



Platelet PGD[®] Test

A. INTENDED USE

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

- pools of up to six (6) units of leukocyte reduced and non-leukocyte reduced whole blood derived platelets that are pooled within four (4) hours of transfusion as a quality control test.

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 platelets for transfusion nor should testing alone be used to extend the shelf life of platelets without consultation with the Center for Biologics Evaluation and Research (CBER) for the appropriate clinical studies.

C. PRINCIPLES OF THE PROCEDURE



The Platelet PGD Test is a single-use, lateral flow, qualitative test comprising Reagents, Controls, Disposables and a Test Device containing two simultaneously run test strips specific for the detection of aerobic and anaerobic GP and GN bacteria. Samples from leukocyte reduced apheresis platelet units and pools of up to six whole blood derived platelets (WBDP) pooled within 4 hours of transfusion 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 | P20C | 20 Tests |
| | REF | P100C | 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

| | <u>Quantity</u> | <u>REF</u> |
|--|-----------------|------------|
| Platelet PGD Controls | 30 Tests | P30C |
| Platelet PGD Test (without Platelet PGD Controls) | 20 Tests | P20 |
| Platelet PGD Test (without Platelet PGD Controls) | 100 Tests | P100 |

Materials Required But Not Provided

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

Optional: Reusable transparent covered enclosure

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 and pools of up to 6 leukoreduced (LR) WBDP and non-leukoreduced (nLR) WBDP pooled within 4 hours of transfusion.
3. The Platelet PGD Test is for quality control use.
4. For optimal sample flow, perform the test at 15 – 30 °C and ≥ 20% relative humidity (RH) in a well-lighted area. Ensure there is minimal airflow across or near the Test Device after addition of sample to the Sample Well. Low RH or airflow across the Test Device may result in extended sample flow times and/or invalid assays. If laboratory environmental conditions are not optimal, a transparent covered enclosure may be used during Test Device incubation.
5. Each operator performing the test must be able to distinguish between the following colors: Green, Yellow, Blue and Red.
6. Do not use materials after their stated expiration dates.
7. The Positive and Negative Controls contain human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents be handled in accordance with the OSHA Standard on Bloodborne Pathogens using Universal Precautions.⁹ Biosafety level 2 or other appropriate bio-safety practices should be used for materials that contain or are suspected of containing infectious agents.
8. The human derived components within the Positive and Negative Controls are non-reactive for Hepatitis B surface Antigen (HBsAg), human immunodeficiency virus type 1 ribonucleic acid (HIV-1 RNA), antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/HIV-2), antibody to hepatitis C virus (anti-HCV) and HCV RNA, West Nile Virus (WNV) RNA and anti-HTLV-I/II when tested by FDA-licensed assays.

Reagent Precautions

Reagents were classified according to OSHA 29 CFR 1910.1030 and 1910.1200 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 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.^{9, 10} Dispose of all test materials as bio-hazardous waste according to your laboratory procedure and applicable regulations.

Handling Precautions

Handle and perform test properly:

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

G. INDICATIONS OF INSTABILITY

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

H. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types and Handling

1. Quality control testing should include each LRAP component of an apheresis collection or a sample obtained from a WBDP pool.
2. For optimal performance, sample LRAP components from 72 hours post-collection through end of day 5 storage. For optimal performance when testing up to 6 WBDP that are pooled within 4 hours of transfusion, sample from 72 hours after collection of the freshest unit in the pool through the end of day 5 storage of the oldest unit in the pool. In pools that will be leukoreduced using a leukoreduction filter, optimal performance may be achieved if the PGD Test is performed on the pool prior to filtration.
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 two hours 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 platelet 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. Strip the segment only one time to avoid activation of platelets.
- While tightly holding the tubing stripper, mix the unit thoroughly by gentle agitation.
- Release the tubing stripper and let tubing segment refill with platelets.
- Create a segment 4-6 inches (10 – 15 cm) long, i.e., sufficient length to yield a 500 µL sample, with heat sealer.
- Cut segment from remainder of tubing with clean cutting instrument that has been wiped with an alcohol pad.
- Drain fresh sample into a sterile secondary sample tube by cutting ends of the segment with a clean cutting instrument.

I. TEST PROCEDURE

Pre-testing Preparation and Notes

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

Control Processing

Note: Reagent 1 is NOT used when testing Controls.

