Reducing septic reactions from platelet transfusions

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Cleveland, OH
Disclosures

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Bacterial Contamination of Platelet Pools
Ohio, 1991

From June 27 through July 30, 1991, four episodes of bacterial contamination of platelet pools occurred in an Ohio hospital and were reported by the hospital through the Food and Drug Administration (FDA) to CDC.

http://www.cdc.gov/mmwr/preview/mmwrhtml/images/ic_wk.gif
January 24, 1992 / 41(03);36-37

Zaza, Tokars, Yomtovian et al. Infect Cont Hosp Epidemiol 1994;15:82-87
**Cases June 27 through July 31, 1991**
**University Hospitals Case Medical Center, Cleveland**

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Presentation</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/F</td>
<td>Cancer</td>
<td>BMT</td>
<td>Rigors, hypotension, death</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>43/F</td>
<td>Cancer</td>
<td>BMT</td>
<td>Rigors</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>83/F</td>
<td>Renal failure</td>
<td>Dialysis</td>
<td>None</td>
<td>Staph. epidermidis</td>
</tr>
<tr>
<td>47/M</td>
<td>ALL</td>
<td>BMT</td>
<td>Rigors</td>
<td>Bacillus cereus</td>
</tr>
</tbody>
</table>

**ALL, acute lymphocytic leukemia**
**BMT, bone marrow transplant**

Bacterial contamination of platelet products

- What is the incidence of bacterial contamination of various platelet products?
- What are the effects on patients of transfusing contaminated products?
- What has been done to date to decrease the risk of bacterial contamination?
- How effective have these measures been?
- What additional steps can be taken to further reduce the risk?
US Platelet Transfusions

• Over 3 million platelet units are transfused in the form of 1.7 million SDP units and 1.5 million RDP units (0.26–0.38 million pools)

• Bacterial contamination rates are similar for SDP and RDP units, but the contamination rate per transfusion, as expected, is 4–6-fold higher for RDP pool transfusions

• Estimated that over 500 bacterially contaminated apheresis units and a similar number of RDP units are transfused each year

• The fatality rate associated with bacterial contamination of platelets is estimated to be ~2 deaths per million units transfused (~6 deaths per year)

• The rate of septic transfusion reactions is estimated to be 10–13 cases per million units transfused (30–40 cases per year)

References:
Transfusion-associated infections


=1,500 per year in USA
Transfusion-related deaths reported to the FDA 2005-2010

- Between 2005 and 2010, microbial infection was the third leading cause of transfusion-related fatalities reported to the FDA, exceeded only by transfusion related acute lung injury (TRALI) and hemolytic transfusion reactions.
- These microbial infections accounted for 11% (35/307) of transfusion-related fatalities.
- Based on 3.5 deaths/y and 2 million platelet transfusions/year, there are ~2 deaths per million units transfused.

Fatalities Reported to FDA Following Blood Collection and Transfusion Annual Summary for Fiscal Year 2010

Transfusion-Transmitted Fatalities Due to Bacterial Contamination of Platelets, USA 1995 to 2010

- Early culture of apheresis units appears to be effective at intercepting most Gram negative contaminants but has not effectively addressed Gram positive contaminants.


Fatals Reported to FDA Following Blood Collection and Transfusion
Annual Summary for Fiscal Year 2010
Testing at issue or outdate – apheresis platelets

Incidence per million units

- Dumont BacT/ALERT: 662
- Jacobs Plate cul: 494
- Yomtovian Gram stain: 889
- Yomtovian >2007 (eBDS tested): 856
- Yomtovian >2007 (BacT tested): 326

Yomtovian R, et al. AABB 2011 abstract SP410
Testing at issue or outdate – RDP or buffy coat pools

