

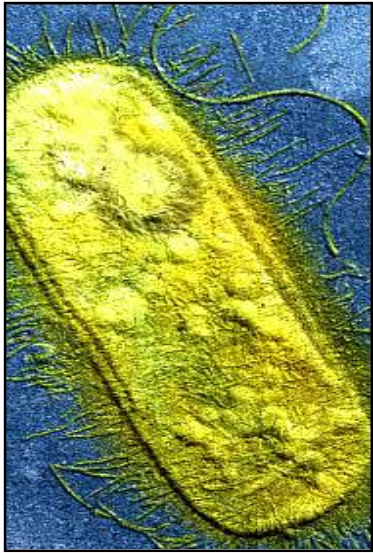
Bacterial Contamination Background



The source of the problem.....

- **skin flora source for bacteria at collection**
- **auto-sterilization in unit is very common**
- **interaction of bacterial strain and nutritive effects of the blood component**

Bacteria pose a unique testing target...



Too few to reliably sample at start of storage

Numerous species implicated

Replicate during storage and at widely differing rates

Understanding the Gap in Detection

Analytical sensitivity

At what concentration are contaminating organisms detected when present in a sample?

Vs.

Clinical sensitivity

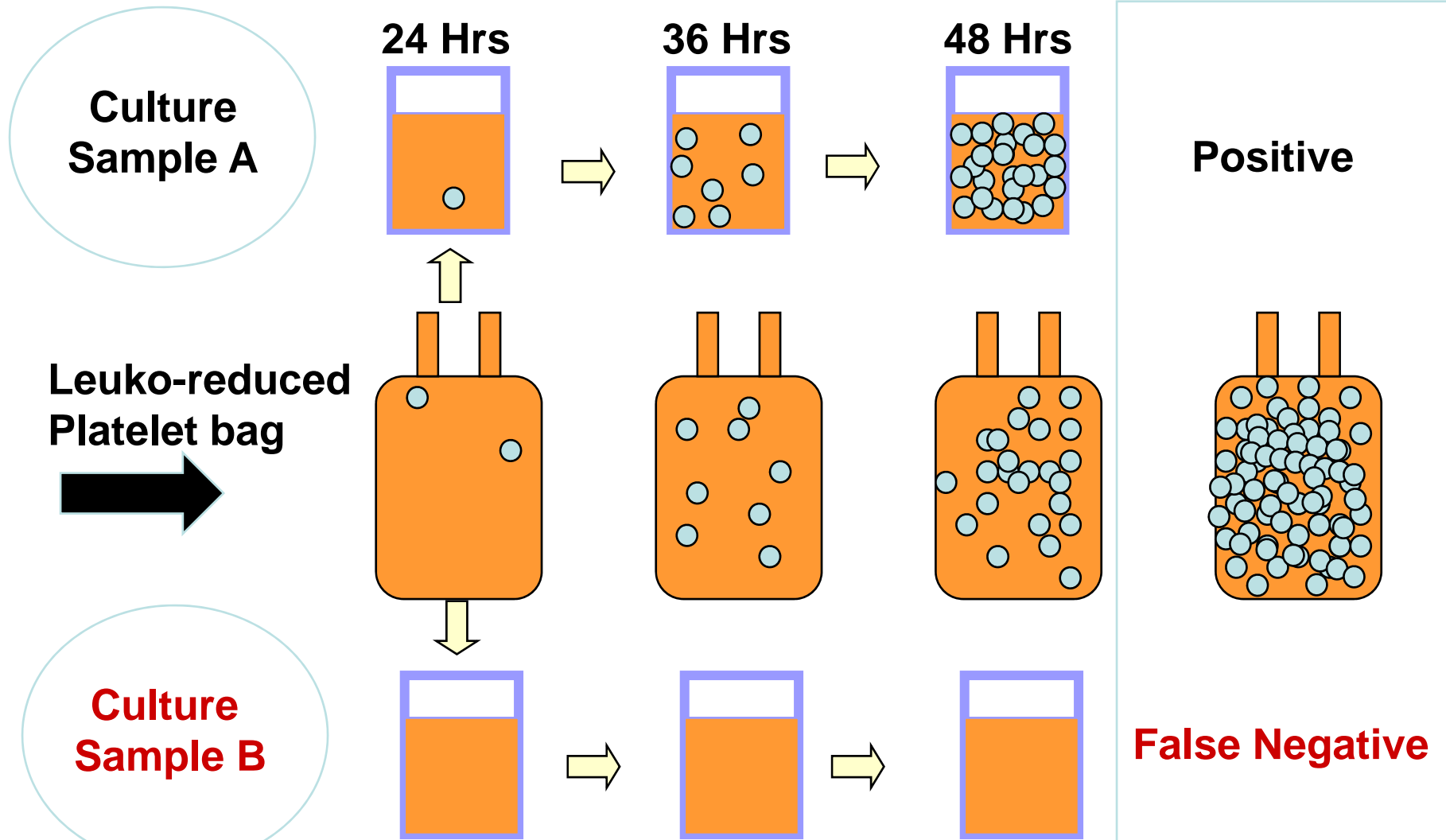
Are contaminated bags detected reliably?