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

Sample Processing

Prior to adding LRAP, pooled LRWBDP or pooled nLRWBDP sample to the Platelet PGD Test Device, perform the following steps for each platelet sample to be assayed.

1. Label a Microfuge Tube to identify the sample being tested.
2. Pipette 500 µL platelet sample into the labeled Microfuge Tube. Properly dispose of pipette tip after transfer.
3. Add 8 drops of Reagent 1 to the Microfuge Tube. Recap the Microfuge Tube and mix by inversion 2 to 3 times. Do not vortex or shake Microfuge Tube. 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. 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 with the tip of a Disposable Pipette to dislodge the pellet from the bottom of the tube. Carefully aspirate and dispense the solution with the Disposable Pipette. The pellet may either immediately dissolve or break into fragments; either outcome is acceptable. Avoid aspirating the solution into the bulb of the pipette. **Do not vortex.** Minimize forming bubbles or foam. Confirm that no fragments are adhered to the exterior or interior of the Disposable Pipette.

Note: A fresh sample must be reprocessed if:

- the pellet has adhered to the disposable pipette
- the processed sample is not blue
- the processed sample is trapped in the pipette bulb

Proceed directly to the next step without pause.

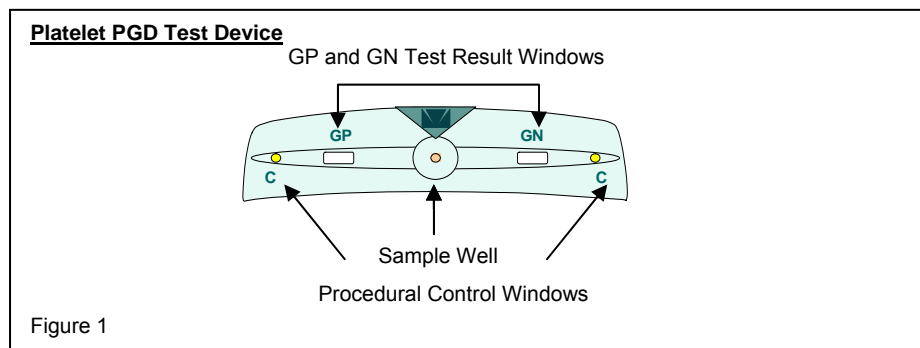
7. Add 4 drops of Reagent 3 to the Microfuge Tube. The processed sample must turn from blue to a pale yellow or straw color upon addition of Reagent 3. Total volume in the Microfuge Tube will be approximately 300 µL. Recap the Microfuge Tube and vortex until the pellet goes into solution. Large cell 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.



2. Place the PGD Test Device on a flat surface at 15 - 30 °C and $\geq 20\%$ relative humidity with minimal airflow. 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 (about 300 μL) in a single action into the Sample Well on the PGD Test Device. See Figure 1. Do not spill or splash sample or reagents on the Test Result or Procedural Control Windows. If this occurs, repeat the test with a fresh sample and PGD Test Device. If laboratory environmental conditions are not optimal, a transparent covered enclosure may be used during Test Device incubation.

Note: The entire Sample Well (300 μL) must be filled for the PGD Test Device to perform properly. Addition of insufficient volume will result in invalid test results.

4. 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. 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 well-lighted area within 60 minutes of sample addition.

Note: The entire Procedural Control Window does not have to change to a blue/purple color before the result can be read and interpreted.

6. Refer to **INTERPRETATION OF RESULTS**. Record your results per your laboratory requirements.
7. After interpretation and recording test results, dispose of used PGD Test Device in a bio-hazardous waste container.

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. Test the Platelet PGD Controls under the following circumstances:

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

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

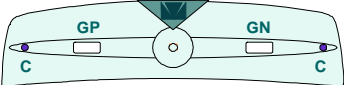
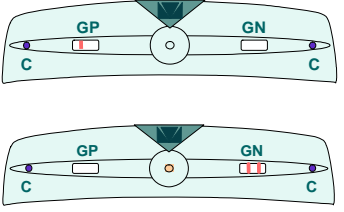
K. INTERPRETATION OF RESULTS

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.

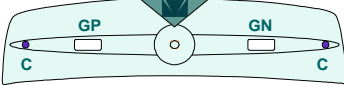
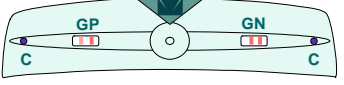
The backgrounds of the GP and GN Test Result Windows must be white or have a light pink homogeneous hue, free from streaks or spots that could interfere with interpretation of the test result. Do not confuse extraneous red spots or streaks with Reactive test results. See Figures 2, 3 and 4.