Incidence per million units

- Murphy APH + B/coat BacT/ALERT 7/8282
- Pearce APH + B/coat BacT/ALERT 6/6438
- Jacobs RPD pools Plate cul 38/15,738
- Yomtovian RDP pools Gram stain 6/7,716 (15 FN)
- Yomtovian >2007 Prepooled (eBDS tested) Plate cul 1/5907
- Yomtovian >2007 Prepooled (BacT tested) Plate cul 2/2128

Yomtovian R, et al. AABB 2011 abstract SP410

Bacterial Contamination of Platelets
University Hospitals Case Medical Center, Cleveland, OH
1991-2010  N=68


Updated to 2010
### Bacterial Contamination of Platelets

Bacterial culture at issue (1991-2006)

<table>
<thead>
<tr>
<th>Surveillance</th>
<th>Active (n=102,998)</th>
<th>Passive (n=135,885)</th>
<th>Odds Ratio (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial contamination</td>
<td>50 1:2,060</td>
<td>2 1:67,942</td>
<td>32.0 (8.0-135.0)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>16 1:6,437</td>
<td>2 1:67,942</td>
<td>10.6 (2.4-45.9)</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>1</td>
<td>1.3 (0.01-21.1)</td>
</tr>
</tbody>
</table>

Jacobs MR, Yomtovian R  CID 2008; 46:1217
Bacterial contamination of platelet products

- What is the incidence of bacterial contamination of various platelet products?
- **What are the effects on patients of transfusing contaminated products?**
- What has been done to date to decrease the risk of bacterial contamination?
- How effective have these measures been?
- What additional steps can be taken to further reduce the risk?
Septic Transfusion Reactions

Definition

- **Clinical criteria**
  - within 4 hours of transfusion,
  - Fever $\geq 39^\circ C$ or a change of $\geq 2^\circ C$,
  - or rigors,
  - or tachycardia $>120$ bpm or a change of $>40$ bpm,
  - or a change of $>30$ mmHg in blood pressure.

- **Microbial confirmation**
  - Identification of the same species of bacteria from the residual platelet product and the patient
    OR
  - Reproducible isolation and quantitation of the same species of bacteria from the platelet product on at least two occasions.

Kuehnert MJ et al, Transfusion 2001
Wendel et al, Transfusion 2005
Jacobs MR, Yomtovian R CID 2008; 46:121
**USA 1998-2000: Passive surveillance: bacteremia reqd.**

172,279 pools of 6 = 1,033,671 units; 1,804,725 apheresis units

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Number</th>
<th>PLT fatalities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive (n = 20)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis*</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Group G Streptococcus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus lugdenensis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B. cereus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>E. faecalis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Gram-negative (n = 14)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens*</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Serratia liquefaciens*</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Enterobacter cloaceae</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P. rettgeri</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Y. enterocolitica</strong></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table includes RBC and platelet transfusions.

6 cases were associated with platelets with fatal reactions:

- Strep agalactiae: 1
- E. coli: 1
- P. rettgeri: 1
- E. cloacae: 1
- S. marcescens: 2

* Associated with RBC transfusions.
## Transfusion reaction grading system

### Table 1. Grading system for septic transfusion reactions.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Reaction type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Patients known to have received a bacterially contaminated platelet transfusion but who did not show either clinical or laboratory evidence of a septic reaction</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>A mild febrile reaction (1–2°C increase in temperature) or an asymptomatic clinical case with positive blood culture result or development of leukocytosis</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>A transient change in vital signs (e.g., fever or hypotension) or clinical status that resolved within 24 h with minimal intervention (e.g., treatment with analgesics or antihistamine) or no intervention</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>A change in vital signs requiring intervention (e.g., intravenous fluid, antibiotic, or vasopressor administration) with resolution without persistent sequelae</td>
</tr>
<tr>
<td>4</td>
<td>Life threatening</td>
<td>A severe reaction with septic shock or impairment of vital organ functions</td>
</tr>
<tr>
<td>5</td>
<td>Fatal</td>
<td>A severe reaction with death partly or fully attributable to the contaminated platelet transfusion</td>
</tr>
</tbody>
</table>