- Culture detection rates indicate that they are not:

True contamination rate: ~ 1:2,000

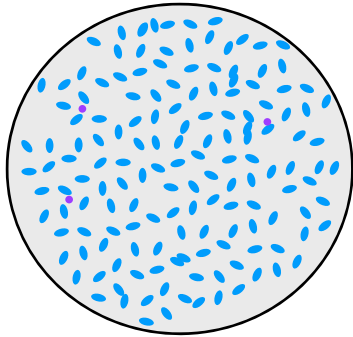
Detected contamination rate: ~ 1:5,000 by culture

Challenge for culture – bacteria in lag phase



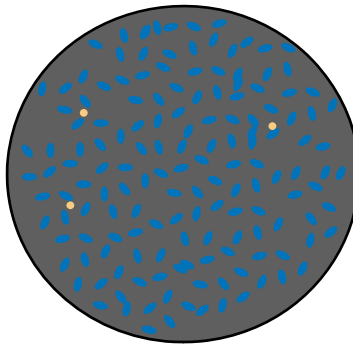
Performance limits of tests for whole blood-derived platelets

Practical? Sensitivity?

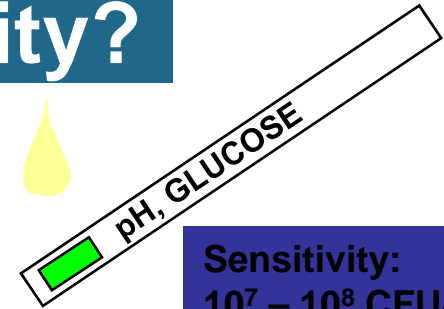


Gram stain sensitivity:
 $10^7 - 10^8$ CFU/mL

Microscopy

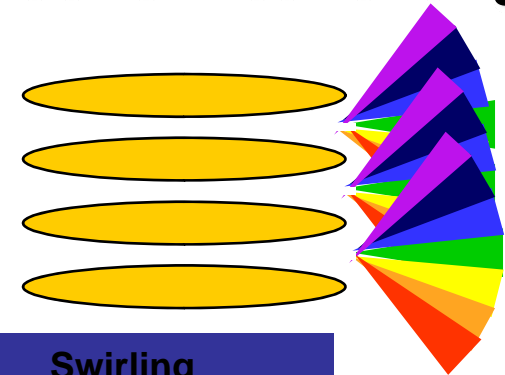


Acridine orange sensitivity:
 $10^5 - 10^7$ CFU/mL



Sensitivity:
 $10^7 - 10^8$ CFU/mL?

Biochemical changes



Swirling
Sensitivity: ? CFU/mL

Wagner S *et al. Transfusion* 1996;36:989-93.

Leach MF *et al. Vox Sang* 1998;74(suppl 1):1180.

A fundamentally different approach

Post-Storage Testing

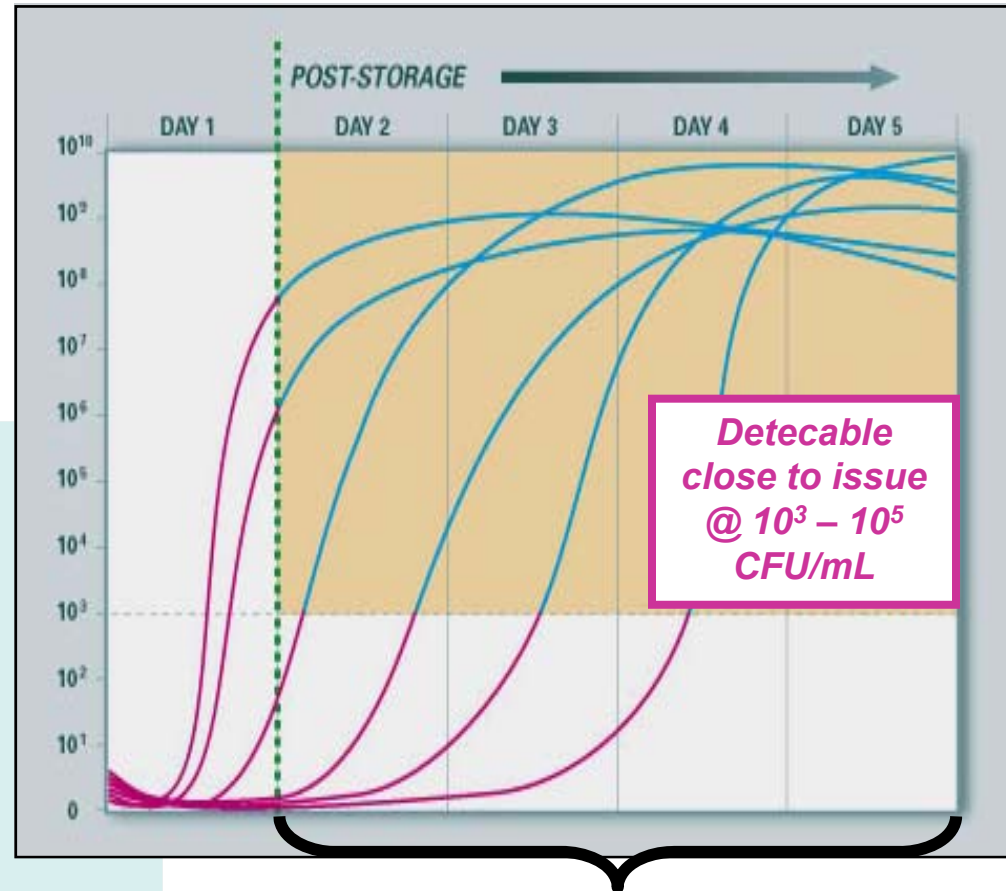
Sampling close to time of issue
Maximizes Clinical Sensitivity

