Detection of Bacterial Contamination in Pooled Whole Blood Derived Platelets With the BacTALERT Automated Culture System

Mark E. Brecher

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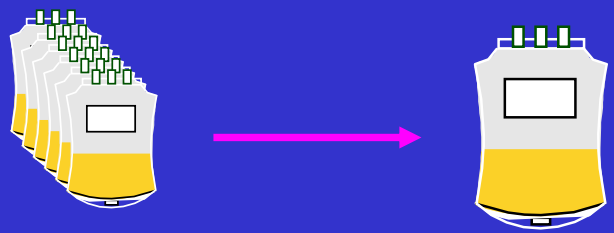


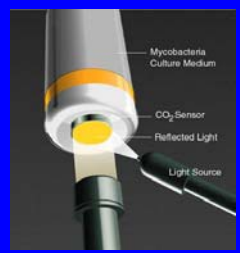
Pre-pooled random platelets

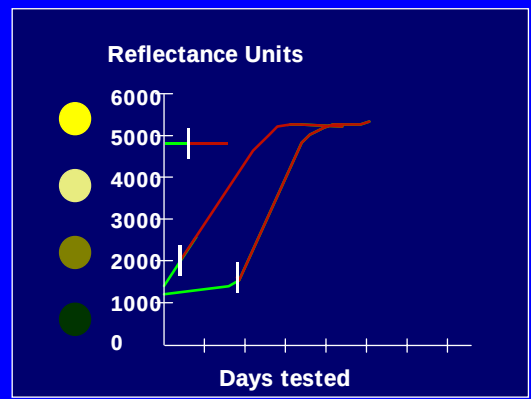
FDA thinking had required

1. CCI studies
2. Release control bacterial detection step

FDA's current thinking regarding pre-storage pooling of WBPCs (aka random platelets) is that such systems can be cleared if culture monitoring QC is performed by tests with analytical sensitivity similar to that cleared for single units

BacT/ALERT Microbial Detection System





TRANSFUSION COMPLICATIONS

Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms

TRANSFUSION PRACTICE

Evaluation of a new generation of plastic culture bottles with an automated microbial detection system for nine common contaminating organisms found in PLT components

TRANSFUSION COMPLICATIONS

Evaluation of a new generation of culture bottle using an automated bacterial culture system for detecting nine common contaminating organisms found in platelet components

M.E. Brecher, D.G. Heath, S.N. Hay, S.J. Rothenberg, and L.C. Stutzman

TRANSFUSION COMPLICATIONS

Validation of BacT/ALERT plastic culture bottles for use in testing of whole-blood-derived leukoreduced platelet-rich-plasma-derived platelets

M.E. Brecher, S.N. Hay, and S.J. Rothenberg

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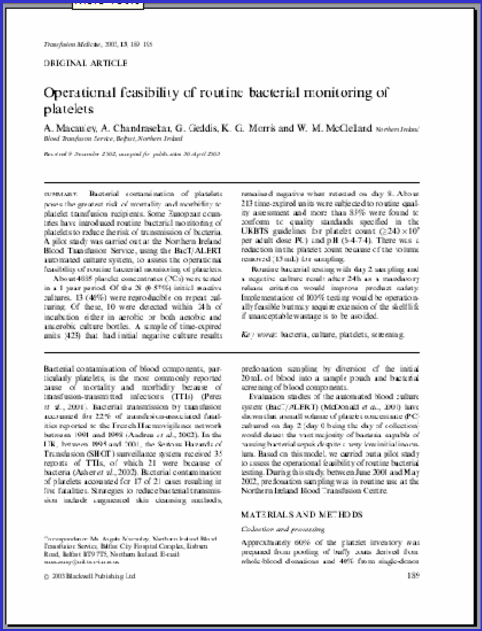
ORIGINAL PAPER

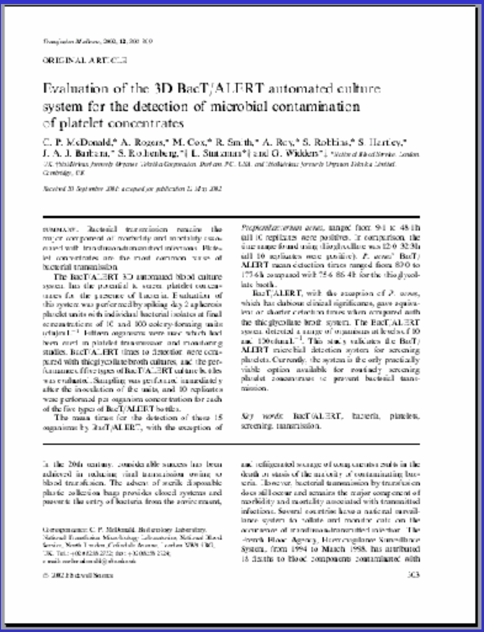
Evaluation of the BacT/Alert automated blood culture system for detecting bacteria and measuring their growth kinetics in leucodepleted and non-leucodepleted platelet concentrates