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.

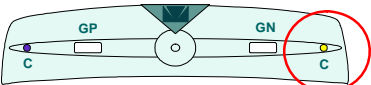
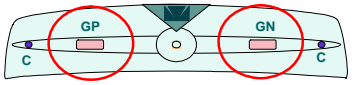
Sample Interpretation

| NON-REACTIVE Sample | REACTIVE Sample |
|--|--|
|  <ul style="list-style-type: none"> No detection lines visible in either the GP or the GN Test Result Windows The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots that might interfere with interpretation of the results <p>Figure 2</p> |  <ul style="list-style-type: none"> One or two detection lines visible in either or both of the GP and GN Test Results Windows No more than two lines present in either Test Result Window |

Control Interpretation

| NEGATIVE Control | POSITIVE Control |
|--|---|
|  <ul style="list-style-type: none"> No detection lines visible in either the GP or the GN Test Result Windows The backgrounds of the GP and GN Test Result Windows are white or have a light pink homogeneous hue, free from streaks or spots that might interfere with interpretation of the results <p>Figure 3</p> |  <ul style="list-style-type: none"> Two detection lines present in BOTH the GP and GN Test Result Windows (total of 4 lines visible) Lines discrete and extend fully from the top to the bottom of the GP and GN Result Windows. (Consider any discrete line within either Test Result Window as reactive, no matter how faint the line.) |

Examples of Invalid Results

| INVALID Test Results | |
|---|--|
|  <ul style="list-style-type: none"> Procedural Control failure where blue/purple failed to appear within 60 minutes |  <ul style="list-style-type: none"> 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 Platelet PGD Test Device.

Figure 4

L. LIMITATIONS

- The Platelet PGD Test is for use during adjunct quality control testing of LRAP following testing with a bacterial detection device cleared by the FDA for quality control testing of LRAP and quality control testing of pools of up to 6 WBDP pooled within 4 hours of transfusion. Performance characteristics for alternate sample types have not been established.
- The Platelet PGD Test must be performed in accordance with the instructions given in the package insert for an accurate test result.
- Interpreting the test results before 20 minutes or after 60 minutes of initiation of sample flow may yield erroneous results.
- Do NOT use the Platelet PGD Test on clumped or coagulated platelet samples. Platelets must be suspended in plasma.
- Processed samples containing fibrin may result in extended sample flow times and/or invalid assays.
- Do NOT use the Platelet PGD Test on refrigerated or frozen samples as inaccurate test results may occur.
- Performance characteristics of the product were established using the following anticoagulants: ACD-A (LRAP), CPD and CP2D (pooled WBDP).
- For REACTIVE test results, intensity of the test line does not correlate to the titer of bacteria in the sample.
- A NON-REACTIVE test result does not mean the unit is sterile or bacteria-free. Non-reactive results may occur if:
 - the samples are not properly obtained or stored
 - the test procedure was improperly followed
 - the concentration of bacteria is below the limit of detection of the test
 - bacterial antigens are present at extremely high concentrations (prozone effect)

Note: Samples ranging in bacterial concentration from 1.1×10^8 CFU/mL (*Clostridium perfringens*) up to 1.1×10^{10} CFU/mL (*Staphylococcus epidermidis*) were tested. The Platelet PGD Test correctly detected the presence of bacteria. There were no false negative results for any of the 10 bacteria tested.

- For potentially interfering substances refer to the appropriate section.

M. PERFORMANCE CHARACTERISTICS

Growth Model Studies for Bacterial Detection in Platelets

LRAP Study 1 Description

The equivalence of the Platelet PGD Test system to BacT/ALERT® for detecting bacterial contamination in LRAP units was evaluated by comparing time to detection of 10 bacterial species. Three sites participated in the study, each using three lots of Platelet PGD Test. All bacterial species were tested with each lot. Platelet units were inoculated with low levels of each bacterial species listed in Table 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 detected 100% of the bacteria-inoculated samples at 48 hours, testing was concluded. If any of the bacteria-inoculated samples were not detected by the Platelet PGD Test at 48 hours, the sampling and testing cycle described above was repeated every 24 hours until there was 100% detection by the Platelet PGD Test.

