VERAX BIOMEDICAL INCORPORATED

Platelet PGD® Test

A. INTENDED USE

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

B. 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*. Bacterial concentrations in contaminated platelet units are very low at the time of collection and may not be reliably detectable by available test methods in samples drawn at that time. During component storage this initial small inoculum of bacteria may grow, but by consequence of the diverse interactions of bacteria, donor unit and environmental conditions, the onset and rate of growth is highly unpredictable. Because of this variability, QC testing for bacterial contamination at a later phase of component storage may serve to maximize the ability to identify contaminated platelet units compared to testing only at an early phase of storage.[1, 2]

A novel Pan Genera Detection® (PGD) technology has been developed that detects the presence of conserved antigens lipoteichoic acid (LTA) and lipopolysaccharide (LPS) found on aerobic and anaerobic GP and GN bacteria, respectively. LTA and LPS targets are located on the surface of their respective bacteria and are primary constituents of the cell walls[3,4]. 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[5,6]. 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[7,8].

The Platelet PGD Test should not be used in determining suitability for release of LRAP for transfusion. Users considering such release should first consult the Center for Biologics Evaluation and Research (CBER) for the appropriate clinical studies.

Performance of the Platelet PGD Test system to detect bacteria in whole blood derived platelets or non-leukocyte reduced platelets is not known since studies were conducted using leukocyte reduced apheresis platelet products. Testing alone should not be used to extend the shelf life of platelets. Users considering such testing should first consult CBER for the appropriate clinical study design.

C. PRINCIPLES OF THE PROCEDURE

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

D. REAGENTS AND MATERIALS

Materials Provided

Platelet PGD Test

REF 01P11-22 20 Tests
REF 01P11-52 100 Tests

Includes the following:

15°C – 30°C	20 Test	100 Test	2°C – 8°C	20 Test	100 Test
PGD Test Device	20 each	100 each	Reagent 1	1 x 6 mL	2 x 12 mL
Disposable Pipettes	20 each	100 each	Reagent 2	1 x 6 mL	2 x 12 mL
Microfuge Tubes	20 each	100 each	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) stabilizer 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	01P11-10
Platelet PGD Test (without Platelet PGD Controls)	20 Tests	01P11-20
Platelet PGD Test (without Platelet PGD Controls)	100 Tests	01P11-50

Materials Required But Not Provided

- Sterile sampling device or tubing stripper, heat sealer and alcohol pad
- Sterile secondary sample tubes with caps, minimum volume 1.5 mL
- 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
- Vortex mixer
- Pipettes, pipettor or other single use device capable of delivering 500 µL
- Sterile disposable 500 µL pipette tips
- Timer
- Personal protective equipment
- Bio-hazardous waste equipment

E. 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 only.
3. The Platelet PGD Test is for quality control use only.
4. Perform the test at 15 – 30 °C, ≥ 20% relative humidity, in a fully lighted area.
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[9]. Bio-safety 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 and West Nile Virus (WNV) RNA when tested by FDA-licensed assays.

Reagent Precautions

Reagents were classified according to OSHA 29 CFR 1910.1030 and 1910.1200 and applicable European Community (EC) Directives. Applicable Classification, Risk (R) and Safety (S) phrases are listed below. Material Safety Data Sheets are available upon request.

Reagent 1 contains methanol and is classified as Toxic (T).

R 10 Flammable.
R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R 39/23/24/25 Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.
S 16 Keep away from sources of ignition - No smoking.
S 35 This material and its container must be disposed of in a safe way.
S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Reagent 2 contains sodium hydroxide and is classified as Irritant (Xi).

R 41 Risk of serious damage to eyes.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 35 This material and its container must be disposed of in a safe way.
S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S 46 If swallowed, seek medical advice immediately and show this container or label.

Reagent 3 contains n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (DDAPS) and is classified as Irritant (Xi).

R 36/38 Irritating to eyes and skin.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 35 This material and its container must be disposed of in a safe way.
S 46 If swallowed, seek medical advice immediately and show this container or label.

Reagents 2 and 3 and the Controls 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.[9] Dispose of all test material including reagents and controls in 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 Reagent or Control bottles.
3. Do not touch exposed dropper tip of Reagent 1, 2, 3 or Controls.
4. Recap Reagent and Control 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 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 incorrect 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.

F. 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 vial.

G. INDICATIONS OF INSTABILITY

1. Inspect Reagent vials 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.

H. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types and Handling

1. Quality control testing should include each LRAP component of an apheresis collection.
2. For optimal performance, sample LRAP units from 72 hours post-collection through end of day 5 storage.
3. 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 30 minutes prior to testing. Discard secondary sample tube in the biohazard waste after use.
4. When opening secondary tubes, ensure caps are not mixed up in order to avoid cross-contamination.
5. 500 µL of platelet sample is required to perform this test.
6. Do not use refrigerated or frozen samples as inaccurate test results may occur.

