Improved Detection of Gram-Negative Platelet Isolates With Next-Generation Platelet PGD Test

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We compared the time to detection of Gram-negative platelet isolates using our current Platelet PGD test and our new prototype PGD* prime *device.

Background:

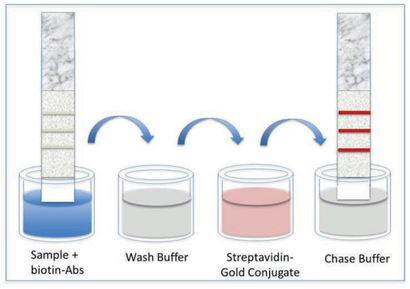
- The Verax Biomedical Platelet PGD Test detects bacterial contamination of platelets.
- We have previously reported the development of improved Gram-negative antibodies designed to provide increased breadth of reactivity across a wide variety of isolates.
- We have now embodied those antibodies in a next generation prototype Platelet PGD Test (PGD *prime*) in a sequential lateral flow assay (LFA) format.
- We compared the time to detection of a variety of Gram-negative (GN) bacteria, that had been isolated from platelet contamination events, using our current PGD test and our prototype next-generation PGD *prime* technology.

Methods:

- All Gram-negative bacteria strains used originated as actual platelet contaminants recovered by collaborating institutions or internal activities and collected by Verax.
- Aphaeresis platelet units were divided into 50 mL single donor platelet bags to create individual test units for inoculation.
- Test units were inoculated with GN platelet isolates at a level of ≈ 10^3 CFU/mL and allowed to grow at controlled room temperature while rocking.
- Mock-inoculated test units derived from the same parent units used for growth were maintained and tested in parallel as negative controls.
- Inoculated bags and mock-inoculated negative controls were sampled in a series of time points, starting at 12 hours post inoculation, and tested by current PGD and new PGD *prime*.
- The PGD *prime* sample preparation procedure eliminates the five minute centrifugation and pellet resuspension steps required by the original Platelet PGD Test.

Initial Evaluation Using Sequential Dipstick Assay

The advantage of using a sequential lateral flow assay (LFA) format was first evaluated using a sequential dipstick format:



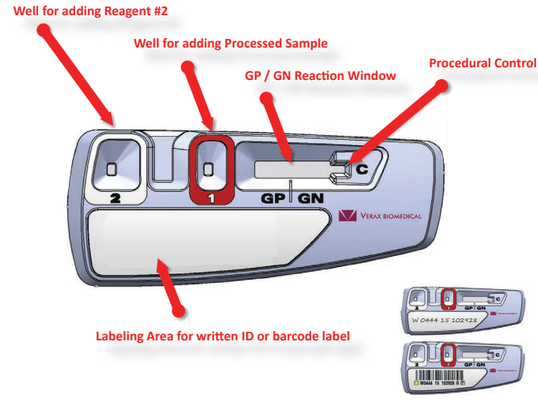
Experiment I: Results with Sequential Dipstick Prototype

- Nine aphaeresis platelet bags were inoculated with wild type platelet isolates.
- Seven of nine inoculated bags grew productively.
- Sequential dipsticks detected the presence of GN platelet isolates earlier than our current PGD test by as much as 18 hours.
- Mock-inoculated negative control bags remained negative throughout the growth study.
- The next-generation PGD *prime* technology in dipstick format demonstrated significantly improved detection of a variety of Gram-negative wild-type platelet isolates.

GN Platelet Isolate	Time to Detection (hours) PGD *prime*	Current PGD	Improvement (hours)
Escherichia coli #4	18	24	6
Escherichia coli #36	12	24	12
Escherichia coli #50	18	36	18
Klebsiella pneumoniae #65	12	24	12
Serratia marcescens #502	24	42	18
Klebsiella pneumoniae #1	12	24	12
Serratia marcescens #31	12	24	12
Escherichia coli #660	(no growth)		
Pseudomonas aeruginosa #5	(no growth)		

Conversion of Sequential Dipstick Assay to an Integrated LFA Device

The sequential dipstick assay was converted to an integrated sequential LFA device with 6 test zones, plus an assay control.



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Experiment II: Results with Prototype PGD *prime* Devices

- Eleven aphaeresis platelet bags were inoculated with wild type platelet isolates.
- All eleven inoculated bags grew productively.
- Prototype PGD *prime* test devices detected the presence of GN platelet isolates up to 24 hours earlier than our current PGD test.
- Mock-inoculated negative control bags remained negative throughout the growth study.
- The prototype PGD *prime* test devices demonstrated significantly improved detection of a variety of Gram-negative wild-type platelet isolates.

GN Platelet Isolate	Time to Detection (hours) PGD *prime*	Current PGD	Improvement (hours)
Citrobacter koseri #53	18	36	18
Escherichia coli #4	24	30	6
Escherichia coli #660	12	42	30
Escherichia coli #36	18	30	12
Escherichia coli #50	18	30	12
Escherichia coli #G	12	24	12
Klebsiella pneumoniae #1	18	30	12
Klebsiella pneumoniae #65	12	24	12
Pseudomonas aeruginosa #5	48	72	24
Serratia marcescens #502	30	42	12
Serratia marcescens #31	12	24	12

Conclusion

- Our next-generation PGD *prime* demonstrates significantly improved detection of a variety of Gram-negative wild-type platelet isolates.
- PGD *prime* is now progressing through the performance verification and validation phase of development.