LRAP Study 1 Results

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

Table 1: LRAP Growth Study 1 Results

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

*One sample detected as both GP and GN

Unless otherwise noted, bacterial strains were blood culture isolates.

LRAP Study 2 Description

The objective of Study 2 was to demonstrate that the Platelet PGD Test was able to detect bacteria missed by culture due to sampling error. Sampling error can occur when bacteria are in lag phase at the time of sampling and therefore are not present at sufficient concentration to be consistently captured in the culture sample.¹¹ This study was performed using three lots of Platelet PGD Test and three bacterial species: a Gram-positive (*Bacillus cereus*), a Gram-negative (*Klebsiella pneumoniae*) and a slower growing organism (*Staphylococcus epidermidis*).

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

For Platelet PGD testing, platelet pellets were prepared by centrifugation from in-date LRAP units. Each platelet pellet was then resuspended in 500 µL drawn from an inoculated HIP bag in order to reconstitute a representative platelet sample and tested using the Platelet PGD Test. Samples collected at 24 hours and every 12 hours thereafter were tested with Platelet PGD Test until reactive results were observed using all three Platelet PGD Test lots. A second culture, including bacterial identification was performed on each bag at the time of the first observed Platelet PGD Test reactive result or at 96 hours if no Platelet PGD Test reactive was observed for a bag. This served to confirm the Platelet PGD Test results and the bacterial growth status of the bag.

LRAP Study 2 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. Platelet PGD Test results for these 5 bags were reactive by 24 to 72 hours after inoculation of the bag. In the single bag which failed to demonstrate bacterial growth as confirmed by a 96 hour culture sample, 9 of 10 of the initial culture samples were positive, indicating that this bag failed to grow or that it autosterilized in spite of initial positive culture results. Platelet PGD Test results were non-reactive 24 to 96 hours after inoculation accurately reflecting the lack of bacterial growth in this bag. Study 2 demonstrated that the Platelet PGD Test, when used following a culture-based test, was able to detect bacterial contamination when an early culture was unable to detect bacteria due to sampling error.

Table 2: LRAP Study 2 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 |
| <i>Bacillus cereus</i> (ATCC 7064) | | | | | | | | | | | |
| 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 |
| <i>Klebsiella pneumoniae</i> | | | | | | | | | | | |
| 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 |
| <i>Staphylococcus epidermidis</i> (ATCC 49134) | | | | | | | | | | | |
| 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 Test non-reactive, R = Platelet PGD Test reactive, POS = Bacterial growth confirmed; study organism confirmed

NT = Not tested

Unless otherwise noted, bacterial strains were blood culture isolates.

WBDPp Study Description

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

Because of the bactericidal properties of fresh platelet-rich plasma, Verax used outdated platelet-derived plasma (OP) as inoculation media. Plasma was recovered by low-speed centrifugation, pooled and placed into empty platelet bags. Following inoculation, the bacterial concentration (CFU/mL)

at the time of inoculation was determined. Approximately 18 hours post-inoculation, initial plate culture was performed to confirm growth in the inoculated unit. For the 9 aerobic bacteria, if bacteria were not detected on the 18 hour plates, the cycle ended for that inoculation. Although 18 hour plates for the 20 *Clostridium perfringens* anaerobic Test units did not detect viable bacteria, the cycles continued to 96 hours post-inoculation.

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

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

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

WBDPp Study Results

Growth studies were conducted using ten bacteria. Table 3 shows the results for the nine aerobic bacteria evaluated in the WBDPp Growth Model Study. All units inoculated with bacteria that supported bacterial growth are included in the table. The table includes the bacterial concentration at Test Unit inoculation, the initial culture results based on sampling 18 hours after inoculation and the time point when six positive test samples were identified by PGD testing as containing bacteria. The shaded cell corresponds to the final use of the unit in the analysis. For all aerobic bacteria except *Staphylococcus epidermidis*, there was 100% detection by the Platelet PGD Test System within 48 hours after inoculation. Of the two units that grew *Staphylococcus epidermidis*, bacteria were detected by the Platelet PGD Test in one unit at 60 hours and in the other 72 hours post-inoculation. APC testing and subsequent bacteria identification confirmed that Platelet PGD results agreed with APC results when bacteria grew (the 9 aerobic bacteria).