Methods for Sample Acquisition

Collect LRAP samples using sterile procedures in order to maintain a closed system.
If sampling with sterile sampling 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.
- While tightly holding the tubing stripper, mix the unit by gentle agitation.
- Release the tubing stripper and let tubing segment refill with platelets.
- Create 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.

I. TEST PROCEDURE

Pre-testing Preparation and Notes

1. Inspect Reagents and Controls for precipitate. Do not use if precipitate is evident.
2. Mix each bottle of Reagent and Control by gentle inversion 2 to 3 times prior to use.
3. Do not allow exposed dropper tips of Reagents or Controls 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 running controls.

1. Label two Microfuge Tubes to identify Controls.
2. Add 2 drops of Positive or Negative Control to the respective Microfuge Tubes. Use caution not to spill any volume.
3. Add 8 drops of Reagent 2 to each Microfuge Tube. Control samples should be blue.
4. Add 4 drops of Reagent 3 to each Microfuge Tube. Cap each Microfuge Tube and briefly vortex. Control samples should be yellow. Proceed directly to *Performing the Test.*

Sample Processing

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

1. Label the provided Microfuge Tube to identify the sample being run.

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. The platelet sample must turn green after addition of Reagent 1 and mixing. The intensity of the color may range from a pale green hue to a dark green color. If sample shows no evidence of color change, discard the sample and repeat. If the repeated sample reacts in the same manner, the sample cannot be run on the Platelet PGD Test Device. 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 carefully decant the plasma into an appropriate waste 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 several times with the tip of a Disposable Pipette to break the pellet into 3 or 4 fragments. Resuspend the pellet by carefully aspirating and dispensing the solution 3 to 4 times with the provided Disposable Pipette. Avoid aspirating the solution into the bulb of the pipette. The pellet may break down into small fragments or go into solution. 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 resuspended pellet. The processed sample should turn from blue to a pale yellow or straw color upon addition of Reagent 3. Total volume in the Microfuge Tube will be ~300 µL. Recap the Microfuge Tube and vortex. Large cell pellet fragments should not be visible. If large fragments are visible, continue to vortex until fragments are 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.

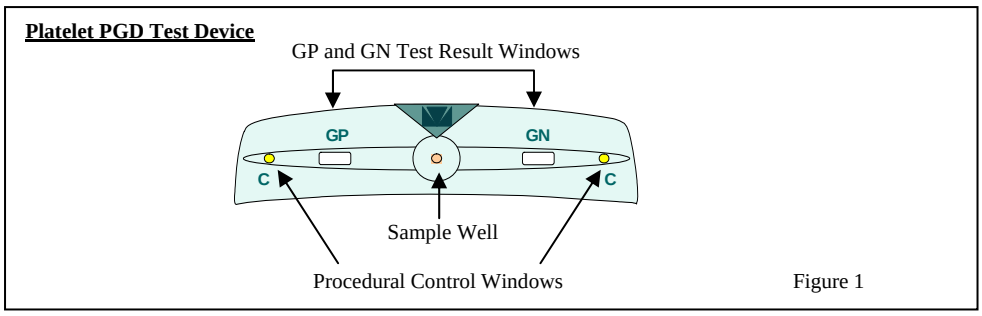


Figure 1

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

3. Pour the entire processed sample or Control (~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.

   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. If sample flow, as indicated by red color movement across the Test Result Windows, does not proceed down the device within 5 minutes, tap the sample pad in the sample well 2 or 3 times with a sterile disposable pipette tip to initiate the sample flow.

5. Once flow has initiated, incubate at least 20 minutes at 15 – 30 °C and ≥ 20% relative humidity.

6. 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 2, 3 and 4. 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, repeat the test with a fresh sample and a new PGD Test Device. Read the results in a fully 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.

7. Refer to **INTERPRETATION OF RESULTS**. Record your results per your laboratory requirements.

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

J. 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 components. Run the Platelet PGD Controls under the following circumstances:

- Each new operator, to establish competency prior to testing LRAP 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 their specific laboratory environment and conditions of use.

K. 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 2, 3 and 4.

The backgrounds of 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 2, 3 and 4.

Evaluate the GP and GN Test Result Windows for the presence or absence of GP and GN detection lines. See Figures 2 and 3. 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 line within either Test Result Window as reactive, no matter how faint the line.

