulting in false negative culture results. PGD*prime* Test results for these five bags were reactive starting 24 hours after inoculation of the bag. The times to detection of the two bags inoculated with *Bacillus cerus* were 24 and 36 hours. Time to detection for the bag inoculated with *Klebsiella pneumoniae* was 36 hours and was 96 hours for the two bags inoculated with *Staphylococcus epidermidis*. The Platelet PGD*prime* Test was able to detect bacterial contamination in LRAP (plasma) units when an early culture did not reliably detect bacteria due to sampling error.

Table 7: Ultra Low Inoculum Study Results – LRAP Suspended in Plasma

Bacteria	Bacterial Concentration at Inoculation (CFU/bag)	Initial Culture Samples Positive	Number of Test Samples Detected by *PGDprime* at Testing Time Point (n = 6)								Second Culture Result
			24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr	108 hr	
Bacillus cereus ATCC 7064											
Bag 1	117.3	5 of 10	6	6							Pos
Bag 2	11.73	3 of 10	0	6	6						Pos
Klebsiella pneumoniae											
Bag 3	1.45	0 of 10	0	6	6						Pos
Staphylococcus epidermidis ATCC 49134											
Bag 4	17.0	0 of 10	0	0	0	0	0	0	6	6	Pos
Bag 5	162.8	7 of 10	0	0	0	0	0	0	6	6	Pos

Ultra Low Inoculum Study—LRAP Suspended in PAS-C and Plasma) Results

Of 10 bags inoculated, four supported bacterial growth (see Table 8). Of 40 initial culture samples taken from these four bags, 16 demonstrated sampling error resulting in false negative culture results. PGD*prime* Test results for these four bags were reactive starting at 36 hours after inoculation of the bag. The time to detection of the bag inoculated with *Bacillus cerus* was 36 hours. Time to detection for the two bags inoculated with *Klebsiella pneumoniae* was 36 hours. For the bag inoculated with *Staphylococcus epidermidis* the time to detection was 96 hours.

The Platelet PGD*prime* was able to detect bacterial contamination in LRAP suspended in PAS-C and plasma when early culture did not reliably detect bacteria due to sampling error.

Table 8: Ultra Low Inoculum Study Results - LRAP Suspended in PAS-C and Plasma

Bacteria	Bacterial Concentration at Inoculation (CFU/bag)	Initial Culture Samples Positive	Number of Test Samples Detected by *PGDprime* at Testing Time Point (n = 6)							Second Culture Result
			24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr	
Bacillus cereus ATCC 7064										
Bag 1	56.1	6 of 10	0	6	6					Pos
Klebsiella pneumoniae										
Bag 2	25.1	5 of 10	0	6	6					Pos
Bag 3	188.4	9 of 10	0	6	6					Pos
Staphylococcus epidermidis ATCC 49134										
Bag 4	66.1	4 of 10	0	0	0	0	0	0	6	Pos

Ultra Low Inoculum Study—Pre-storage Pools of LR WBDP Suspended in Plasma – Study Results

Of 10 bags inoculated, four supported bacterial growth (see Table 9). Of 40 initial culture samples taken from these four bags, 29 demonstrated sampling error resulting in false negative culture results. Prior to testing, samples were prepared by combining one volume of inoculated platelet with five volumes of uninoculated pooled platelets. PGD*prime* Test results for these four bags were reactive starting at 36 hours after inoculation of the bag. Time to detection of both bags inoculated with *Bacillus cereus* was 36 hours. The time to detection of *Klebsiella pneumoniae* was 36 hours, while time to detection of *Staphylococcus epidermidis* was 96 hours. The The Platelet PGD*prime* Test was able to detect bacterial contamination in pre-storage pools of LR WBDP suspended in plasma when early culture did not reliably detect bacteria due to sampling error.