Vox Sanguinis


ORIGINAL ARTICLE

Operational feasibility of routine bacterial monitoring of platelets

A. Macauley, A. Chandrasekar, G. Geddis, K. G. Morris and W. M. McClelland Northern Ireland Blood Transfusion Service, Belfast, Northern Ireland


ORIGINAL ARTICLE

Evaluation of the 3D BacT/ALERT automated culture system for the detection of microbial contamination of platelet concentrates

C. P. McDonald,* A. Rogers,* M. Cox,* R. Smith,* A. Roy,* S. Robbins,* S. Hartley,* J. A. J. Barbara,* S. Rothenberg,† L. Sutaman*† and G. Widders*‡

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Pooled Leukoreduced PRP Platelets
10 and 100 CFU/mL

SCD

5X

4 mLs per bottle

0.5 mL per Plate x 2

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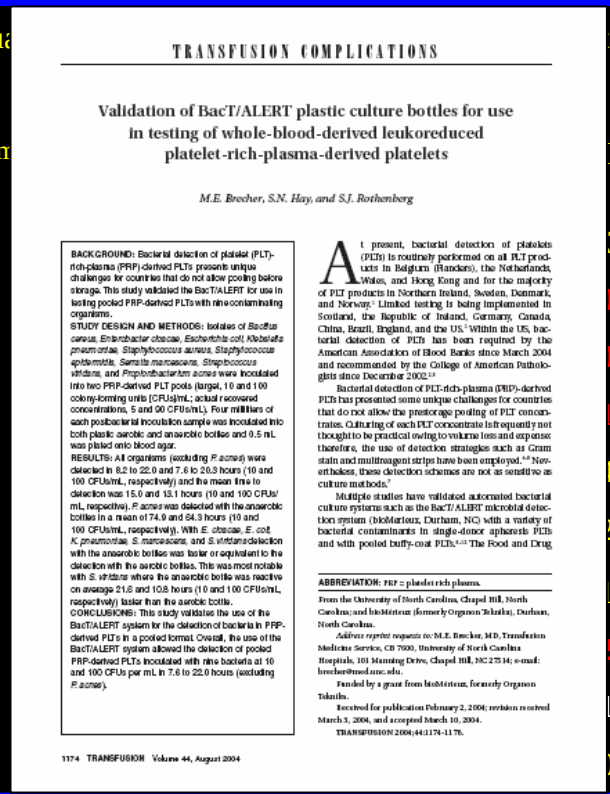
Pooled Leukoreduced PRP Platelets

Organism	Inocul CFU/mL	Actua CFU/m	andard anaerobic bottles - Hours ean	SD	Min	Max
B. cereus	4	5	3	0.6	8.2	9.7
E. cloacae	20	17	.7	0.1	10.6	10.9
E. coli	13	6	.6	0.1	10.5	10.6
K. pneumo	12	2	.8	0.1	11.6	11.9
P. acnes	10	<2	.9	1.1	74.4	76.8
S. aureus	12	4	.7	0.3	12.4	13.1
S. epiderm.	5	3	.2	0.7	20.6	22.4
S. marces.	20	2	.0	0.1	11.8	12.0
S. viridans	4	3	.4	0.6	20.7	22.0
Mean Total	11	5	.5	19.9	N/A	N/A



TRANSFUSION COMPLICATIONS

Validation of BacT/ALERT plastic culture bottles for use in testing of whole-blood-derived leukoreduced platelet-rich-plasma-derived platelets

M.E. Brecher, S.N. Hay, and S.J. Rothenberg

BACKGROUND: Bacterial detection of platelet (PLT)-rich-plasma (PRP)-derived PLTs presents unique challenges for countries that do not allow pooling before storage. This study validated the BacT/ALERT for use in testing pooled PRP-derived PLTs with nine contaminating organisms.

STUDY DESIGN AND METHODS: Isolates of *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Streptococcus viridans*, and *Propionibacterium acnes* were inoculated into two PRP-derived PLT pools (target, 10 and 100 colony-forming units [CFUs]/mL; actual recovered concentrations, 5 and 90 CFUs/mL). Four milliliters of each postbacterial incubation sample was inoculated into both plastic aerobic and anaerobic bottles and 0.5 mL was plated onto blood agar.