Although no bacteria were detected by culture at 18 hours post-inoculation, PGD testing was performed for the anaerobe *Clostridium perfringens* starting at 36 hours and continuing until 96 hours post-inoculation. Culture was performed again on all 96 hour samples. Platelet PGD results agreed with culture results; neither the Platelet PGD Test nor APC detected bacteria at 96 hours post-inoculation, thus demonstrating auto-sterilization. Additional studies demonstrated that *Clostridium perfringens* was detected through a mid-10⁵ CFU/mL concentration in 6-member LRWBDP pools prepared by spiking a single unit with bacteria before pooling and testing with the PGD Test.

Table 3: WBDPp Study Results

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

D = Bacteria detected

Unless otherwise noted, bacterial strains were blood culture isolates.

Limit of Detection (Analytical Sensitivity)

Study Description

The Platelet PGD Test's limit of detection (LoD) was determined for each of the 10 organisms listed in Table 4. Testing was performed using 3 lots of Platelet PGD Test with multiple operators and samples withdrawn from multiple LRAP units and tested in replicates of 10. Dilution plate counting was used to assign a CFU/mL concentration. The CFU/mL value of the sample when the Platelet PGD Test achieved 10/10 detection was defined as the assay's LoD. For WBDPp testing a three level challenge panel (bracketing the LoD) was prepared and tested using three lots of the Platelet PGD Test. The panel was tested with LRAP, LRWBDPp and nLRWBDPp. The Platelet PGD Test detected bacteria equally across all three platelet types and all three lots. Table 5 shows the organisms and panel member concentration in CFU/mL at which the Platelet PGD Test detected bacteria in all three platelet types. Note: Preparation of panel members to achieve specific CFU/mL concentrations (e.g., LoD) is not possible.

Results

Study results are shown in Tables 4 and 5.

**Table 4: Limit of Detection in LRAP
(Analytical Sensitivity)**

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

* Anaerobe

Unless otherwise noted, bacterial strains were blood culture isolates.

Table 5: Confirmation of Detection in LRAP and WBDPp

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

Reproducibility

Reproducibility of the Platelet PGD Test was assessed using LRAP and pools of WBDP (WBDPp).

LRAP Study Description

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

LRAP Results

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

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

Table 6: Reproducibility Panel (LRAP)

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

Unless otherwise noted, bacterial strains were blood culture isolates.

For the 120 valid results from negative specimens, 100% gave the expected result of non-reactive. For the 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 Platelet PGD Test lots at either level ($p = 1.0$ for MID level and $p = 0.29$ for LOW level) using Fisher's exact test. To determine whether there were any differences in detection rates among the three product lots, Fisher's exact test was performed for each level using the total detected and not detected. The Platelet PGD Test accurately and reliably detected low- and mid-level panel members from a diverse set of GP and GN bacteria and accurately and reproducibly identified negative panel members as non-reactive.

WBDDP Study Description

Reproducibility was evaluated in 6-unit pools of leukocyte reduced and non-leukocyte reduced platelets (LRWBDDP and nLRWBDDP, respectively) using a 24-member panel tested over a 6 day period. The panel comprised 4 negative panel members (no bacteria present) and 20 positive panel members, 2 positive panel members for each of the 10 bacteria listed in Table 7. Positive panel members were present in low and mid level concentrations (approximately 0.5 and 1.5 log above LoDs, respectively). A total of four operators (2 operators for LRWBDDP and 2 operators for nLRWBDDP) at three sites performed the reproducibility study using three Platelet PGD Test lots. Each panel member was tested in a single platelet type by one operator at each test site on 6 different days using the three Platelet PGD Test lots on each of two days. Each panel member was tested a total of 18 times using LRWBDDP and 18 times using nLRWBDDP. Platelet PGD Controls were assayed on each day of testing.

WBDDP Study Results

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

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

See Table 7.

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

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

Table 7: Reproducibility Panel (WBDPp)

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

* Unless otherwise noted, bacterial strains were blood culture isolates

Specificity

Specificity of the Platelet PGD Test was assessed for LRAP and pools of up to 6 LR and nLRWBDP.

LRAP 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.

LRAP Study Results

Of the 610 samples tested, 608 were non-reactive. Two initially reactive samples were observed, but upon retest in duplicate only one sample was repeatedly reactive. Repeat testing included resampling and retesting by culture to confirm the absence of bacterial contamination in both samples. The specificity of the Platelet PGD Test when testing 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%.

