PALL Medical

Current issues of bacteria detection; the number one threat of infectious complications associated with transfusion of platelets

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for BSAC April 7, 2004

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AGENDA

Potential availability problems with implementation of CAP & AABB 5.1.5.1

Practical issues of bacteria detection

The ‘Pool & Store’ solution

***E*nhanced performance with *e*BDS**

STATEMENT OF THE PROBLEM

- Unintended consequences of bacteria detection
  - Implementation of bacteria detection may encourage greater reliance on single donor and abandon random donor because of higher cost of supplies and personnel (each unit in pool is tested separately)
  - Concerns over availability of platelets (RDPC) prompted HHS to ask AABB to delay implementation of bacteria detection for platelets
  - Hospitals may implement alternative methods of bacteria detection that will allow contaminated platelets be transfused e.g. dipsticks, pH meters, etc.
- Implication
  - There is a ‘tradeoff’ between safety and availability that can be resolved with the approval of ‘pool & store’

Comparison of Products

Concerns	Apheresis	Whole blood-derived Pool & Store Formerly 5-10 units Currently 4-6 units
COST	√	√
LOGISTICS	√	√
SAFETY & AVAILABILITY	√	√

‘EQUIVALENT’

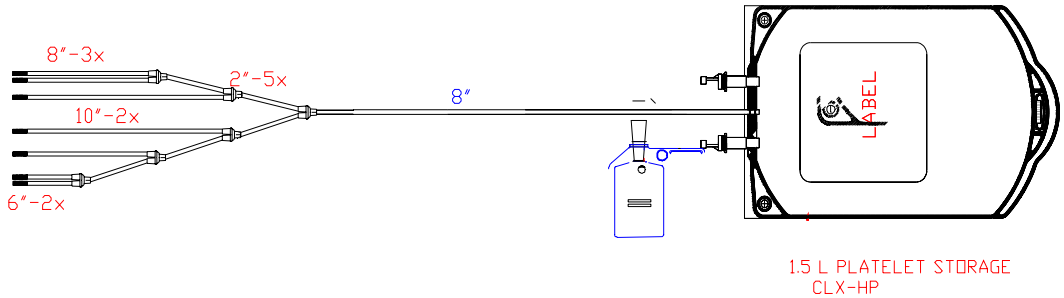
The solution is in 'POOL & STORE'

POOL & STORE

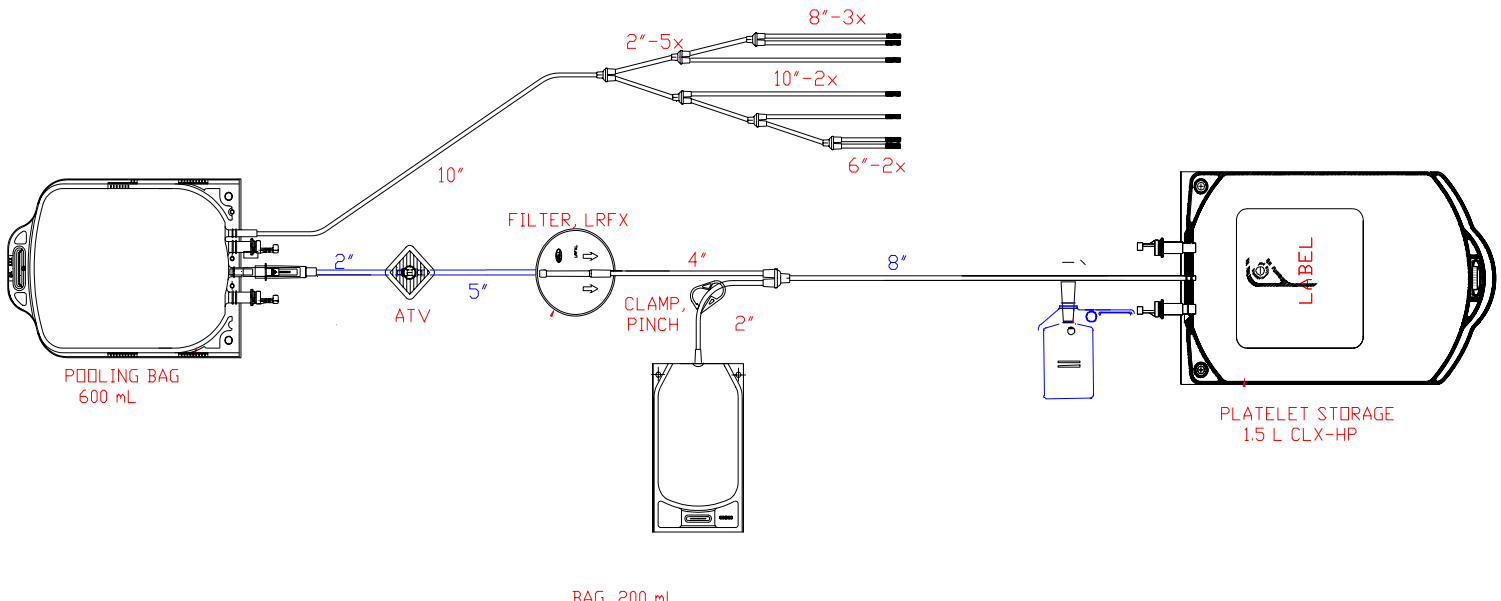
- **Current Pall Status**
  - **Pool & Store - 24 hour CCI data submitted, meeting with FDA on Apr 15 to discuss**
  - **The literature is replete with comparisons between 1 and 24 hr and the direction of the effect is proportion with 1 hr data about twice that of 24 hr**
  - **Pall 'Pool & Store' systems have been in routine use in Europe for years**