Bacterial load and transfusion reaction, n=46

Bacterial load and transfusion reaction
Apheresis and random donor units

No significant differences between contamination rates and bacterial load of apheresis and random units

Bacterial load and transfusion reaction
Virulence of contaminant

<table>
<thead>
<tr>
<th>Reaction grade</th>
<th>Bacterial count (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10^0</td>
</tr>
<tr>
<td>Mild</td>
<td>10^1</td>
</tr>
<tr>
<td>Moderate</td>
<td>10^2</td>
</tr>
<tr>
<td>Severe</td>
<td>10^3</td>
</tr>
<tr>
<td>Life-threatening</td>
<td>10^4</td>
</tr>
<tr>
<td>Fatal</td>
<td>10^5</td>
</tr>
</tbody>
</table>

The graph shows the relationship between bacterial load and transfusion reaction grade, with different bacterial species represented by various symbols.

- **More virulent species**:
  - OR 3.5 (1.9-6.2) for any reaction
  - OR 8.5 (2.0-36.6) for severe reaction

Bacterial contamination of platelet products

• What is the incidence of bacterial contamination of various platelet products?
• What are the effects on patients of transfusing contaminated products?
• What has been done to date to decrease the risk of bacterial contamination?
• How effective have these measures been?
• What additional steps can be taken to further reduce the risk?
Early detection systems

Three testing systems are licensed in the United States for platelet quality control

- BacT/ALERT 3D automated microbial detection system (bioMérieux, Durham, NC): Culture-based (shelf-life incubation)
- Enhanced bacterial detection system (eBDS; Pall Corp., East Hills, NY): Culture-based (24 h incubation)
- ScanSystem (Hemosystem, Marseille, France): Fluorescent staining of bacteria with detection by scanning
Early detection systems – apheresis and prepooled RDP

Incidence per million units

<table>
<thead>
<tr>
<th>Apheresis</th>
<th>BacT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eder</td>
<td>166</td>
</tr>
<tr>
<td>Dumont</td>
<td>195</td>
</tr>
<tr>
<td>Yomtovian</td>
<td>132</td>
</tr>
<tr>
<td>Yomtovian</td>
<td>61</td>
</tr>
</tbody>
</table>

Yomtovian R, et al. AABB 2011 abstract SP410
Sensitivity of culture at production vs outdate or at-issue

Yomtovian R, et al. AABB 2011 abstract SP410
Growth of bacteria in platelets based on one viable organism in a 400 ml unit (0.0025 organisms/ml)

Days after collection: 0 1 2 3 4 5 6 7


- no lag, 8-h gen time
- no lag, 4-h gen time
- no lag, 1-h gen time
- auto-sterilization
- 1-day lag
- 3-day lag
Limitations of Culture Testing

**Target Captured**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image of sample" /></td>
<td><img src="image2.png" alt="Image of incubation" /></td>
</tr>
</tbody>
</table>

**POSITIVE**

**Target Missed**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image of sample" /></td>
<td><img src="image2.png" alt="Image of incubation" /></td>
</tr>
</tbody>
</table>

**FALSE NEGATIVE**

Too few targets to reliably capture a bug in sample
Limitations of Early Culture Testing

Modeling the effect of concentration on bacterial detection when a 300 mL unit is contaminated with 0-300 CFUs (0-1 CFU/mL). The figure shows the probability curves for an 8-mL sample divided into two culture bottles.

Probability of a single viable bacterium in an 8 ml sample of a 300 ml product:

- 100% 0.6 CFU/ml ~180 CFU/product
- 90% 0.28 CFU/ml ~80 CFU/product
- 50% 0.09 CFU/ml ~27 CFU/product
- 25% 0.04 CFU/ml ~1 CFU/product

Accrediting agency recommendations

• College of American Pathologists (CAP) recommended in December 2002 that a method for bacterial detection in platelet units be put in place.