Eliminate false negatives

Avoid quarantines

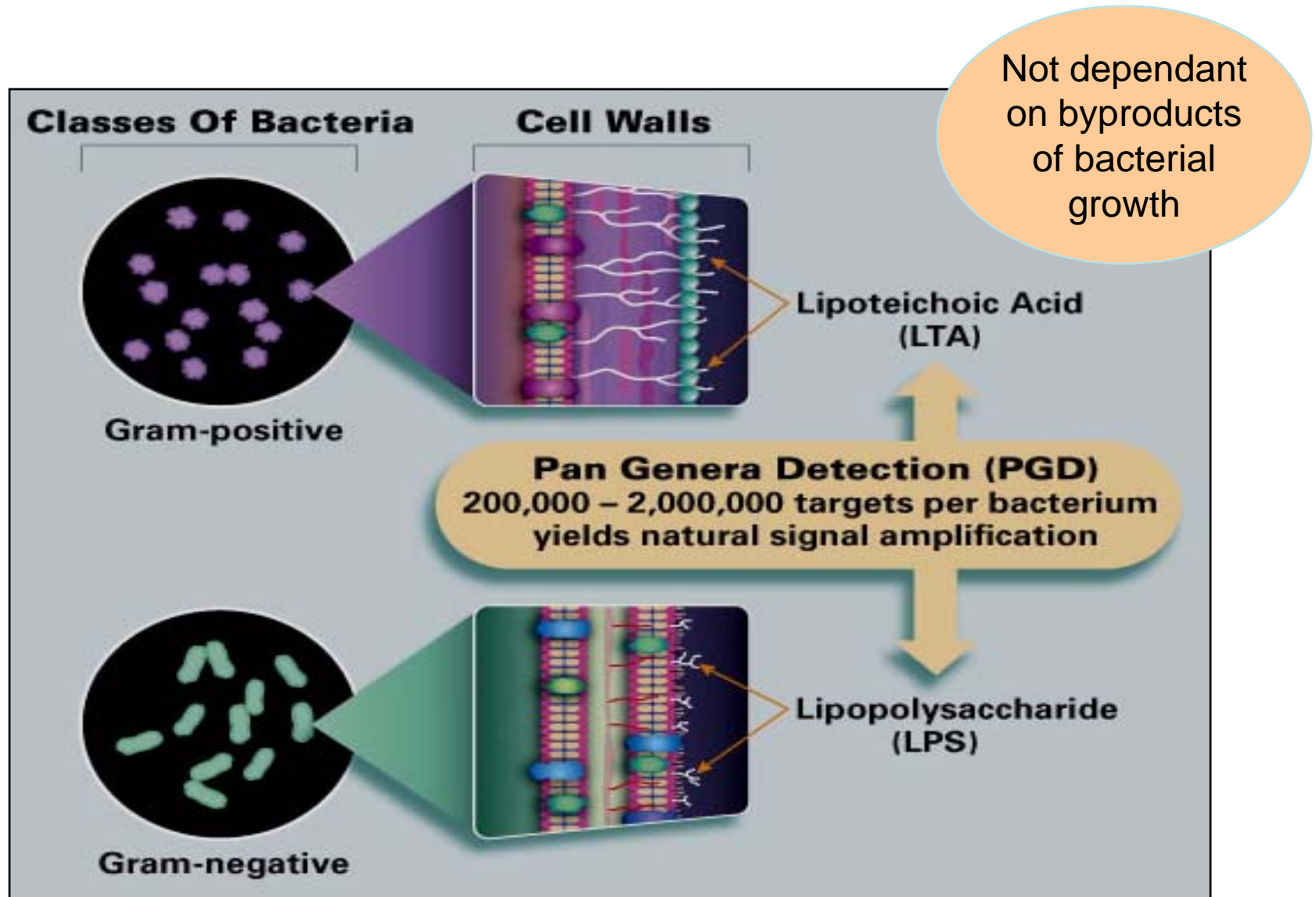
Eliminate call-backs

Simplify inventory management



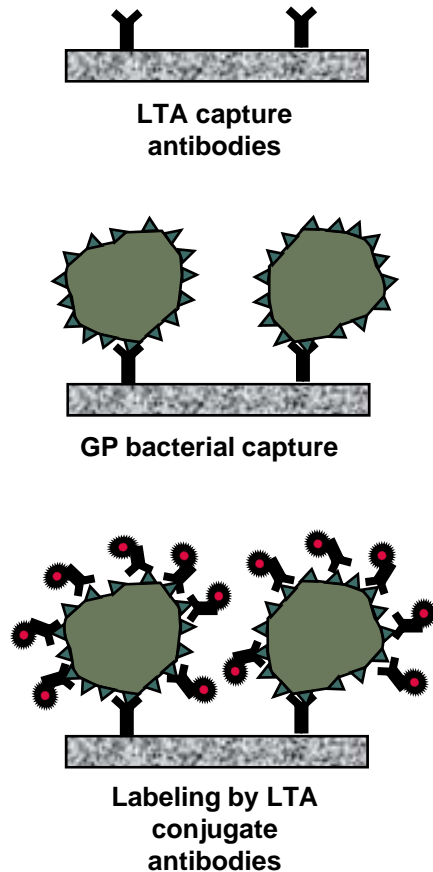
Run rapid test at time of issue

Patented Pan Genera Detection (PGD[®]) Technology

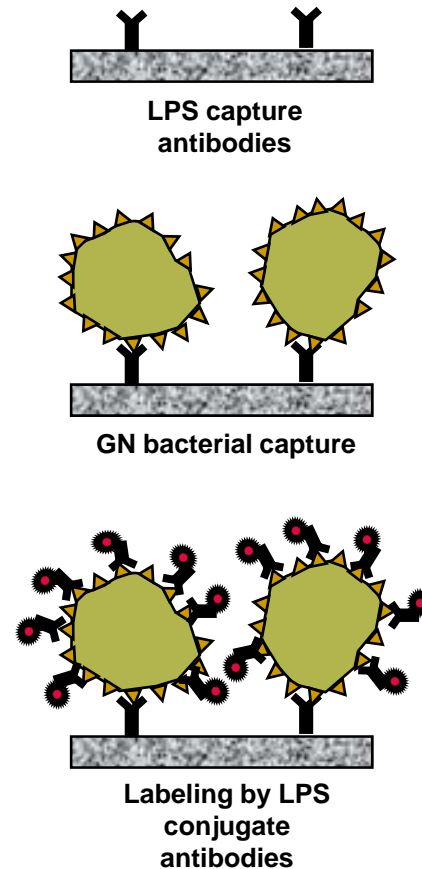


PGD* Immunoassay Format: Two Tests Run in Parallel

Gram positive Sandwich Immunoassay



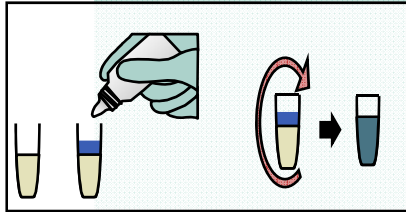
Gram negative Sandwich Immunoassay



Platelet PGD®* Test Procedure