Pool & Store System Designs

System 1. *for leukoreduced (LR) RDPC*



System 2. *for non-LR RDPC*



Studies completed to date

- In vitro (5 and 7 days) and in vivo (5 day) studies have been performed for both systems:
  - No effect of pre storage pooling on lymphocyte (IL-6, IL-8, MLR, etc.) and plasma activation (complement, coag factors, etc) during storage.
  - Satisfactory in vivo (5 days) and in vitro (5 and 7 days) storage quality

Reflection of the Problem

Table 1. Summary of organisms identified in the BACON, SHOT, and BACTHEM studies

Organism	United States	United Kingdom	France	Total
Gram positive				
Bacillus cereus	1	4 (1)	2	7(1)
Coagulase negative *Staphylococcus*	9	6 (1)	5	20 (1)
Streptococcus species	3 (1)	2		5 (1)
Staphylococcus aureus	4	2 (1)		6 (1)
Propionibacterium acnes			3	3
Subtotal	17 (1 or 6%)	14 (3 or 21%)	10 (0 or 0%)	41 (4 or 10%)
Gram negative				
Klebsiella species			2 (1)	2 (1)
Serratia species	2 (2)		1 (1)	3 (3)
Escherichia coli	5 (1)	2 (1)	1	8 (2)
Acinetobacter species			1	1
Enterobacter species	2 (1)	1 (1)	1	4(2)
Providencia rettgeri	1 (1)			1(1)
Yersinia enterocolitica	1			1
Subtotal	11 (5 or 45%)	3 (2 or 67%)	6 (2 or 33%)	20 (9 or 45%)
Total	28 (6 or 21%)	17 (5 or 29%)	16 (2 or 13%)	58 (11 or 19%)

Numbers of fatalities and the percent of the total are reported in parenthesis.
BACON—Bacterial Contamination of Blood; BACTHEM—French matched case-control study assessing transfusion-associated bacterial contamination determinants; SHOT—Serious Hazards of Transfusion.
(*From* Kuehnert *et al.* [6], Perez *et al.* [7], and Ness *et al.* [8••].)