Table 9: Ultra Low Inoculum Study Results – Pools of LR WBD Platelets Suspended in Plasma

Bacteria	Bacterial Concentration at Inoculation (CFU/bag)	Initial Culture Samples Positive	Number of Test Samples Detected by *PGDprime* at Testing Time Point (n = 6)							Second Culture Result
			24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr	
Bacillus cereus ATCC 7064										
Bag 1	8.1	1 of 10	0	6	6	6				Pos
Bag 2	75.4	9 of 10	0	6	6	6				Pos
Klebsiella pneumoniae										
Bag 3	24.8	1 of 10	0	0	6	6	6	6	12†	Pos
Staphylococcus epidermidis ATCC 49134										
Bag 4	65.5	0 of 10	0	0	0	0	0	0	12†	Pos

† Operator ran a second bag sample on a second set of devices to confirm reactivity status at last time point. All results were consistently reactive.

Potentially Interfering Substances

Study Description

This study evaluated substances or sample conditions that might interfere with the ability of the Platelet PGD*prime* Test to correctly identify bacterial-negative platelet samples as Non-reactive and bacteria-positive samples as Reactive. The substances and conditions are listed in Table10. All testing was performed using a 12-member panel comprising 11 bacteria-positive and 1 bacteria-negative members. Three lots of the Platelet PGD*prime* Test were used. Leukocyte reduced platelets (LRAP and LR WBDP/LR WBDPp) and nLR WBDP/nLR WBDPp were tested. At least five examples of each potentially interfering condition was tested for each platelet type, with the exception of RF and HAMA, where ten samles were tested.

Table 10: Potentially Interfering Substances Tested

	Substance Tested	Substance Level
Donor Conditions	Autoimmune antibodies	ds DNA (10 - 360 IU/mL)
		ANA (Positive, qualitative test)
		RF: 16.9 - 272 IU/mL
		Human anti-mouse antibody (HAMA): 10.5 – 182 ng/mL
	Hypergammaglobulinemia	IgA (505 - 744 mg/dL)
		IgG (2105 to 3901 mg/dL)
		IgM (461-933 mg/dL)
	Lipemia	305-553mg/dL
	Hypercholesterolemia	352 to 1230 mg/dL
	Hyperproteinemia	≥ 9 g/dL
	Hypoproteinemia	2.84-3.98 g/dL
Sample Conditions	Hemolysis	0 - 350 µg/dL
	pH	5.5 - 8.5
	Platelet concentration (% normal / native)	50% - 200% average concentration
	Red blood cells (concentration in %)	0% -0.7%
	White blood cells	~ 4×10^4 – 4×10^5 cells/mL for WBDP
	Platelet Additive Solution	0 and 100% for LRAP

Results

With the exception of one elevated IgM sample, there were no effects of the substances/conditions tested on the performance of the Platelet PGD*prime* Test when testing bacteria-positive panel members. One elevated IgM sample yielded a repeatable false negative result with *Escherichia. coli*, while all other bacteria were detected in the presence of this IgM sample. A possible explanation for this result is that the sample contains a high titer of antibodies specific to *Escherichia coli*.

The potential interferents purchased for use in evaluating donor conditions were not provided as sterile materials from the sample supplier. Six HAMA samples and one ANA sample produced reactive results on single Gram-negative lines, results that are consistent with the presence of bacterial

antigens. One ds-DNA sample and two hyperproteinemia samples produced signals on multiple capture lines, indicative of non-specific reactions. All other samples representing donor conditions and all samples representing sample conditions produced Non-reactive results when tested in platelet samples absent bacterial panel members. One elevated IgM, one elevated IgG and four hyperproteinemia samples did not flow in the assay and yielded Invalid test results.

Prozone (Hook Effect)

Study Description:
A high titer bacterial panel was prepared from stocks of 11 bacterial strains to assess whether excess bacterial antigen would yield false Non-reactive results. See Table 11. Three lots of Platelet PGD*prime* were used in this study.