Sample Interpretation

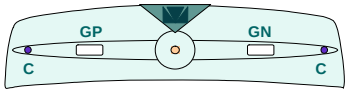
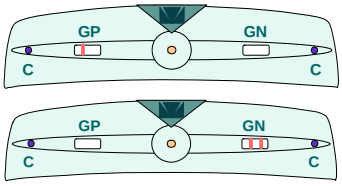
NON-REACTIVE SAMPLE	REACTIVE SAMPLE
	
• No detection lines visible in either the GP or the GN Test Result Windows • The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots	• One or two detection lines will be visible in either or both of the GP and GN Test Results Windows • No more than two lines will be present in either Test Result Window

Figure 2

Control Interpretation

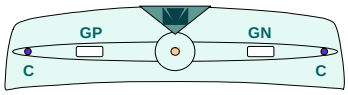
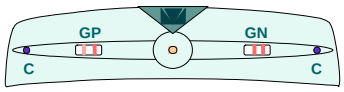
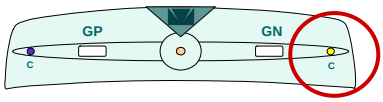
NEGATIVE CONTROL	POSITIVE CONTROL
	
• No detection lines visible in either the GP or the GN Test Result Windows • The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots	• There must be two detection lines present in BOTH the GP and GN Test Result Windows (total of 4 lines visible) • The lines should be discrete and extend fully from the top to the bottom of the GP and GN Result Windows. Consider any discrete line within either Test Result Window as reactive, no matter how faint the line

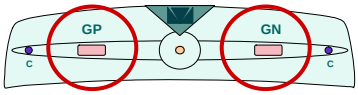
Figure 3

Examples of Invalid Results

INVALID Test Results



Procedural Control failure where blue/purple failed to appear within 60 minutes



Failure of GP and GN Result Windows to clear to white or light pink

An INVALID result cannot be interpreted.
When INVALID results occur, repeat the test using a new sample and new PGD Test Device.

Figure 4

L. LIMITATIONS

1. The Platelet PGD Test may be used only as an adjunct quality control test following testing with a bacterial detection device cleared by FDA for quality control testing of LRAP.
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 erroneous results.
4. The Platelet PGD Test is for use during quality control testing of LRAP units. Performance characteristics for alternate sample types have not been established. The product must not be used with non-leukocyte reduced apheresis platelets.
5. Do NOT use the Platelet PGD Test on clumped or coagulated platelet samples. Platelets must be suspended in plasma.
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 ACD-A anti-coagulant leukocyte-reduced platelet products.
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 level of detection of the test
    d. bacterial antigens are present at extremely high concentrations (prozone effect) . Note: Samples ranging in bacterial concentration from $4.7x10^8$ CFU/mL (*Bacillus cereus*) up to $9.2x10^9$ CFU/mL (*Escherichia coli*) were tested and showed no prozone effect.

10. LRAP samples with Rheumatoid Factor (RF) ≥ 16.9 IU/mL may produce a False Reactive test result.

M. PERFORMANCE CHARACTERISTICS

Growth Model Studies for Bacterial Detection in Platelets

Study 1 Description
The equivalence of the Platelet PGD Test 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 1. 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 48 hours 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 System 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.

Results
All types of tested bacteria were detectable by the Platelet PGD Test System 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 System 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 PGD Test Device and the result was non-reactive.

Table 1. Growth Study Results

Bacteria	Site	Bacterial Concentration (CFU/mL) in LRAP at unit inoculation	BacT /ALERT (Hours after inoculation of LRAP unit for a positive result)		PGD (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 (Isolate)	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 (Isolate)	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 (Isolate)	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 (Isolate)	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

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Study 2 Description

The objective of the second study 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[10]. This study was performed using three 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*).

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 4mL 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), PGD testing was performed on that bag.

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

Results

Of 6 bags inoculated, 5 supported bacterial growth (see Table 2). Of 50 initial culture samples taken from these 5 bags, 40 demonstrated sampling error resulting in false negative culture results. These 5 bags tested reactive by Platelet PGD 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 autosterilized in spite of initial positive culture results. Platelet PGD was nonreactive 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 2. Low Titer Growth Study Results

Bacteria	Bacterial Concentration CFU / Bag*	Initial Culture Samples Positive	PGD 24 hr	PGD 36 hr	PGD 48 hr	PGD 60 hr	PGD 72 hr	PGD 84 hr	PGD 96 hr	Second Culture	
										Sample Time	Result
Bacillus cereus											
Bag 1	45	5 of 10	NR	R	R	--	--	--	--	36 hrs	Pos
Bag 2	<4.5	0 of 10	NR	R	R	--	--	--	--	36 hrs	Pos
Bag 3	~1	0 of 10	NR	R	R	--	--	--	--	36 hrs	Pos
Klebsiella pneumoniae											
Bag 4	174	9 of 10	NR	NR	NR	NR	NR	NR	NR	96 hrs	Neg**
Bag 5	<17.4	0 of 10	R	R	--	--	--	--	--	24 hrs	Pos
Staphylococcus epidermidis											
Bag 6	26	5 of 10	NT	NT	NR	NR	R	R	--	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.
NR = Platelet PGD non-reactive
R = Platelet PGD reactive
NT = Not tested

Limit of Detection (Analytical Sensitivity)

Study Description
The Platelet PGD Test's limit of detection (LoD) was determined for each of the 10 organisms listed in Table 3. Platelet PGD testing was performed using 3 lots of Platelet PGD 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 achieved 10/10 detection was defined as the assay's LoD.