Brecher & Hay. Curr Hematol Reports 2004;3:121-7

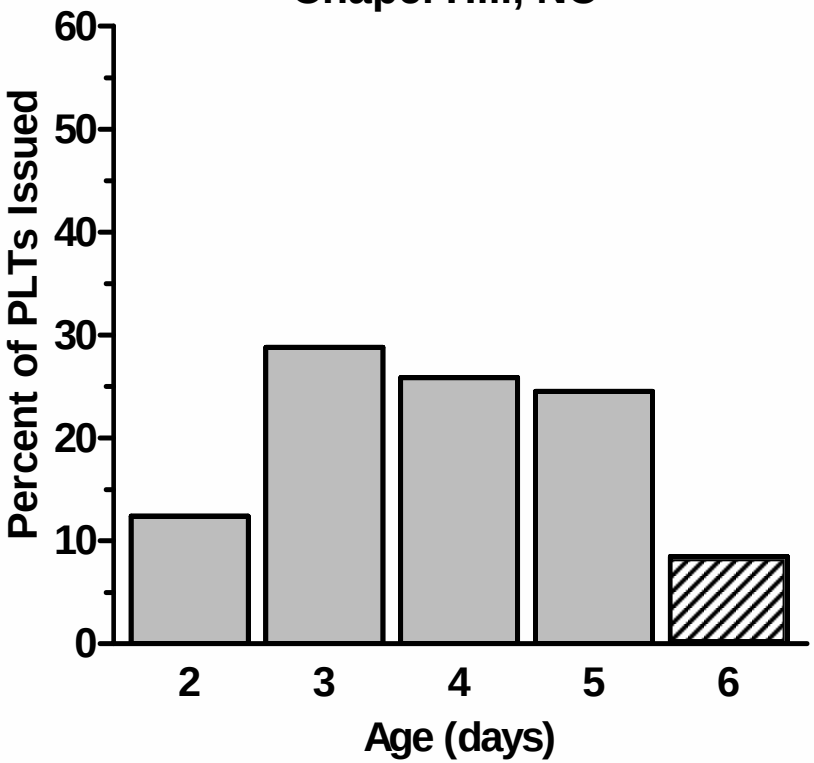
The BaCon study shows PLT 2 to 4 days old are associated with Mortality

Fatal transfusion reactions reported in BaCON study from 1998-2000		
Implicated Component	**Organism**	**Storage (days)**
SDP	*Group B beta-Strep*	4
SDP	*E coli*	2
SDP	*P rettgeri*	3
SDP	*E cloacae*	3
Pooled PLT	*S marcescens*	2
Pooled PLT	*S marcescens*	2

Kuehnert *et al.* Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. Transfusion 2001;40:1493-1499.

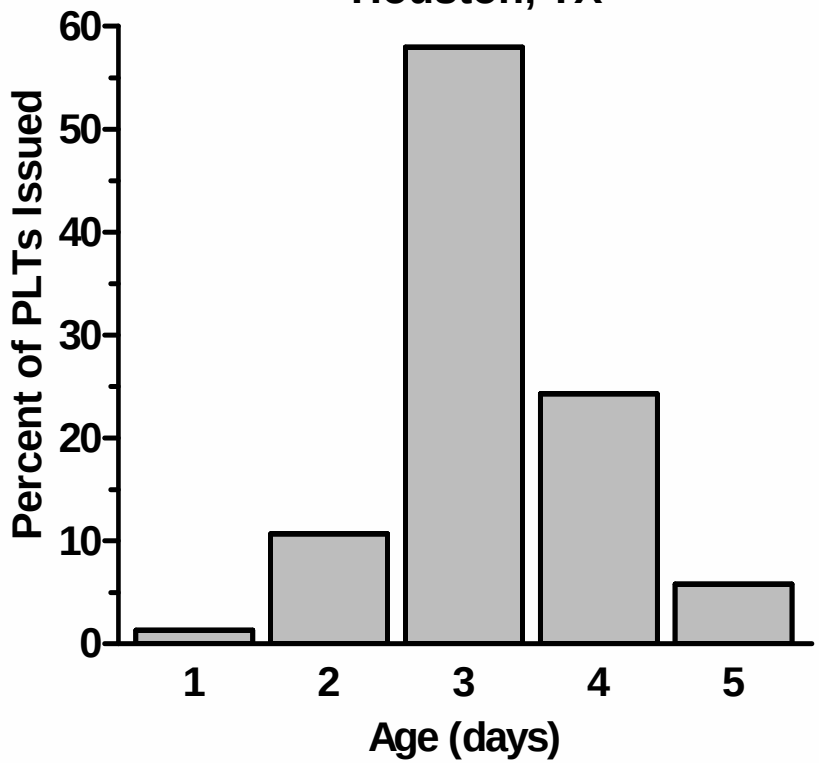
Practical Use of 2-4 day old PLTs

University of North Carolina
Chapel Hill, NC



Brecher ME, *et al.* Transfusion 2003;43:974-8.