RESULTS: All organisms (excluding *P. acnes*) were detected in 8.2 to 22.0 and 7.6 to 20.3 hours (10 and 100 CFUs/mL, respectively) and the mean time to detection was 15.0 and 13.1 hours (10 and 100 CFUs/mL, respectively). *P. acnes* was detected with the anaerobic bottles in a mean of 74.9 and 64.3 hours (10 and 100 CFUs/mL, respectively). With *E. cloacae*, *E. coli*, *K. pneumoniae*, *S. marcescens*, and *S. viridans* detection with the anaerobic bottles was faster or equivalent to the detection with the aerobic bottles. This was most notable with *S. viridans* where the anaerobic bottle was reactive on average 21.6 and 10.8 hours (10 and 100 CFUs/mL, respectively) faster than the aerobic bottle.

CONCLUSIONS: This study validates the use of the BacT/ALERT system for the detection of bacteria in PRP-derived PLTs in a pooled format. Overall, the use of the BacT/ALERT system allowed the detection of pooled PRP-derived PLTs inoculated with nine bacteria at 10 and 100 CFUs per mL in 7.6 to 22.0 hours (excluding *P. acnes*).

At present, bacterial detection of platelets (PLTs) is routinely performed on all PLT products in Belgium (Flanders), the Netherlands, Wales, and Hong Kong and for the majority of PLT products in Northern Ireland, Sweden, Denmark, and Norway.[1] Limited testing is being implemented in Scotland, the Republic of Ireland, Germany, Canada, China, Brazil, England, and the US.[1] Within the US, bacterial detection of PLTs has been required by the American Association of Blood Banks since March 2004 and recommended by the College of American Pathologists since December 2002.[2,3]

Bacterial detection of PLT-rich-plasma (PRP)-derived PLTs has presented some unique challenges for countries that do not allow the prestorage pooling of PLT concentrates. Culturing of each PLT concentrate is frequently not thought to be practical owing to volume loss and expense; therefore, the use of detection strategies such as Gram stain and multireagent strips have been employed.[4-6] Nevertheless, these detection schemes are not as sensitive as culture methods.[7]

Multiple studies have validated automated bacterial culture systems such as the BacT/ALERT microbial detection system (bioMérieux, Durham, NC) with a variety of bacterial contaminants in single-donor apheresis PLTs and with pooled buffy-coat PLTs.[8-13] The Food and Drug

ABBREVIATION: PRP = platelet-rich plasma.

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Pooled Leukoreduced PRP Platelets
10 and 100 CFU/mL

SCD

10X

4 mLs per bottle

0.5 mL per Plate x 2

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Pooled Leukoreduced PRP Platelets 100 CFU/mL (1+5)

Organism	Single Inocul CFU/mL	Single Actual CFU/mL	# plates	Aerobic bottles - Hours Mean	SD	Min	Max	Anaerobic bottles - Hours Mean	SD	Min	Max
B. cereus	30.0	41	20	8.6	0.1	8.5	8.7	9.6	0.3	9.1	10
Clos perf	1.9	<2	2					10.9	0.4	10.3	11.9
E. Cloacae	238	405	14	11.9	0.2	11.6	12.1	10.5	0.2	10.4	10.9
E. coli	135	190	20	10.6	0.2	10.3	10.8	10.0	0.2	9.8	10.5
K. pneumo	171	30	13	11.4	0.2	10.9	11.8	11.2	0.2	10.9	11.6
P. acnes	61.1	60	20					74.9	26.8	64.8	151.2
S. aureus	170	49	19	11.9	0.2	11.7	12.2	12.6	0.2	12.3	13
S. epiderm.	78.6	38	20	16.6	0.2	16.3	16.8	19.6	0.6	18.7	20.5
S. marces.	81.4	25	8	12.7	0.2	12.5	12.9	12.7	0.1	12.7	13
S. viridans	240	75	15	**30**	1.7	27	32.3	19.7	0.3	19.1	20.3
Mean Total	120.7	91.5	15.1	14.2	0.4	13.6	14.7	19.2	2.9	17.8	27.3

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Pooled Leukoreduced PRP Platelets 10 CFU/mL (1+5)