• AABB Standard 5.1.5.1
  – The blood bank or transfusion service shall have methods to limit and detect bacterial contamination in all platelet components.
  – All AABB accredited blood banks were required to meet Standard 5.1.5.1 by March 1, 2004.

• AABB Bulletin 03-12: Use a method to detect bacterial contaminants that remain and grow during storage of components. The more sensitive the detection method, the better the assurance that the majority of clinically significant contaminants will be detected prior to transfusion. Procedures that are currently available and will meet AABB Standards include culture (Pall BDS and BacT/ALERT methods), microscopy (Gram, Acridine Orange and Wright stains), pH and glucose.


Bulletin 04-07: Actions Following an Initial Positive Test for Possible Bacterial Contamination of a Platelet Unit. AABB, Bethesda, MD, 2004.

AABB standard 5.1.5.1 (effective March 2004)
The blood bank or transfusion service shall have methods to limit and detect or inactivate bacterial contamination in all platelet components

- **Apheresis**
  - Collection facilities adopted culture
  - FDA cleared culture-based QC tests
  - Culture at 24hrs, release 12-24hrs later

- **WBD**
  - Culture not practical for WBD units
  - Hospitals validate non FDA cleared tests
History of AABB PBC Guidance

Since 1996 the AABB has issued ten Association Bulletins on Platelet Bacterial Contamination to:

(1) educate the blood bank community regarding the scope and magnitude of this ongoing problem
(2) promulgate standards to detect and reduce the incidence in both apheresis and whole blood derived platelets
(3) develop standardized protocols for identification of true positive results and donor management
(4) support industry efforts to provide technology to improve detection and reduction strategies
## History of AABB PBC Guidance

<table>
<thead>
<tr>
<th>YEAR</th>
<th>BULLETIN</th>
<th>SUBJECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>AB 96-06</td>
<td>Initial Information PBC</td>
</tr>
<tr>
<td>2002</td>
<td>AB 02-08</td>
<td>Proposed Standards 5.1.5.1 &amp; 5.6.2</td>
</tr>
<tr>
<td>2003</td>
<td>AB 03-07</td>
<td>Methods to limit PBC</td>
</tr>
<tr>
<td>2003</td>
<td>AB 03-10</td>
<td>Guidance on implementing 5.1.5.1 on 3/1/2004</td>
</tr>
<tr>
<td>2003</td>
<td>AB 03-12</td>
<td>Further guidance on methods to detect PBC; swirling out</td>
</tr>
<tr>
<td>2004</td>
<td>AB 04-07</td>
<td>Actions following an initial positive test for PBC; provision of standardized definitions of positive results</td>
</tr>
<tr>
<td>2005</td>
<td>AB 05-02</td>
<td>Managing blood donors who test positive for PBC</td>
</tr>
<tr>
<td>2009</td>
<td>AB 09-04</td>
<td>Reviews PBC detection gap for WBD &amp; alerts membership to development of new rapid qualitative immunoassay</td>
</tr>
<tr>
<td>2010</td>
<td>AB 10-02</td>
<td>Final wording interim Standard 5.1.5.1.1</td>
</tr>
<tr>
<td>2010</td>
<td>AB 10-05</td>
<td>Guidance to implement 5.1.5.1.1 &amp; PBC detection in WBD platelets</td>
</tr>
</tbody>
</table>
Bacterial detection methods for whole-blood derived platelets in US Hospitals, 2004

Proportion of Labs Using Method (%)

- Dipstick
- Analyzer
- Dipstick
- Analyzer
- Swirling
- Culture
- Microscopy
- Other
- None

College of American Pathologists 2004 J-B Survey
AABB Interim standard 5.1.5.1.1

Interim standard 5.1.5.1.1, as approved by the AABB Board of Directors, reads as follows:

• **5.1.5.1** The blood bank or transfusion service shall have methods to limit and to detect or inactivate bacteria in all platelet components. Standard 5.6.2 applies.