MD Anderson Cancer Center
Houston, TX



Werch JB, *et al.* Transfusion 2002;42:1027-31

Observations about bacteria growth in PCs

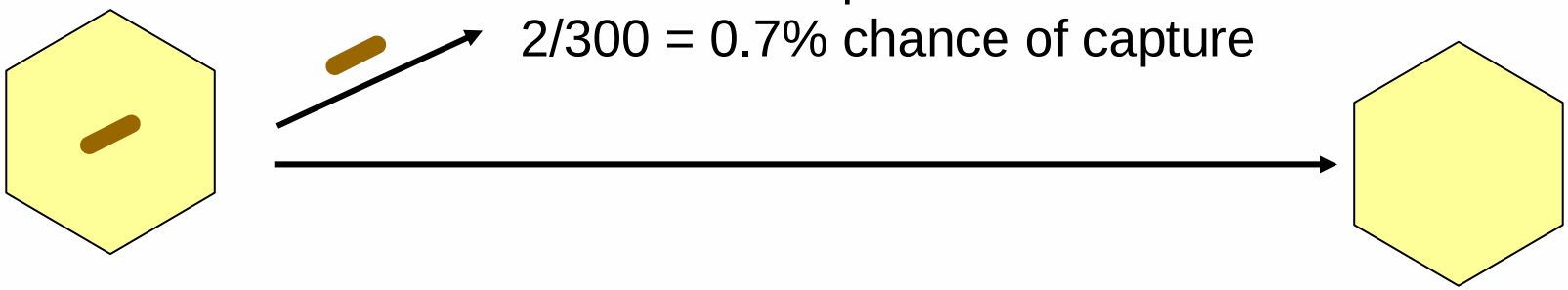
- **Bioburden usually low (rarely >10 CFU/mL)**
  - Buchholz DH, Young VM, Friedman NR, Reilly JA, Mardiney MR Jr. Bacterial proliferation in platelet products stored at room temperature. Transfusion-induced Enterobacter sepsis. N Engl J Med. 1971 Aug 19;285(8):429-33.
  - Buchholz DH, Young VM, Friedman NR, Reilly JA, Mardiney MR Jr. Detection and quantitation of bacteria in platelet products stored at ambient temperature. Transfusion. 1973 Sep-Oct;13(5):268-75.
  - Cunningham M, Cash JD. Bacterial contamination of platelet concentrates stored at 20 degrees C. J Clin Pathol. 1973 Jun;26(6):401-4.
  - Arnow PM, Weiss LM, Weil D, Rosen NR. Escherichia coli sepsis from contaminated platelet transfusion. Arch Intern Med. 1986 Feb;146(2):321-4.

- **Concentrations less than 5 CFU/mL are often complicated by inconsistent growth ('autosterilization')**
  - Myhre BA, Walker LJ, White ML. Bacteriocidal properties of platelet concentrates. Transfusion. 1974 Mar-Apr;14(2):116-23.
  - Heal JM, Singal S, Sardisco E, Mayer T. Bacterial proliferation in platelet concentrates. Transfusion. 1986 Jul-Aug;26(4):388-90.
  - Kim DM, Brecher ME, Bland LA, Estes TJ, McAllister SK, Aguero SM, Carmen RA, Nelson EJ. Prestorage removal of Yersinia enterocolitica from red cells with white cell-reduction filters. Transfusion. 1992 Sep;32(7):658-62.
  - Brecher ME, Boothe G, Kerr A. The use of a chemiluminescence-linked universal bacterial ribosomal RNA gene probe and blood gas analysis for the rapid detection of bacterial contamination in white cell-reduced and nonreduced platelets. Transfusion. 1993 Jun;33(6):450-7.