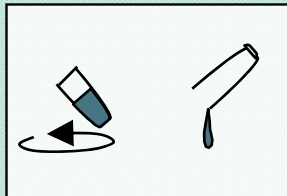
Centrifugation

A.



Add **500 uL** platelet sample to and **8 drops** of Reagent

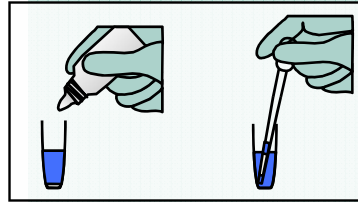
1



Spin for 5 minutes @ 13,400 RPM Decant supernatant

Resuspension

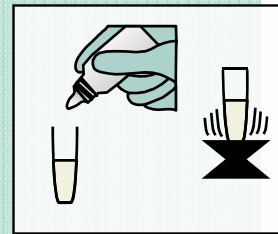
B.



Add **8 drops** of Reagent

2

Solution should Be BLUE

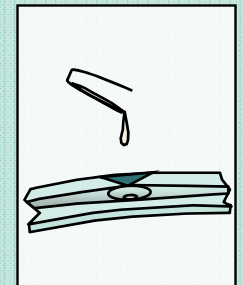


3

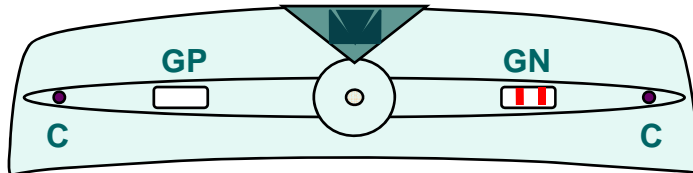
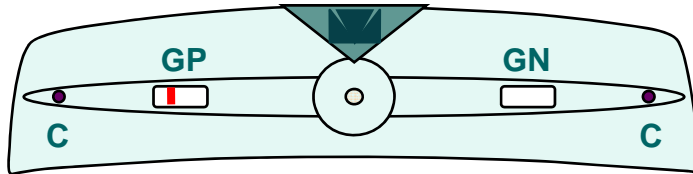
Reading

C.

