Validation of the Analytical Sensitivity of Commercial Scale Lots of the Verax PGD*prime*® Test for Bacteria in Platelets

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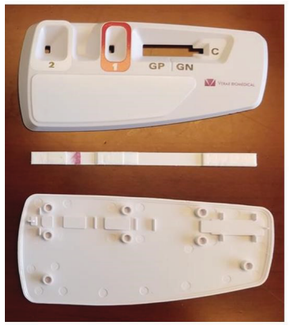
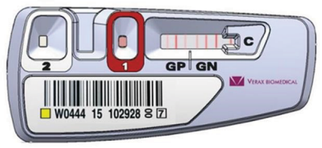
Background

- The PGD*prime* rapid test for bacteria in platelets was developed as an improvement of the PGD® test currently used as a Safety Measure for platelet transfusion.
- It was designed to provide a simpler test procedure compared to the current product and improved specificity through the use of intact IgG for capture and $F(ab')_2$ fragments for detector antibodies for 5 out of 6 test lines.
- Using 3 sequential cGMP lots, the Analytical Sensitivity of the PGD*prime* test was 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.

Methods

Phase 1: Sensitivity in Apheresis Platelets

- A blinded, frozen 32-member bacterial test panel was prepared. The panel comprised 3 levels of each PGD bacterial claim strain so that, after reconstitution in platelet samples, one member was below the limit of detection (LoD) of the PGD assay ("Lower"), one member was at or slightly above the LoD of the assay ("Middle"), and one was above the LoD of the PGD assay ("Upper"). Two negative samples were included.
- Panel members were thawed and added 1:21 to 10 in-date apheresis platelets in plasma.
- The reconstituted panel members were tested on one lot of PGD and on 3 lots of PGD*prime.* Bacteria included *B. cereus, C. perfringens, S. aureus, S. epidermidis, S. agalactiae, E. aerogenes, E. coli, K. pneumoniae, P. aeruginosa,* and *S. marcescens.*

Bacterial Test Panels

Bacterial ID	Level	PGD LoD Claim CFU/mL	Panel CFU/mL	Log ratio to LoD
Bacillus cereus	Upper	1.2E+04	7.8E+04	0.8
	Middle		2.7E+04	0.4
	Lower		3.4E+03	-0.5
Clostridium perfringens	Upper	8.9E+04	7.8E+05	0.9
	Middle		2.4E+05	0.4
	Lower		3.6E+04	-0.4
Staphylococcus aureus	Upper	8.2E+03	7.7E+04	1.0
	Middle		1.8E+04	0.3
	Lower		2.1E+03	-0.6
Staphylococcus epidermidis	Upper	9.2E+03	4.9E+04	0.7
	Middle		2.7E+04	0.5
	Lower		1.9E+03	-0.7
Streptococcus agalactiae	Upper	5.5E+04	5.1E+05	1.0
	Middle		1.6E+05	0.5
	Lower		1.8E+04	-0.5
Enterobacter aerogenes	Upper	1.0E+04	5.6E+04	0.8
	Middle		3.3E+04	0.5
	Lower		1.1E+03	-0.9
Escherichia coli	Upper	2.8E+04	1.2E+05	0.6
	Middle		5.6E+04	0.3
	Lower		4.2E+03	-0.8
Klebsiella pneumoniae	Upper	2.0E+04	1.8E+05	1.0
	Middle		6.1E+04	0.5
	Lower		2.4E+03	-0.9
Pseudomonas aeruginosa	Upper	8.2E+03	3.0E+04	0.6
	Middle		1.7E+04	0.3
	Lower		2.6E+03	-0.5
Serratia marcescens	Upper	8.6E+05	7.6E+06	0.9
	Middle		2.5E+06	0.5
	Lower		1.7E+05	-0.7
Negative Control	Neg	0.0E+00	0.0E+00	0
	Neg		0.0E+00	0

Phase 2: Sensitivity in Other Platelet Types

An LoD Challenge Study in other platelet types was conducted by spiking the mid-level panel members into 6 units each of the following platelet types:

- pre-storage pools of leukocyte-reduced random donor platelets (PSP)
- post-storage pools of non-LR random donor platelets (NLR pools)
- apheresis platelets in platelet additive solution (PAS)
- An apheresis platelet unit was also spiked with the mid-level panel members as a control.
- *S. oralis* was included as a new detection target for PGD*prime*. It was tested at a level typically associated with previously reported serious septic events with platelets.

Results/Findings

Phase 1: Apheresis Platelets Spiked with Bacterial Panels

LoD Comparison (Reactive Results/Units Tested)			
Bacteria	Level (mid=LoD)	PGD	PGD*prime* (3 Lots)
B. cereus	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	26 / 30
S. aureus	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	22 / 30
S. epidermidis	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	30 / 30
S. agalactiae	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	30 / 30
C. perfringens	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	1 / 10	29 / 30
E. aerogenes	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	2 / 30
P. aeruginosa	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	4 / 30
E. coli	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	2 / 30
S. marcescens	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	1 / 10	30 / 30
K. pneumoniae	Upper	10 / 10	30 / 30
	Middle	10 / 10	30 / 30
	Lower	0 / 10	0 / 30
Negative		0 / 20	0 / 60*

**2 initial reactives, no repeat reactives*

Phase 2: LoD Challenge in Other Platelet Types

Bacteria	Platelet Type Tested	PGD*prime* (3 Lots)
B. cereus	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
S. aureus	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
S. epidermidis	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
S. agalactiae	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
C. perfringens	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
E. aerogenes	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
P. aeruginosa	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
E. coli	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
S. marcescens	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
K. pneumoniae	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
S. oralis	PSP	30/30
	NLR pool	18/18
	apheresis in PAS	21/21
Negative	PSP	0/20
	NLR pool	0/18
	apheresis in PAS	0/21

Conclusions

- All samples at and above the LoD of PGD were detected by PGD and by all 3 lots of PGD*prime* in apheresis platelets.
- For some strains, PGD*prime* detected bacterial concentrations lower than the LoD of PGD.
- In the LoD challenge study, PGD*prime* detected all species at the LoD of PGD in all platelet types.

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