• **5.1.5.1.1** Detection methods shall either be approved by the FDA or validated to provide sensitivity equivalent to FDA-approved methods.

• This interim standard has an effective date of January 31, 2011.
Decreasing the risk of bacterial contamination

• Prevention
  – Disinfection of venepuncture site
  – Diversion of initial blood flow
  – Pathogen inactivation technology
  – Use as early as possible

• Detection
  – At time of production
  – At time of use
Prevention – pathogen inactivation

• Several pathogen inactivation technologies have been developed, including methylene blue, riboflavin and solvent-detergent treatment. In addition, a number of photodynamic processes use psoralen-based chemicals followed by ultraviolet light to inactivate bacteria and viruses by preventing replication and transcription of nucleic acids.

• The main disadvantage of pathogen inactivation is that no method is equally effective for all organisms and these methods may be ineffective against spore-forming organisms.

• In Europe the INTERCEPT Blood System for Platelets (Cerus Europe BV, Leusden, The Netherlands) using amotosalen received the CE Mark for pathogen inactivation of platelets in 2002 and is currently in clinical use in several European countries.

• There may no longer be a need for a bacterial detection system if a pathogen inactivation system is used.


Detection of bacterial contamination

- Culture
  - Early
  - Late
- Microscopy
  - Gram stain, acridine orange stain
  - Fluorescent stain with detection by scanning
- Biochemical markers – pH and glucose
- Rapid tests for use at or near time of issue
  - Endotoxin detection by limulus lysate test
  - Antibody-based lateral flow device for detection of bacteria
  - Detection of conserved regions of bacterial 23S rRNA and a heat shock protein by real-time, reverse transcriptase PCR assay
  - Detection of bacterial 16S ribosomal RNA with oligonucleotide probes
  - Rapid Bioluminescent Bacterial Detection System using luciferase
  - Bacterial lipoteichoic acid and lipopolysaccharide detection by lateral flow immunoprecipitation
  - Bacteriological Biosensor using engineered spores as fluorogenic nanodetectors
  - Bacterial peptidoglycan chromogenic immunoassay

Detection by Gram stain, prospective, N=16,477
University Hospitals Case Medical Center, 1991-1999

Datapoints not labeled are coagulate negative staphylococci

Serratia

Viridans strep.

S. aureus

Interdicted

Not interdicted

Application of early culture to whole-blood derived platelet pools: Pall Acrodose PL System

510(k) Number: BK050074
Device Name: Pall Acrodose PL System

Indications For Use: The Pall Acrodose PL System is indicated for pooling of ABO identical, leukocyte-reduced, whole-blood-derived platelet concentrates and subsequent storage for up to 5 days after blood collection when coupled with a device cleared by FDA for detection of bacterial contamination in pooled leukoreduced whole-blood-derived platelets.

The Pall Acrodose PL System should be used with whole-blood-derived platelet concentrates collected in CP2D anticoagulant and leukoreduced using the Leukotrap RC PL or Leukotrap PL Filtration Systems. Each CLX HP extended storage bag can store 2.2 - 5.8 x 10^{11} platelets, from 4 to 6 platelet concentrates, at a platelet concentration of < 2.0 x 10^6 µL, in a volume of 180 - 420 mL.

http://www.fda.gov/cber/seltr/k050074L.htm
At-issue detection systems

One system is currently licensed in the United States for platelet quality control:

PGD Test (Verax Biomedical, Worcester, Massachusetts)

It has received 510(k) clearance from the FDA as a rapid, qualitative immunoassay for the detection of aerobic and anaerobic Gram-positive and Gram-negative bacteria in leukocyte reduced apheresis platelets (LRAP) as an adjunct quality control test following testing with a bacterial detection device cleared by the FDA for quality control testing of LRAP and pooled RDP