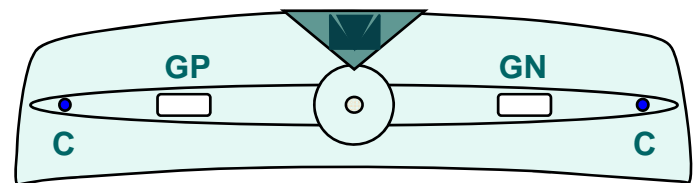
Fill well and Incubate at room temperature until procedural controls change color (~ 20 min)



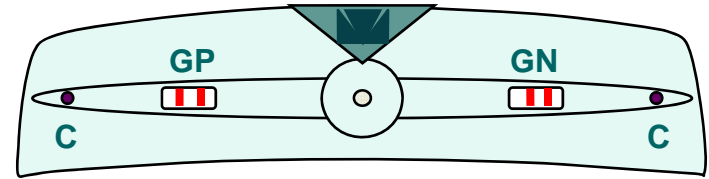
Examples of PGD* Test Results



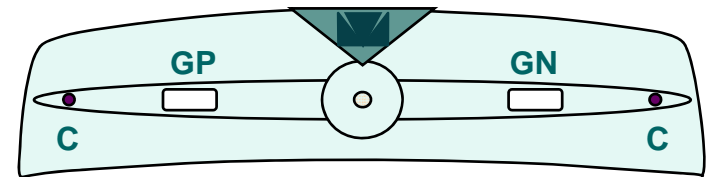
Reactive results



Non-Reactive result



Positive Control result



Negative Control result

Platelet PGD Test: Clinical Performance (LRAP)

- **Specific**

99.7% specificity (N=610 apheresis units) confirmed with plate culture

- **No prozone**

No false negative test results with bacteria $>1E09$ CFU/ml

- **Reproducible**

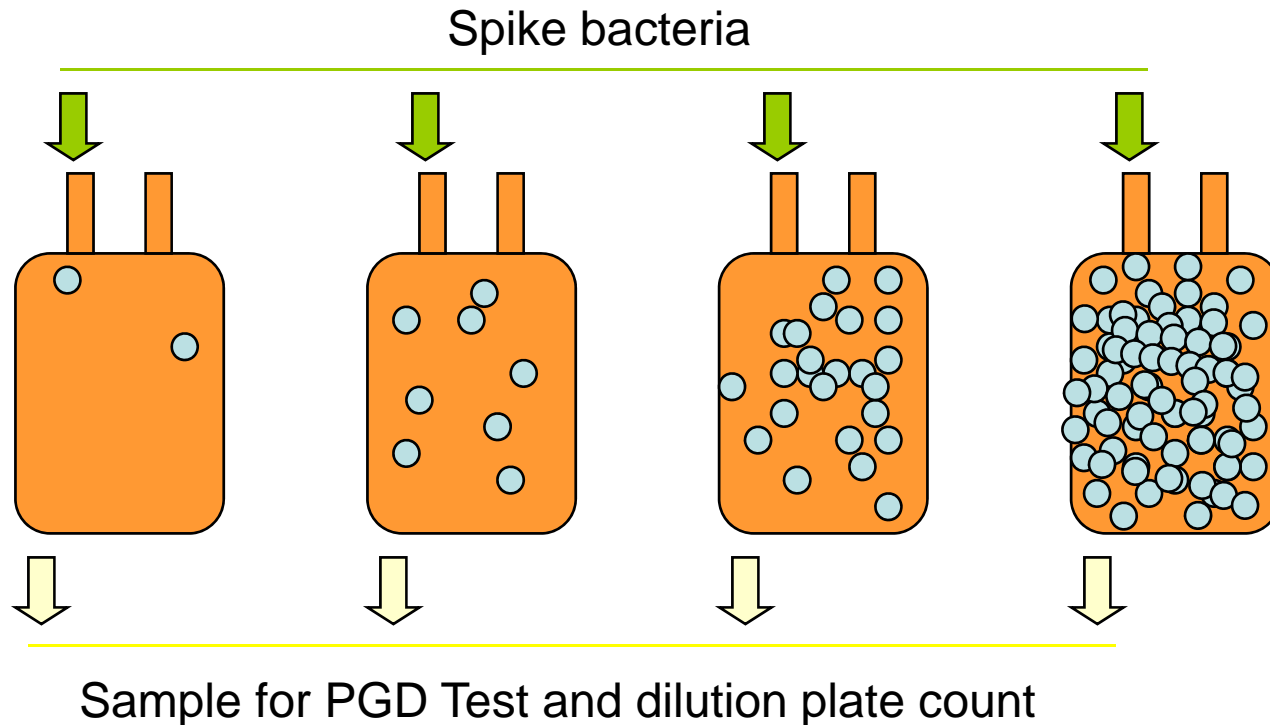
98.8% concordance of expected results on low-titer panel at 3 clinical sites