Sampling Error

1 bug/300 mL SDP bag

Sample 2 mL
2/300 = 0.7% chance of capture



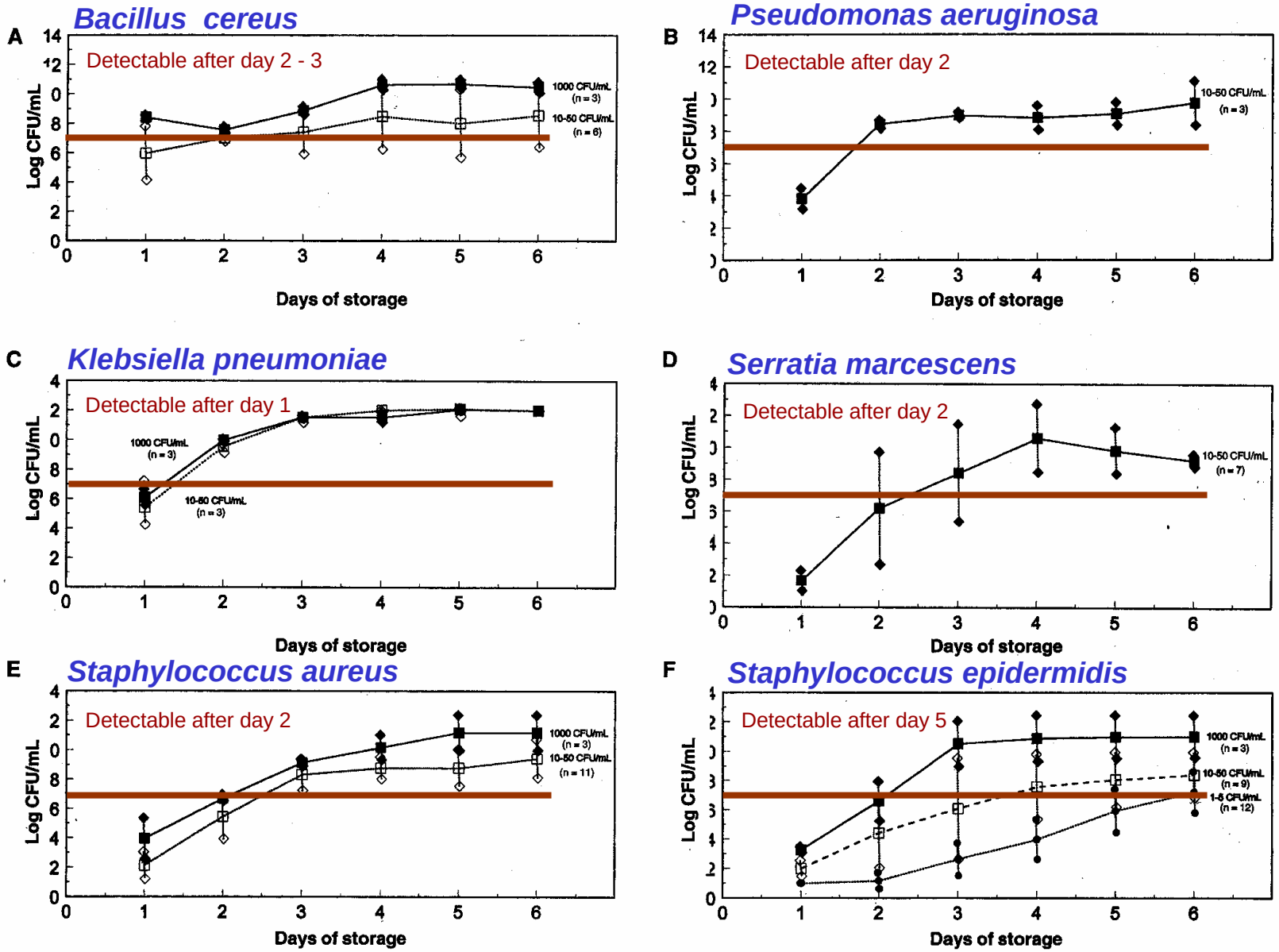
If bug <u>is</u> capture it leaves the platelet bag sterile and the result is a false positive

If bug is <u>not</u> captured, the result is a false negative

- Allow organisms time to grow to sufficient levels to avoid sampling error (12-24 hrs)
- Applicable to SDP and RDPC alike
- Hard to imagine its avoidance regardless of method of detection