Results

Table 3. Limit of Detection (Analytical Sensitivity)

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

*Anaerobe

Reproducibility

Study Description:
The reproducibility of the Platelet PGD Kit 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 4. Positive panel members were present in a 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 PGD lots on each of two days. For each day of reproducibility testing, two Platelet PGD controls were tested.

Results:
The Platelet PGD Controls passed on all days. All strips tested 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 4. Reproducibility Panel Members

Bacteria Panel Member	GP or GN	Level	Logs Above LoD	Number Detected (N = 30)	Detection Rate
Bacillus cereus Isolate	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 Isolate	GN	Low	0.4	30	100%
		Mid	1.1	30	100%
Escherichia coli Isolate	GN	Low	0.2	29	97%
		Mid	1.0	30	100%
Klebsiella pneumoniae Isolate	GN	Low	0.4	30	100%
		Mid	0.9	30	100%
Pseudomonas aeruginosa Isolate	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 Isolate	GP	Low	0.5	28	93%
		Mid	0.6	29	97%
Staphylococcus epidermidis Isolate	GP	Low	0.4	30	100%
		Mid	1.2	30	100%
Streptococcus agalactiae Isolate	GP	Low	0.0	30	100%
		Mid	0.6	30	100%

For the 120 valid results from negative specimens, 100% gave the expected result of non-reactive. For the panel members that were missed, 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 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 between the three kit 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.

Specificity

Study 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.

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 Verax PGD Test when testing negative LRAP samples was 99.7% (lower one-sided 95% confidence limit = 99.0%) when initially reactive results were used. The observed specificity based on repeatedly reactive results was 99.8%.

Potentially Interfering Substances

Study Description
All testing was performed using 3 lots of the Platelet PGD Test and multiple operators. Non-reactive, Gram-positive and Gram- negative samples were tested with the potential interferents listed in Table 5 below.

Table 5. Potentially Interfering Substances Tested

Donor Conditions	Sample Conditions
Autoimmune antibodies	Hemolysis
ds DNA (10 - 252 IU/mL)	0 – 350 µg/dL
ANA (Positive, qualitative test)	
RF (13.3 – 773 IU/mL)	
Heterophile antibodies	pH
Positive (qualitative test)	5.5 – 8.5
Human anti-mouse antibody (HAMA)	Platelet concentration (% normal)
11.4 – 105.0 ng/mL	50% -200% normal concentration
Hypergammaglobulinemia	Red blood cells (concentration in %)
IgA (522 - 2470 mg/dL)	0% - 0.35% hematocrit
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	

Results
With the exception of Rheumatoid Factor (RF) samples, there were no effects of the substances/conditions tested on performance of the Platelet PGD Test. All PGD non-reactive samples remained non-reactive in the presence of the substances. All reactive samples remained reactive in the presence of the substances. 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 PGD results

Prozone (Hook Effect)

Study Description
Verax tested high titer, bacteria-inoculated LRAP samples in order to assess whether excess bacterial antigen would yield false non-reactive results, see Table 6. Dilution plate counting was used to confirm the sample concentration in CFU/mL. A total of 218 assays were performed using three lots.

Table 6. Prozone

Bacteria	Maximum Concentration (CFU/mL)
Bacillus cereus Isolate	4.7×10^8
Clostridium perfringens ATCC 13124	1.8×10^9
Enterobacter aerogenes Isolate	8.9×10^9
Escherichia coli Isolate	9.2×10^9
Klebsiella pneumoniae Isolate	1.8×10^9
Pseudomonas aeruginosa Isolate	1.1×10^9
Serratia marcescens ATCC 43862	2.0×10^9
Staphylococcus aureus Isolate	2.1×10^9
Staphylococcus epidermidis Isolate	8.2×10^9
Streptococcus agalactiae Isolate	7.7×10^9

Results
At concentrations above 4.7×10^8 and up to 9.2×10^9 (the highest concentrations tested), the Platelet PGD Test correctly detected the presence of bacteria. There were no false negative results for any of the 10 bacteria tested.

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Keys to Symbols Used

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

VERAX BIOMEDICAL INCORPORATED
377 Plantation Street
Worcester, MA 01605 USA

Distributed by:
Abbott Laboratories Inc.
Abbott Park, IL 60064 USA
Abbott Diagnostics

For Customer Service:
Abbott Diagnostics Customer Service
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