LRWBDPp and nLRWBDPp Study Description

Specificity was evaluated by testing samples from 428 unique whole blood derived platelet pools, representing 2568 platelet donations, using at least three lots of the Platelet PGD Test. Samples were cultured at the time of Platelet PGD testing; samples that were culture-negative were included in the determination of Platelet PGD Test specificity. Age of the pooled platelets sampled and tested ranged from Day 2 through Day 5 post-collection.

LRWBDPp and nLRWBDPp Study Results

Of 428 confirmed culture-negative pools, 425 were non-reactive in the Platelet PGD Test. Three samples were repeatedly reactive. The specificity of the Platelet PGD Test when testing culture-negative samples of pooled WBDP was 99.3% (lower one-sided 95% confidence limit = 98.2%).

Potentially Interfering Substances

LRAP Study Description

All testing was performed using LRAP samples, 3 lots of the Platelet PGD Test and five operators. Non-reactive, Gram-positive and Gram-negative samples were tested with all potential interferents listed in Table 8 except WBC concentration.

LRWBDPp and nLRWBDPp Study Description

Testing was performed using LRWBDPp and nLRWBDPp, 2-3 lots of the Platelet PGD Test and five operators. Non-reactive, Gram-positive and Gram-negative samples were tested with the potential interferents as noted in Table 8.

Table 8: Potentially Interfering Substances Tested

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

Results

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

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

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

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

Prozone (Hook Effect)Study Description

High titer, bacteria-inoculated platelet samples were tested in the Platelet PGD Test in order to assess whether excess bacterial antigen would yield false non-reactive results; see Table 9. Dilution plate counting was used to confirm the sample concentration in CFU/mL. A total of 218 assays were performed using LRAP and three Platelet PGD Test lots. For WBDP, 584 assays were performed across three Platelet PGD Test lots.

Table 9: Prozone

| Bacteria | Maximum Concentration (CFU/mL) | |
|--|--------------------------------|----------------------|
| | LRAP | WBDPp |
| <i>Bacillus cereus</i> | 4.7×10^8 | 1.8×10^8 |
| <i>Clostridium perfringens</i> ATCC 13124 | 1.8×10^9 | 1.1×10^8 |
| <i>Enterobacter aerogenes</i> | 8.9×10^9 | 4.0×10^9 |
| <i>Escherichia coli</i> | 9.2×10^9 | 1.5×10^9 |
| <i>Klebsiella pneumoniae</i> | 1.8×10^9 | 2.4×10^9 |
| <i>Pseudomonas aeruginosa</i> | 1.1×10^9 | 1.7×10^9 |
| <i>Serratia marcescens</i> ATCC 43862 | 2.0×10^9 | 1.3×10^9 |
| <i>Staphylococcus aureus</i> | 2.1×10^9 | 2.1×10^9 |
| <i>Staphylococcus epidermidis</i> | 8.2×10^9 | 1.1×10^{10} |
| <i>Streptococcus agalactiae</i> | 7.7×10^9 | 7.0×10^9 |

Unless otherwise noted, bacterial strains were blood culture isolates.

Results

At concentrations above 1.1×10^8 and up to 1.1×10^{10} (the highest concentrations tested that yielded consistently valid assays*), the Platelet PGD Test correctly detected the presence of bacteria. There were no false negative results for any of the 10 bacteria tested. (*During WBDPp testing, samples containing $> 1.2 \times 10^{10}$ CFU bacteria/mL were obviously viscous, cloudy and milky and resulted in invalid assays.)

N. REFERENCES


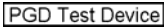

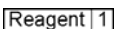

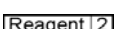



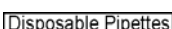

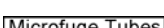

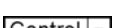



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Keys to Symbols Used

| | | | |
|---|---|---|--------------------------|
|  | List Number |  | Platelet PGD Test Device |
|  | <i>In vitro</i> diagnostic medical device |  | Reagent 1 |
|  | Lot Number |  | Reagent 2 |
|  | Expiration Date |  | Reagent 3 |
|  | Temperature limitations |  | Disposable Pipettes |
|  | Manufacturer |  | Microfuge Tubes |
|  | Consult instructions for use |  | Negative Control |
|  | Attention, see instructions for use |  | Positive Control |
|  | Do not re-use | | |



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