Ultra-Low Inoculum Growth Study to Validate Commercial Scale Lots of the PGD*prime*® Rapid Test for Detection of Bacteria in Platelets

David LaVerda, Johny Lisitu, Lisa Shinefeld, Nancy Best, Remo Vallejo

Verax Biomedical Incorporated, Marlborough, MA

Presented at the AABB Annual Meeting, San Antonio, TX. October 2019

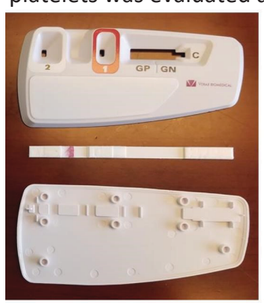
Ultra-Low Inoculum Growth Study to Validate Commercial Scale Lots of the PGD*prime*® Rapid Test for Detection of Bacteria in Platelets

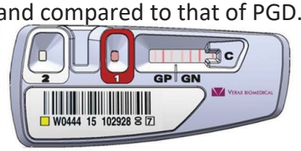
David LaVerda, Johny Lisitu, Lisa Shinefeld, Nancy Best, Remo Vallejo

Verax Biomedical Incorporated, Marlborough, MA

Background

- The PGD*prime* rapid test for bacteria in platelets is an improvement of the Platelet PGD® test currently used as a Safety Measure for platelet transfusion.
- PGD*prime* detects bacterial contamination prior to transfusion and can overcome the limitations of primary culture testing.
- PGD*prime* offers a simplified test procedure.
- Multiple commercial scale lots have now been manufactured under cGMP.
- Using 3 sequential lots and 3 representative bacteria, an Ultra-Low Inoculum (ULI) Growth study was undertaken to simulate extremely low bacterial counts in platelet units that could lead to false negatives for primary culture testing due to sampling errors.
- The ability of the test to detect the proliferation of bacteria from these extremely low levels of contamination in platelets was evaluated and compared to that of PGD.
- In a separate Analytical Growth Study, the ability of the test to detect the proliferation of 9 claim strain bacteria from initially low levels of contamination in apheresis platelets was evaluated and compared to that of PGD.





- 6 capture lines
- 3 GP, 3 GN
- 5 polyclonal, 1 monoclonal Ab pairs
- Sequential assay on a single test strip
- Cartridge designed for bar coding and integration with readers

PGD*prime* Test

PGD Procedure

1. Add Reagent 1 (lysing agent) to platelet sample
2. Centrifuge
3. Decant supernatant
4. Add Reagent 2 (base) to pellet
5. Disrupt pellet and mix
6. Add Reagent 3 (neutralizer) and vortex
7. Transfer to PGD test device.

PGD*prime* Procedure

1. Add Reagent 1A (base) to platelet sample. Invert to mix.
2. Add Reagent 1B (neutralizer) to sample. Invert to mix.
3. Transfer to test device.
4. Add Chase buffer (Reagent 2).

- No centrifuge is required.

ULI Study Methods

- The ULI Study involved individually growing 3 representative bacteria, *B. cereus, K. pneumoniae* and *S. epidermidis*, from initial inocula of <1 CFU/mL in each of 3 platelet types: apheresis platelets in plasma, apheresis platelets in Platelet Additive Solution (PAS-C), and pre-storage pools of leukoreducedwhole blood derived platelets (LRWBDPp).
- To confirm the low level of inoculation, ten 4 mL samples were cultured from each spiked bag. For a bag to continue with testing, at least one of the 10 samples from that bag must show no bacterial growth.
- At 24 hours after inoculation, and every 12 hours thereafter, each bag was sampled and tested as blinded samples with 3 lots of PGD*prime* and 1 lot of PGD, all in duplicate.
- Testing for each bag continued until sufficient growth occurred for all 6 PGD*prime* tests and 2 PGD tests to be reactive for 2 consecutive time points.
- The times to initial reactivity with PGD*prime* and PGD were compared.

Analytical Growth Study Methods

- In the Analytical Growth Model Study, the following bacteria were inoculated into in-date apheresis platelet units to a target concentration range of 1-30 CFU/mL: *B. cereus, S. aureus, S. epidermidis, S. agalactiae, E. aerogenes, E. coli, K. pneumoniae, P. aeruginosa,* and *S. marcescens*.
- The level of viable bacteria in each bag was quantified by dilution plate count.
- At 24 hours after inoculation, and every 12 hours thereafter, each bag was sampled and tested as blinded samples using 3 lots of PGD*prime* and 1 lot of PGD, all in duplicate.
- Testing for each bag continued until sufficient growth occurred for all 6 PGD*prime* tests and 2 PGD tests to be reactive for 2 consecutive time points.
- The times to initial reactivity with PGD*prime* and PGD were compared.

ULI Growth Study Results/Findings

Bacteria	CFU/Bag at Inoculation	CFU/mL at Inoculation	Initial Reactivity Hours after inoculation (number detected/number tested)	
			PGD (Duplicates from 1 lot)	PGD*prime* (Duplicates from 3 lots)
Apheresis				
Bacillus cereus	117.3	0.514	24 (2/2)	24 (6/6)
	11.73	0.051	36 (2/2)	36 (6/6)
Klebsiella pneumoniae	1.45	0.007	36 (2/2)	36 (6/6)
Staphylococcus epidermidis	17	0.075	96 (2/2)	96 (6/6)
PAS-C				
Bacillus cereus	56.1	0.301	36 (2/2)	36 (6/6)
Klebsiella pneumoniae	25.1	0.078	84 (2/2)	36 (6/6)
	188.4	0.78	60 (2/2)	36 (6/6)
Staphylococcus epidermidis	66.1	0.234	96 (2/2)	96 (6/6)
LRWBDPp				
Bacillus cereus	8.1	0.029	48 (2/2)	36 (6/6)
	75.4	0.29	48 (2/2)	36 (6/6)
Klebsiella pneumoniae	24.6	0.099	96 (2/2)	48 (6/6)
Staphylococcus epidermidis	65.5	0.23	96 (2/2)	96 (6/6)

Analytical Growth Study Results/Findings

Bacteria	Initial CFU/mL in bag	Initial Reactivity Hours after inoculation (no. detected/no. tested)	
		PGD	PGD*prime*
B. cereus	18.8	24 (2/2)	24 (6/6)
E. aerogenes	6.3	48 (2/2)	60 (6/6)
	5.8	72 (2/2)	72 (6/6)
E. coli	9.8	96 (2/2)	96 (6/6)
K. pneumoniae	6.5	36 (2/2)	36 (6/6)
P. aeruginosa	21	84 (2/2)	84 (6/6)
S. marcescens	3.8	48 (2/2)	48 (6/6)
S. aureus	17.3	48 (2/2)	48 (6/6)
S. epidermidis	16.3	96 (2/2)	84 (6/6)
S. agalactiae	13.8	120 (2/2)	108 (6/6)

We observed that some of the platelet units inhibited growth much longer than in previous studies. This is not an unexpected observation as bacterial growth rates will vary from donor sample to donor sample due to variations in individual levels of inhibitory and immune factors.

Conclusions

In growth model studies, PGD*prime* detects bacteria growing in platelet units after initially low levels of contamination at elapsed times comparable to or better than the Verax Platelet PGD Test

VERAX BIOMEDICAL