- **Minimal Interferents**

Specificity and sensitivity not affected by common plasma interferents (e.g. HAMA, hyperlipemia, etc)

Analytical Sensitivity (LRAP)

METHODS:



Criteria for Limit of Detection: 10/10 replicates detected by PGD Test

Analytical Sensitivity (LRAP)

Table 9.1.1.4-1: Platelet PGD Limits of Detection

Organism	Source	LoD
<i>Bacillus cereus</i>	Isolate*	1.2e4
<i>Clostridium perfringens</i>	ATCC 13124	8.9e4
<i>Enterobacter aerogenes</i>	Isolate*	1.0e4
<i>Escherichia coli</i>	Isolate*	2.8e4
<i>Klebsiella pneumoniae</i>	Isolate*	2.0e4
<i>Pseudomonas aeruginosa</i>	Isolate*	8.2e3
<i>Serratia marcescens</i>	ATCC 8100	8.6e5
<i>Staphylococcus aureus</i>	Isolate*	8.2e3
<i>Staphylococcus epidermidis</i>	Isolate*	9.2e3
<i>Streptococcus agalactiae</i>	Isolate*	5.5e4

*blood culture Isolate

Low Titer Inoculation Growth Study in Fresh LRAP Platelets

CULTURE EQUIVALENCY STUDY: A GROWTH MODEL SYSTEM FOR EVALUATION BACTERIA DETECTION TESTS IN PLATELETS

DAY POST-COLLECTION>>>>>	0	1	2	3	4	5
Pt UNIT ACTIVITIES	COLLECT	HOLD OR SHIP	SHIP, HOLD OR TRANSFUSE	TRANSFUSE	TRANSFUSE	TRANSFUSE
DAY OF CE STUDY>>>>>	0	1	2	3	4	5
	INOCULATE	TEST by BacT/ALERT	PGD TEST	PGD TEST	PGD TEST	PGD TEST

BACTERIA USED IN SUDY	INOCULATION AVE. CFU/ml	POS BacT/ALERT hr post-inoculation	PGD Test at 48hr	PGD Test at 72hr
<i>Klebsiella pneumoniae</i>	6.3	32.0	POS (2/3)	POS (1/3)
<i>E coli</i>	8.8	30.5	POS	
<i>Pseudomonas spp</i>	4.3	34.3	POS	
<i>Serratia marcescens</i>	5.6	31.3	POS	
<i>Enterobacter spp</i>	6.6	33.3	POS	
<i>S. aureus</i>	6.7	31.6	POS	
<i>Bacillus spp</i>	1.6	28.0	POS	
<i>Streptococcus spp</i>	3.4	31.0	POS	
<i>S. epidermidis</i>	10.7	34.6	NEG	POS

**STUDY PERFORMED AT THREE CLINICAL SITES

Ultra-Low Titer Inoculation Growth Study in Plasma from Platelets

ULTRA-LOW INOCULATION STUDY: A GROWTH MODEL SYSTEM FOR DEMONSTRATION OF ADDED VALUE OF POST-STORAGE BACTERIAL DETECTION TESTS IN PLATELETS

Bacteria	INOCULATION CFU/bag*	NUMBER OF TIME 0 CULTURES = POS**	SAMPLES TESTED POST-INOCULATION							TERMINAL CULTURE	
			PGD 24hr	PGD 36hr	PGD 48hr	PGD 60hr	PGD 72hr	PGD 84hr	PGD 96hr	SAMPLE TIME (hr)	RESULT
<i>Klebsiella pneumoniae</i>	174	9 of 10	NR	NR	NR	NR	NR	NR	NR	96	Neg
	17	0 of 10	R	R						24	Pos
<i>Serratia marcescens</i>	9	1 of 10	NR	R	R					36	Pos
	1	0 of 10	NR	R	R					36	Pos
<i>Enterobacter spp</i>	43	8 of 10	R	R						24	Pos
	8	2 of 10	NR	R	R					36	Pos
<i>Pseudomonas spp</i>	65	9 of 10	NR	NR	R	R				48	Pos
	8	2 of 10	NR	NR	R	R				48	Pos

*Based on number of observed colonies from ten 8ml samples at Time 0 plating after inoculation into 250-300ml unit.