Bacteria growth in PC

Implications for sensitivity of detection methods

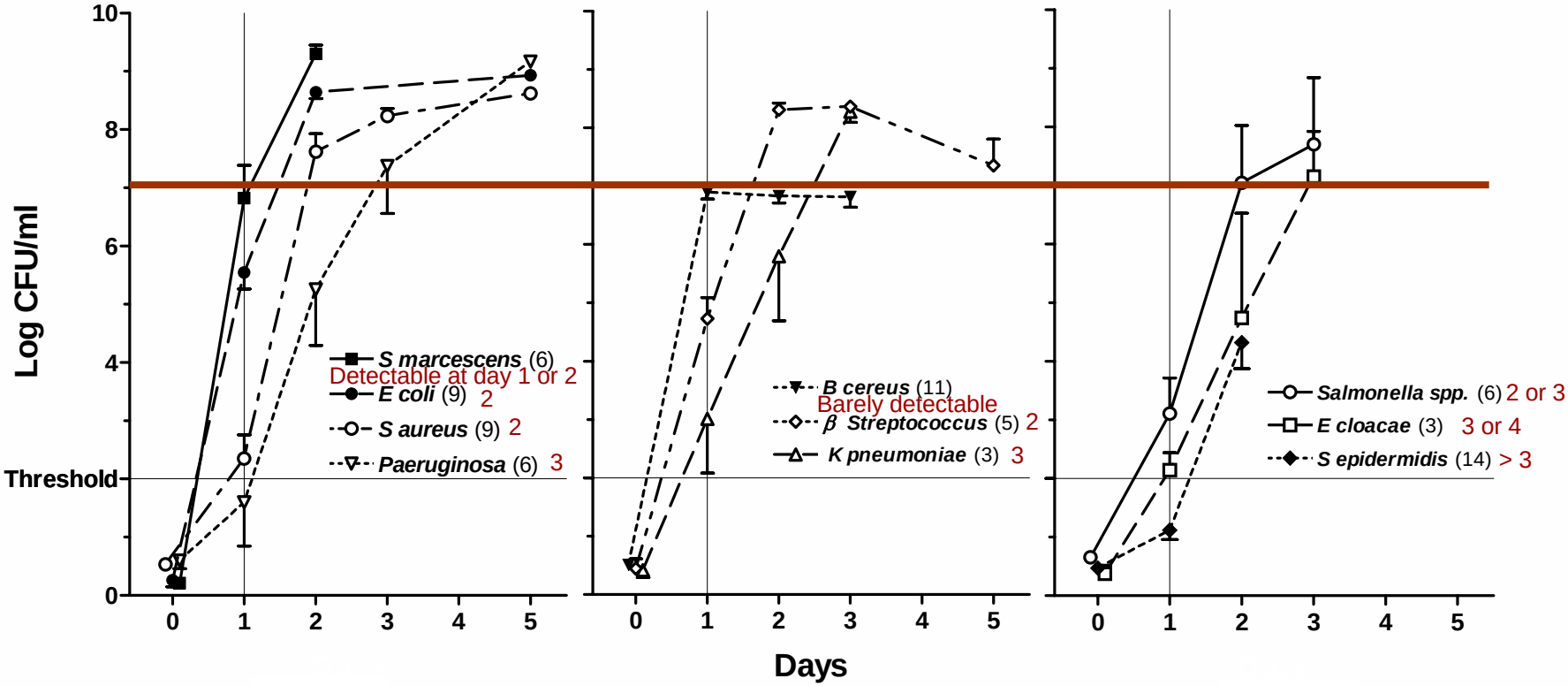


Brecher ME, *et al*. Transfusion. 2000 Nov;40(11):1308-12.

Corroborating data

Bacteria Growth Curves

Leukoreduced Platelet Concentrate 22 C



1-5 CFU/mL at time zero in fresh LR-RDPC (n=)

Ortolano GA, *et al*. Detection of bacteria in WBC-reduced PLT concentrates using percent oxygen as a marker for bacteria growth. Transfusion. 2003 Sep;43(9):1276-85

Culture-based methods like eBDS

- Take a sample

- Foster growth of bacteria in the sample to get to detection sooner than measuring microbes in the platelet product could provide

Pall *e*BDS System

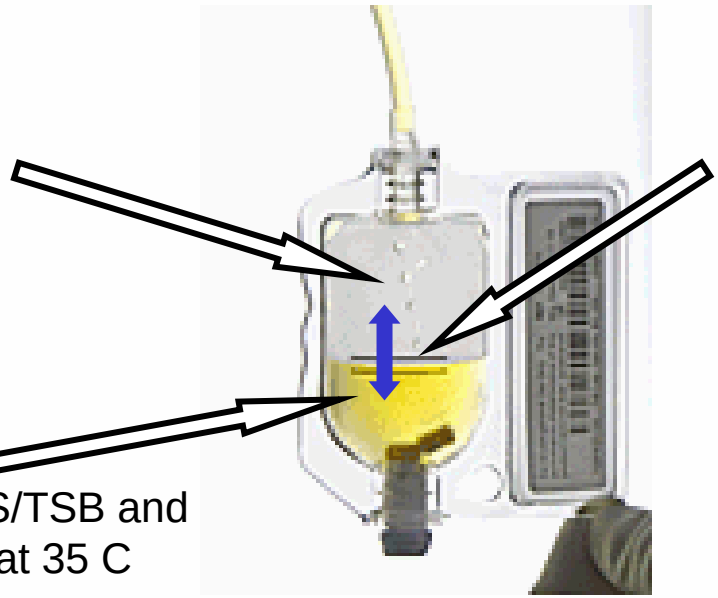
Sampling Set

Equipment

Technical approach for bacterial detection

The Pall BDS, and now *e*BDS, use oxygen as a surrogate marker of bacterial growth