Panel members were provided as stock solutions that were diluted 1:10 into five units of each of the following platelet types: LRAP suspended in PAS-C and plasma, pools of nLR WBDP, pre-storage pools of LR WBDP; one post-storage pool of LR WBDP and 6 units of LRAP in plasma. If any Invalid results or Non-reactive results were obtained, further 1:10 dilutions were made until valid and Reactive test result(s) were obtained.

Results
A total of 231 assays were performed using three lots of Platelet PGD*prime* Test. Table 11 shows the highest bacterial concentration platelet sample that provided a valid and reactive result. At concentrations above 8.3×10^6 and up to 2.6×10^{10} CFU/mL (the highest concentrations tested that yielded consistently valid assays*), the Platelet PGD*prime* Test correctly detected the presence of bacteria. There were no false negative results for any of the 11 bacteria tested.

(*During testing, many samples containing $>10^{10}$ CFU bacteria/mL, samples of *B. cereus* at 8.3×10^8 and *C. perfringens* at 1.2×10^9 were obviously viscous, cloudy and milky and resulted in invalid assays.)

Table 11: Prozone

Bacteria	Maximum Concentration (CFU/mL) that Provided a Valid Result for All Tests, by Platelet Type			
	LRAP (plasma) *n = 6*	**LRAP (PAS-C and plasma)** *n = 5*	**nLR-WBDPp plasma)** *n = 5*	**Pre-storage Platelet Pools (plasma)** *n = 5*
Bacillus cereus	8.3×10^7	8.3×10^7	8.3×10^6	8.3×10^7
Clostridium perfringens ATCC 13124	1.2×10^8	1.2×10^8	1.2×10^8	1.2×10^8
Staphylococcus aureus	5.3×10^9	5.3×10^8	5.3×10^8	5.3×10^8
Staphylococcus epidermidis	1.5×10^{10}	1.5×10^{10}	1.5×10^{10}	1.5×10^{10}
Streptococcus agalactiae	2.6×10^{10}	2.6×10^{10}	2.6×10^{10}	2.6×10^{10}
Escherichia coli	1.8×10^9†	1.8×10^9†	1.8×10^9†	1.8×10^9
Klebsiella aerogenes	1.6×10^9	1.6×10^9	1.6×10^9	1.6×10^9
Klebsiella pneumoniae	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9
Pseudomonas aeruginosa	2.1×10^9	2.1×10^9	2.1×10^9	2.1×10^9
Serratia marcescens ATCC 8100	2.2×10^9	2.2×10^9	2.2×10^9	2.2×10^9
Streptococcus oralis	5.4×10^9	5.4×10^8‡	5.4×10^8‡	5.4×10^9

Unless otherwise noted, bacterial strains were isolates from blood cultures or recovered from platelet contamination events.

† At 1.8×10^{10} CFU/mL, 5 of 6 LRAP samples, 4 of 5 LRAP suspended in PAS-C and plasma samples and 4 of 5 nLR WBD platelet pools yielded valid PGD*prime* results and were detected; one sample per platelet type resulted in an invalid test.
‡ At 5.4×10^9 CFU/mL, 4 of 5 samples of LRAP (PAS-C and plasma) and 4 of 5 samples of pre-storage pools of platelets suspended in plasma yielded valid PGD*prime* results and were detected; one sample per platelet type resulted in an invalid test.

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Keys to Symbols Used

REF	List Number	**PGD*prime* Test Device**	Platelet PGD*prime* Test Device
LOT	Lot Number	Reagent 1A	Reagent 1A
	Expiration Date	Reagent 1B	Reagent 1B
	Temperature limitations	Reagent 2	Reagent 2
	Manufacturer	Sample Pipettes	Sample Pipettes
	Consult instructions for use	Processing Tubes	Processing Tubes
	Attention, see instructions for use	Transfer Pipettes	Transfer Pipettes
	Do not re-use	Control –	Negative Control
		Control +	Positive Control

VERAX BIOMEDICAL

148 Bartlett Street
Marlborough, MA 01752 USA

For Technical Support
Verax Biomedical Technical Support
1.866.948.3729