**Based on 1 or more colonies counted per 8ml sample applied to culture.

Ultra-Low Titer Inoculation Growth Study in Plasma from Platelets

ULTRA-LOW INOCULATION STUDY: A GROWTH MODEL SYSTEM FOR DEMONSTRATION OF ADDED VALUE OF POST-STORAGE BACTERIAL DETECTION TESTS IN PLATELETS

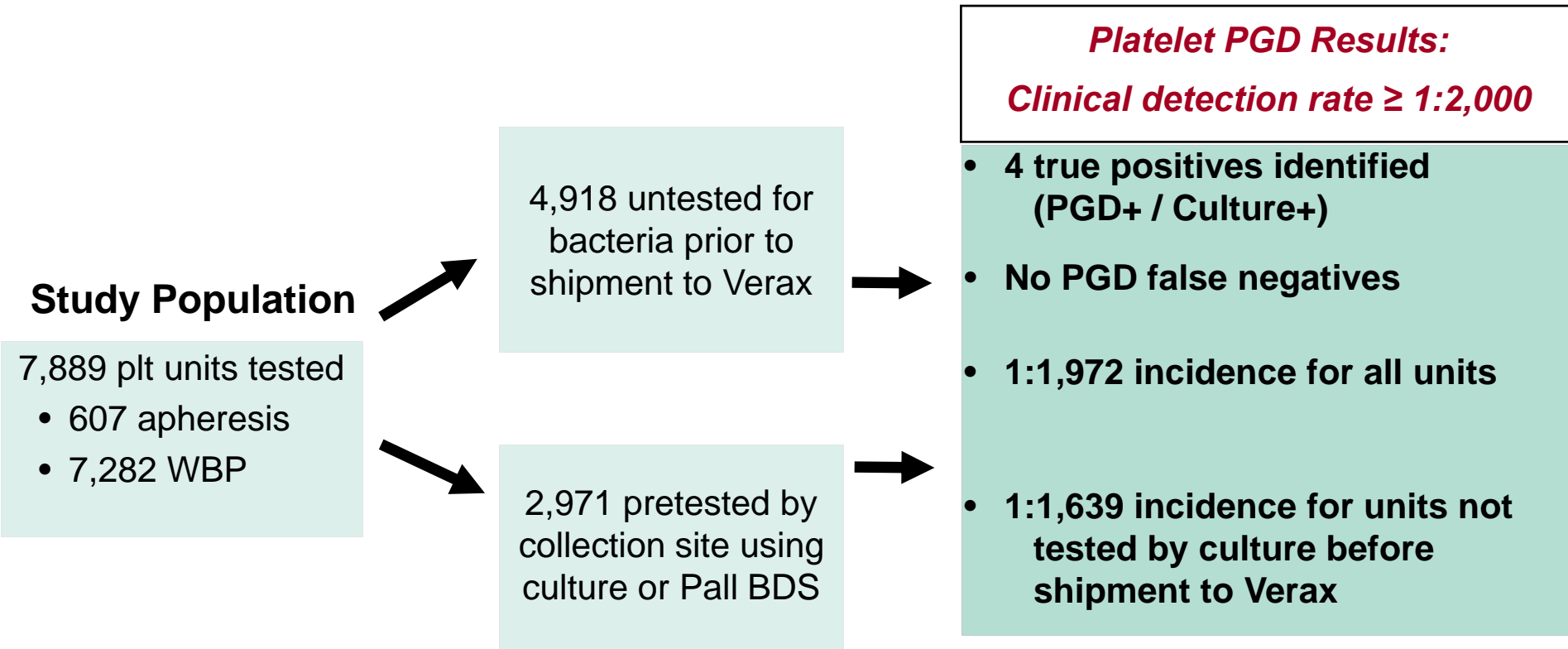
Bacteria	INOCULATION CFU/bag*	NUMBER OF TIME 0 CULTURES = POS**	SAMPLES TESTED POST-INOCULATION							TERMINAL CULTURE	
			PGD 24hr	PGD 36hr	PGD 48hr	PGD 60hr	PGD 72hr	PGD 84hr	PGD 96hr	SAMPLE TIME (hr)	RESULT
<i>Bacillus spp</i>	45	5 of 10	NR	R	R					36	Pos
	5	0 of 10	NR	R	R					36	Pos
	1	0 of 10	NR	R	R					36	Pos
<i>S. epidermidis</i>	26	5 of 10	NT	NT	NR	NR	R	R		72	Pos
<i>Streptococcus spp</i>	100	9 of 10	NR	R	R					36	Pos
	10	3 of 10	NR	R	R					36	Pos
<i>S. aureus</i>	60	8 of 10	NR	NR	R	R				48	Pos
	6	2 of 10	NR	NR	R	R				48	Pos

*Based on number of observed colonies from ten 8ml samples at Time 0 plating after inoculation into 250-300ml unit.