Stage 3:
Pall *e*BDS measures oxygen in headspace and compares to predetermined threshold limit



Stage 2: Oxygen equilibration between air and plasma

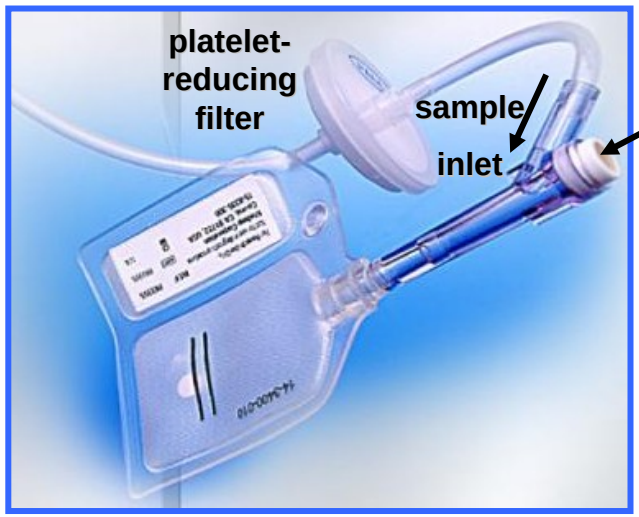
Stage 1:
Incubate in SPS/TSB and agitate sample at 35 C

Bacteria consume oxygen in plasma

*e*BDS Operational Enhancements

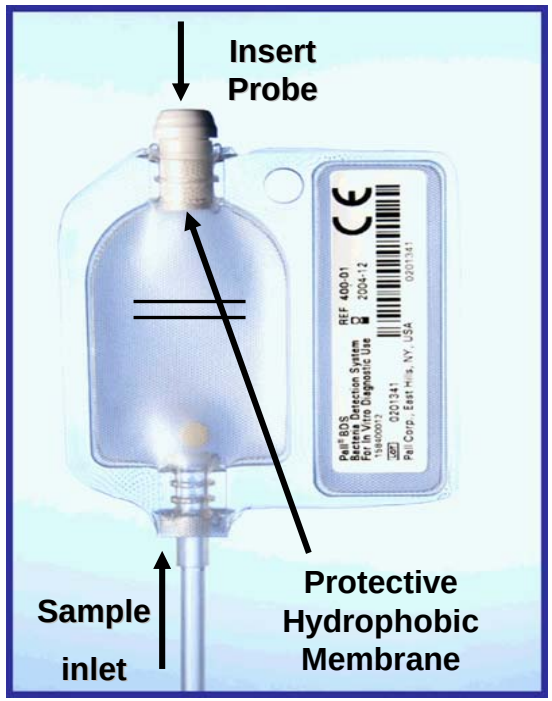
Removed filter to eliminate bacteria retention in filter and

re-designed sampling port to prevent aspiration of plasma that used to cause a probe to block (increased delta P) requiring change and re-sample



Original (Pall BDS)

Platelet reducing filter
Sample inlet and probe port on same side



New (Pall *e*BDS)

ZERO TIME SAMPLING NOT IN OUR IFU BUT DATA REFLECTS *e*BDS ROBUSTNESS

Low Level Spiking Studies

Table 1. *Performance Summary with Sampling Taken at the Time of Inoculation*

	Bacteria Level Immediately After Inoculation and Sampling (Sample Time = 0 hrs)				**Number Detected out of Total Tested** (Detection with Sampling at 0 hrs)
	≤5 CFU/mL	6-15 CFU/mL	16-50 CFU/mL	>51 CFU/mL	
S. epidermidis	4	1			5 of 5
S. agalactiae	5	4	2		11 of 11
S. aureus		5	4		9 of 9
P. aeruginosa		11			8 of 11
S. choleraesuis	4	2	5		11 of 11
E. coli	1	6			7 of 7
E. cloacae		6			6 of 6
B. cereus	2	6	4		12 of 12
K. pneumoniae	3	7	1		11 of 11
S. marcescens		5			5 of 5
TOTAL	**19**	**53**	**16**	**0**	**85 of 88 (96.6%)**