Organism	Single Inocul CFU/mL	Single Actual CFU/mL	# plates	Aerobic bottles - Hours Mean	SD	Min	Max	Anaerobic bottles - Hours Mean	SD	Min	Max
B. cereus	3	<2	7	9.5	0.2	9.1	9.8	11.4	0.7	10.3	12.3
*Clos perf**	0.2	<2	1					13.3	2.9	10.6	16.4
E. Cloacae	24	13	20	12.8	0.2	12.6	13.1	11.6	0.2	11.4	11.9
E. Coli	13.5	9	7	11.8	0.2	11.5	12.0	11.1	0.2	10.80	11.4
K. Pneu*	17	3	0	12.3	0.2	12.0	12.7	12.2	0.2	12.0	12.5
P. Acnes	6.1	6	2					74.4	1.6	72	76.8
S. Aureus	17	8	4	13.2	0.1	13	13.3	13.9	0.3	13.3	14.3
S. epiderm.	7.9	2	8	18.0	0.3	17.5	18.5	22.1	0.7	21.1	22.8
S. marces*	8.1	<2	1	14.9	0.8	14.2	16	16.9	4.1	14.5	23
S. virid*	24	<2	1	**48.4**	6.8	37.6	55.2	23.7	1.5	21.3	25.4
Mean Total	12.1	4.9	5.1	17.6	1.1	15.9	18.8	21.1	1.2	19.7	22.7

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Pooled Leukoreduced PRP Platelets composite (1+5)

Organism	Single Inocul CFU/mL	Single Actual CFU/mL	# plates	Aerobic bottles - Hours Mean	SD	Min	Max	Anaerobic bottles - Hours Mean	SD	Min	Max
B. cereus	3	<2	7	9.5	0.2	9.1	9.8	11.4	0.7	10.3	12.3
Clos perf	1.9	<2	2					10.9	0.4	10.3	11.9
E. Cloacae	24	13	20	12.8	0.2	12.6	13.1	11.6	0.2	11.4	11.9
E. Coli	13.5	9	7	11.8	0.2	11.5	12.0	11.1	0.2	10.80	11.4
K. pneumo	171	30	13	11.4	0.2	10.9	11.8	11.2	0.2	10.9	11.6
P. Acnes	6.1	6	2					74.4	1.6	72	76.8
S. Aureus	17	8	4	13.2	0.1	13	13.3	13.9	0.3	13.3	14.3
S. epiderm.	7.9	2	8	18.0	0.3	17.5	18.5	22.1	0.7	21.1	22.8
S. marces.	81.4	25	8	12.7	0.2	12.5	12.9	12.7	0.1	12.7	13
S. viridans	240	75	15	**30**	1.7	27	32.3	19.7	0.3	19.1	20.3
Mean Total	56.6	16.8	8.6	14.9	0.4	14.3	15.5	19.9	0.5	19.2	20.6

All bottles reactive (anaerobic bottles only for strict anaerobies)

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Pooled Leukoreduced PRP Platelets composite (1+5)

Organism	Single Inocul CFU/mL	Single Actual CFU/mL	# plates	Aerobic bottles - Hours Mean	SD	Min	Max	Anaerobic bottles - Hours Mean	SD	Min	Max
B. cereus	3	<2	7	9.5	0.2	9.1	9.8	11.4	0.7	10.3	12.3
Clos perf	1.9	<2	2					10.9	0.4	10.3	11.9
E. Cloacae	24	13	20	12.8	0.2	12.6	13.1	11.6	0.2	11.4	11.9
E. Coli	13.5	9	7	11.8	0.2	11.5	12.0	11.1	0.2	10.80	11.4
*K. pneum**	17	3	0	12.3	0.2	12.0	12.7	12.2	0.2	12.0	12.5
P. Acnes	6.1	6	2					74.4	1.6	72	76.8
S. Aureus	17	8	4	13.2	0.1	13	13.3	13.9	0.3	13.3	14.3
S. epiderm.	7.9	2	8	18.0	0.3	17.5	18.5	22.1	0.7	21.1	22.8
S. marces.	81.4	25	8	12.7	0.2	12.5	12.9	12.7	0.1	12.7	13
*S. virid**	24	<2	1	**48.4**	6.8	37.6	55.2	23.7	1.5	21.3	25.4
Mean Total	19.6	7.2	5.9	17.3	1.0	15.7	18.4	20.4	0.6	19.5	21.2

2 bottle set reactive (excepting strict anaerobes)

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	Klebs. Pneumoniae 3 CFU/m: **(0/20 plates)**		Serratia. Marcescens <2 CFU/mL **(1/20 plates)**		Strep. Viridans <2 CFU/mL **(1/20 plates)**	
Bottle Set	BPA	BPN	BPA	BPN	BPA	BPN
1	R		R	R	R	R
2	R			R	R	R
3	R	R	R		R	R
4	R	R	R		R	R
5	R	R		R	R	R
6	R	R		R	R	R
7	R	R	R		R	R
8	R	R			R	
9	R	R				R
10	R	R			R	R

Conclusion

BacTAlert reliably detects a contamination Level of 10 CFU/mL in a single unit after Pooling/dilution with 5 other units.

Such detection may facilitate FDA approval of pre-pooled whole blood derived platelets.