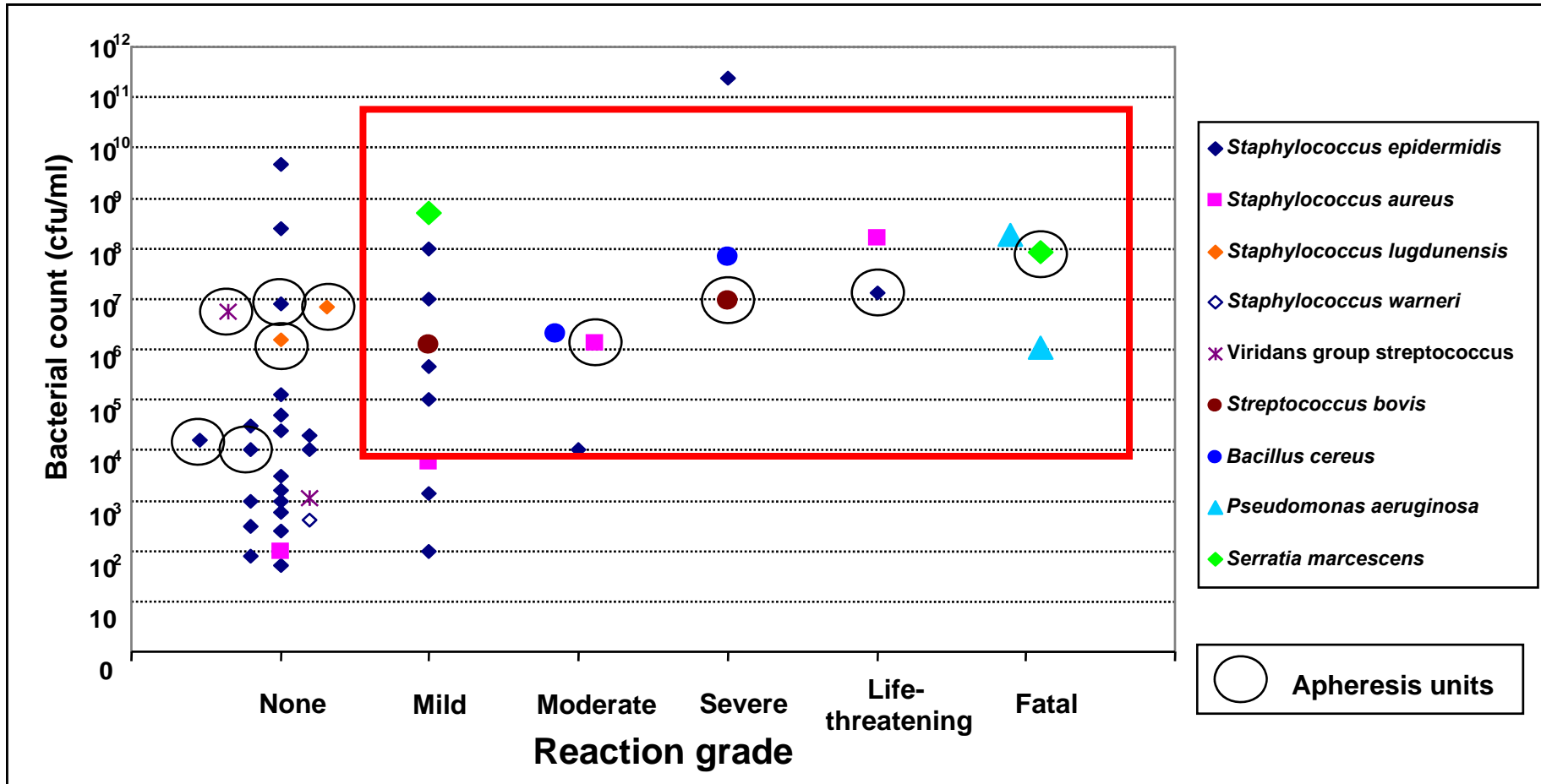
**Based on 1 or more colonies counted per 8ml sample applied to culture.

NT = not sampled/tested

Preclinical data indicates that testing near issue will work...



Bacteria Titer Correlated with Transfusion Reactions....



Jacobs, M. R., C. E. Good, H. M. Lazarus, R. A. Yomtovian. 2008. Relationship between bacterial load, species virulence and transfusion reaction with transfusion of bacterially contaminated platelets. Clin Infect Dis in press.

Yomtovian / Jacobs: BPAC Meeting, 2006

An at issue detection system with a sensitivity of:

~10⁵ CFU/ml would have prevented:

- all fatal reactions
- 91% of serious reactions
- 79% of all reactions

~10³ CFU/ml would have prevented:

- all fatal and serious reactions
- 95% of all reactions

Extension of PGD Test to additional sample types:

- Buffy coat platelets
- Leukoreduced whole blood-derived platelet pools
- Non-leukoreduced whole blood-derived platelet pools

Post-storage testing for bacteria in LRAP....a different approach



*FDA-approved as adjunct QC test for leukoreduced apheresis platelets only. Performance not validated for use in other platelet samples.