The U.S. Food and Drug Administration in September 2011 cleared a new indication for the PGD test - the PGD test was approved to be labeled and marketed as a “safety measure,” the first time the agency has used this designation for a medical device
Bacterial contamination of platelet products

• What is the incidence of bacterial contamination of various platelet products?
• What are the effects on patients of transfusing contaminated products?
• What has been done to date to decrease the risk of bacterial contamination?
• How effective have these measures been?
• What additional steps can be taken to further reduce the risk?
Effect of Inlet-line Diversion and skin prep on BacT/ALERT Cultures

For Prestorage-pooled platelets:
- No Diversion
- Diversion

O.R. 0.46; 95% C.I. 0.2 - 0.95

For Apheresis platelets:
- Povidone-iodine
- Chloraprep

O.R. 0.47, 95% C.I. 0.21 - 1.03

Effect of Inlet-line Diversion and skin prep on septic reactions with apheresis platelets

Passive Reporting (per distributed product) with 100% inlet-line diversion and 8 ml aerobic cultures:

- Risk of Sepsis: ~1:108,000 (16/year)
- Risk of Fatality: ~1:500,000 (3.4/year)

Bacterial contamination of platelet products

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• What has been done to date to decrease the risk of bacterial contamination?
• How effective have these measures been?
• What additional steps can be taken to further reduce the risk?
Additional steps to further reduce the risk

1. Whole-blood derived platelets should be prepooled and cultured early to bring their level of testing up that of apheresis platelets.

2. The optimal volume and conditions to provide the most cost-effective method for detection of bacterial contamination by early culture need to be clarified to improve sensitivity.

3. The real incidence and significance of bacterial contamination need to be studied by QUANTITATIVE culture at time of issue to assess the value of prevention and detection methods.

4. The clinical efficacy of point-of-issue assays by hospital transfusion services needs to be determined.

5. The safety, efficacy and cost-effectiveness of pathogen inactivation in the eradication of bacterial contamination need to be determined.
Multi-site Study of 27,682 Doses Using the Verax Pan Genera Detection Assay

- Study performed at 18 study sites by over 160 technologists on apheresis units previously tested by early culture (BacT/ALERT or eBDS) and released as culture negative
- Doses tested by Platelet PGD test on day of issue (16 sites) or shortly after issue (2 sites) according to the manufacturer’s directions
- Positive PGD results repeated in duplicate and aerobic and anaerobic plate cultures performed
- Concurrent aerobic plate cultures were also performed on 10,430 units at three of the study sites, with quantitation of positives at one study site

University Hospitals
Case Medical Center
SCHOOL OF MEDICINE
Case Western Reserve UNIVERSITY
As not all bacterially contaminated platelet units are detected by prestorage culture testing, the goal was to determine if the PGD Test could detect and interdict such breakthrough cases.

The study was a multi-hospital study of the PGD test used on the day of platelet transfusion to identify bacterially contaminated SDP doses that had been released as culture-negative.

The sample size required to detect approximately 10 PGD true positive doses was estimated to be between 22,830 and 50,000 doses based on rate for units with bacterial loads of >10^5 CFU/mL (the detection limit of the PGD Test) being 438 per million units (1:2,283)*

A proportion of the total SDP doses tested would also be evaluated by concurrent culture at the time of PGD testing to assess the sensitivity of the PGD Test, with the goal of testing 10,000 doses in this arm of the study.

