Validation of the Specificity of the PGD*prime*® Test for Bacteria in Platelets with Commercial Scale Lots

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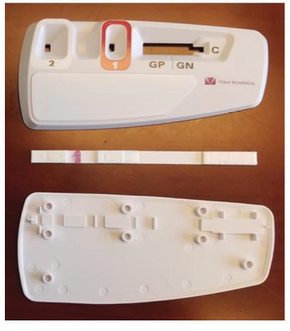
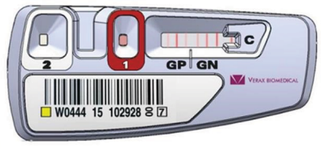
Background

- 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.
- Multiple commercial scale lots have now been manufactured under cGMP and evaluated for Specificity.





- 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

- The specificity of the new test was evaluated in a multi-site study using 3 consecutive manufacturing lots of PGD*prime* devices and reagents. Three sites tested a total of 3802 individual in-date platelet doses.

Platelet types evaluated:

1. Leukocyte-reduced whole blood derived platelets (LR WBD)
2. Post-storage pools of LR whole blood derived platelets (LR WBDP)
3. Leukoreduced apheresis platelets in plasma (LRAP)
4. Non-leukocyte-reduced whole blood derived platelets (NLR WBD)
5. Post-storage pools of NLR whole blood derived platelets (NLR WBDP)
6. Leukocyte-reduced apheresis platelets in platelet additive solution (PAS)
7. Pre-storage pools of LR platelets (PSP)

- Any platelet sample with an initially reactive (IR) result was retested in duplicate using two additional PGD*prime* devices. If one or both of the two retests yielded a reactive result, the sample was classified as repeat reactive (RR) by PGD*prime*.
- If both retests were non-reactive, the final interpretation was non-reactive (NR). All samples were tested using aerobic and anaerobic plate culture to determine true bacterial status.
- Single repeat testing was performed for samples with initial invalid results (INV).
- If a sample was culture-positive but non-reactive by PGD*prime*, the PGD*prime* result was classified as a False Negative (FN). If a sample was culture-negative but PGD*prime* RR, it was classified as a False Positive (FP).
- To be included in the Specificity Study, each platelet sample required a PGD*prime* result and a culture result.
- Traditional agar plate culture (APC) was used to determine the true status of each sample. Units were deemed negative if no colonies were detected under either aerobic or anaerobic conditions after 3-7 days.
- Many of the LR WBD and NLR WBD units were tested as individual units and then combined into 6-member pools.
- Although some doses were sampled and tested at multiple times, only the first interpretation generated at the initial time point has been included in the specificity calculations so that all summary results are based on unique doses.

Table 1. Distribution of Doses Tested by Platelet Type and Test Site

Platelet Type	Site 1	Site 2	Site 3	All
LR WBD	110		501	611
LR WBDP			75	75
LRAP	441	1160		1601
NLR WBD			503	503
NLR WBDP			65	65
PAS		42	255	297
PSP	251	399		650
All	802	1601	1399	3802

Table 2. Distribution of Unit Age by Platelet Type

Type	Age at First Test					All
	Day 2	Day 3	Day 4	Day 5	Day 6	
LR WBD	308 (50%)	125 (20%)	163 (27%)	15 (2%)	0 (0%)	611
LR WBDP	46 (61%)	7 (9%)	21 (28%)	1 (1%)	0 (0%)	75
LRAP	13 (1%)	427 (27%)	365 (23%)	733 (46%)	63 (4%)	1601
NLR WBD	399 (79%)	27 (5%)	48 (10%)	29 (6%)	0 (0%)	503
NLR WBDP	54 (83%)	3 (5%)	3 (5%)	5 (8%)	0 (0%)	65
PAS	39 (13%)	93 (31%)	120 (40%)	45 (15%)	0 (0%)	297
PSP	0 (0%)	151 (23%)	229 (35%)	270 (42%)	0 (0%)	650
All	859 (23%)	833 (22%)	949 (25%)	1098 (29%)	63 (2%)	3802

Results/Findings

Table 3. PGD*prime* Interpretation by Platelet Type

Platelet Type	Final result				All
	Inv	IR	NR	Ind	
LR WBD	0 (0%)	0 (0%)	611 (100%)	0 (0%)	611
LR WBDP	0 (0%)	0 (0%)	75 (100%)	0 (0%)	75
LRAP	2 (0.1%)	1 (0.1%)	1,598 (99.8%)	0 (0%)	1,601
NLR WBD	0 (0%)	1 (0.2%)	501 (99.6%)	1 (0.2%)*	503
NLR WBDP	0 (0%)	1 (1.5%)	64 (98.5%)	0 (0%)	65
PAS	0 (0%)	2 (0.7%)	295 (99.3%)	0 (0%)	297
PSP	0 (0%)	0 (0%)	650 (100%)	0 (0%)	650
All	2 (0.1%)	5 (0.1%)	3,794 (99.8%)	1 (0.0%)	3,802

* One sample classified as Ind (Indeterminate) because the IR for that sample was only followed up by a single additional test making it impossible to interpret as either NR or RR.

There were a total of 15 initially INV results (0.39%). Of these, 13 were NR on repeat testing, 2 were INV on 3 repeat tests. These 2 were excluded from the final statistical analyses.

Table 4. PGD*prime* Final Result Summary by Platelet Type

Unit Type	Results				All	Specificity	
	Initially Reactive/Rate	Repeat Reactive/Rate	Non-Reactive	Indeterminate		Observed	Lower 1-Sided 95% CL
LR WBD	0/0%	0/0%	611	0	611	100.0%	99.6%
LR WBDP	0/0%	0/0%	75	0	75	100.0%	96.5%
LRAP	1/0.06%	0/0%	1598	0	1599	100.0%	99.8%
NLR WBD*	1/0.2%	0/0%	501	1	503	99.8%	99.1%
		0/0%			502	100.0%	99.5%
NLR WBDP	1/1.5%	0/0%	64	0	65	100.0%	96.0%
PAS	2/0.7%	0/0%	295	0	297	100.0%	99.1%
PSP	0/0%	0/0%	650	0	650	100.0%	99.6%
All*	5/0.13%	0/0%	3794	1	3800	100.0%	99.9%
						100.0%	99.9%

*Reported both with (N=503) and without (N=502) the sample classified as Ind (indeterminate) because the Initially Reactive result for that sample was only followed by a single additional test making it impossible to interpret as either Non-Reactive or Repeat Reactive. (Note: The single repeat test performed was Non-Reactive.)

Conclusions

Using 3 cGMP lots to test 3800 unique units of different types of platelets, PGD*prime* showed 5/3800 initial reactives(0.13% IR), **0/3800 RR (0% False Positive results),**and **0% False Negative results** based on culture results.

The estimated Specificity based on 3800 test results was 100% with a Lower 1-sided 95% confidence limit of 99.9%.

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