Low Level Spiking Studies

Table 2. *Performance Summary with Sampling Taken 24 Hours After Inoculation*
(Number of Bacteria Detected with 24 Hour Incubation)

	Bacteria Level at the Time of Inoculation	**Bacteria Level After 24 Hours Storage** (Sample Time = 24 hrs)				**Number Detected out of Total Tested** (Detection with Sampling at 24 hrs)
	Median (range) CFU/mL	≤5 CFU/mL	6-15 CFU/mL	16-50 CFU/mL	>51 CFU/mL	
S. epidermidis	7 (2-52)	1	15	8	3	27 of 27
S. agalactiae	5 (2-20)	3	7	9	9	28 of 28
S. aureus	8 (2-51)			5	24	29 of 29
P. aeruginosa	9 (1-15)		1	4	19	24 of 24
S. choleraesuis	8 (1-55)	6		2	16	24 of 24
E. coli	6 (2-15)				27	27 of 27
E. cloacae	8 (2-13)	4	4	4	16	28 of 28
B. cereus	13 (3-27)			2	31	33 of 33
K. pneumoniae	5 (1-17)	12	9	3	9	33 of 33
S. marcescens	9 (1-16)	2			25	27 of 27
TOTAL		**28**	**36**	**37**	**179**	**280 of 280 (100%)**

Summary of Limitations to BDS
all addressed by *e*BDS

BDS Limitations

- Sensitivity: variability in threshold of detection, particularly with slow growing organisms
- Ease of Use: Frequent probe changes due to probe blockage
- System errors (Error 6.2 – “zero” oxygen reading)
- Platelet volume loss: 7 mls of platelets required

eBDS Solutions

- Eliminate requirement for cell-removing filter allowing improved capture of low level bacteria contamination
- New sample site design virtually eliminates probes blockage
- Software programming change
- New set design requires only 4 mls

Alternative Point of Issue Test Methods

Multi-reagent strips (dipsticks)
pH meter or pH by blood gas analyzer
glucose by automated analyzer
(glucose oxidase assay)

Salient Feature	Study
Minimum CFU/mL just before postive detection by either pH, glucose or swirling was, for 7 different organisms, about 10^6 and averages of approximately 10^8	Wagner & Robinette. Transfusion 1996; 36:989-93
Glucose detects *S aureus* at 10^3 CFU/mL, *K pneumoniae* at 10^5 (2 days after inoc) and *S epi* not detected through 5 days	Burstain et al. Transfusion 1997; 37:255-8
Glucose for *S aureus* on day 2 at 10^3 CFU/mL; *Salmonella* on days 3, 4 or 5 at 10^{10} and *Staph* on day 2 at 10^3	Leach MF, *et al.* Transfusion 1998;38(Suppl):89S
Glucose & pH on day 2 *K pneunoniae* at 10^9 CFU/mL, day 3 for *S marcescens* at 10^9, day 4 *S aureus* at 10^8 and *S epi* not detected through day 5	Werch JB, *et al.* Transfusion 2002;42:1027-31

Most Complete Study to Date

- *B cereus* at 10^6 CFU/mL on day 2 of storage, 2 of 3 + with glucose with dipstick but not detected with pH or swirling
- *S aureus* at 10^5 CFU/mL on day 3 of storage, 2 of 3 + glucose with dipstick, 3/3 detected with pH but none for swirling
- *S epi* at 10^4 CFU/mL on day 4 of storage, 0 of 3 detected with any method (dipstick pH and glucose, analyzer glucose, analyzer pH or swirling)

Hay & Brecher manuscript – *in press*

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Conclusion

- Bacteria contamination occurs with significant morbidity and mortality
- There are two QC approved methods that are based upon long-standing and well-understood principles of standard culture technology
- The preferential use of approved QC bacteria detection methods is limited to SDP because of cost in applying this to multiple RDPC
- The reliance upon SDP may present availability problems
- Cost issues for RDPC can drive health care to use inferior methods of bacteria detection which may compromise safety
- *Pool and Store* is a solution

Whatever BSAC can do to reduce the latency to approval of pool and store will likely promote both platelet safety and availability