### Study sites

<table>
<thead>
<tr>
<th>Adventist Hinsdale Hospital, Hinsdale, IL</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Children’s Hospital of Philadelphia, Philadelphia, PA</td>
</tr>
<tr>
<td>Indiana University Health, Indianapolis, IN</td>
</tr>
<tr>
<td>Cornell University, New York, NY</td>
</tr>
<tr>
<td>Johns Hopkins Hospital, Baltimore, MD</td>
</tr>
<tr>
<td>Lahey Clinic, Burlington, MA</td>
</tr>
<tr>
<td>North Shore-Long Island Jewish Health System, Manhasset, NY*</td>
</tr>
<tr>
<td>Oregon Health and Science University, Portland, OR</td>
</tr>
<tr>
<td>University of Colorado Hospital, Aurora, CO</td>
</tr>
<tr>
<td>University of California, San Diego Medical Center, Thornton Hospital, San Diego, CA</td>
</tr>
</tbody>
</table>

| University of California, San Francisco Medical Center, San Francisco, CA |
| University Hospitals Case Medical Center, Cleveland, OH* |
| University of Illinois at Chicago, Chicago, IL |
| University of Minnesota, Minneapolis, MN |
| University of North Carolina Hospital, Chapel Hill, NC |
| University of Southern California – Los Angeles County, Los Angeles, CA |
| University of Virginia, Charlottesville, VA |
| Vanderbilt University Medical Center, Nashville, TN* |

*Concurrent culture sites*
Verax Pan Genera Detection (PGD) Assay

- Single-use, qualitative test for the detection of aerobic and anaerobic Gram positive and Gram negative bacteria
- Detects the presence of conserved bacterial surface cell wall antigens, lipoteichoic acid and lipopolysaccharide, using specific antibodies

![Bar chart showing analytic sensitivity in LRAP for various bacterial species.](chart.png)
Verax Biomedical Platelet PGD Test procedure

**Centrifugation**

A. Add 8 drops of Reagent to 500uL Platelet Sample. Solution Turns Green.

Spin for 5 Minutes & Decant.

**Resuspension**

B. Add 8 drops of Reagent and Resuspend Pellet. Solution Turns BLUE.

**Reading**

C. Transfer to well and Wait for results. Interpret results.

Negative

Positive

Invalid

Verax Insert P00583 Rev. D Nov 09
Definitions

- Reactive (R) = 2 of 3 PGD tests were Reactive
- False Positive (FP) = any R PGD result not confirmed positive by culture
- True positive (TP) = Reactive (R) PGD result with bacterial contamination confirmed by culture
- True Negative (TN) = PGD Non-reactive (NR) result on culture-negative sample*
- False negative (FN) = PGD Non-reactive (NR) result on culture-positive sample*

*only applicable to sites performing concurrent culture
Study design

SDP doses with valid PGD test results

Reactive PGD test

- Culture positive
- Culture negative

Non-reactive PD test

Concurrent Culture at 3 sites

- Culture positive
- Culture negative
Results

• All SDP units had been tested for bacterial contamination and released as culture-negative by collection centers (BacT/ALERT used for 81%, eBDS for 16%)

• 27,682 individual SDP doses, derived from 22,371 apheresis collections, were tested at 18 hospitals during 2008-2010

• Hospitals tested between 72 and 7,672 doses
Results

Valid PGD test results
N=27,620

Reactive
N=151

Culture
Positive
N=9

Culture
Negative
N=142
## Platelet Bacterial Contamination Detection by Platelet PGD Test – true positive results

<table>
<thead>
<tr>
<th>Bacterial species isolated by culture at issue</th>
<th>Age of unit (days)</th>
<th>Confirmation method*</th>
<th>Bacterial load (CFU/ml)**</th>
<th>Transfusion status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp; P. acnes</td>
<td>3</td>
<td>BC</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS</td>
<td>3</td>
<td>PC, GS</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS</td>
<td>3</td>
<td>PC, GS</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3</td>
<td>PC, GS</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS; Peptostrep</td>
<td>4</td>
<td>PC, BC, GS</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS</td>
<td>4</td>
<td>PC</td>
<td>NT</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS</td>
<td>5</td>
<td>PC, GS</td>
<td>1.3 x 10e6</td>
<td>Tx – no rxn</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>5</td>
<td>PC, GS</td>
<td>1 x 10e7</td>
<td>Not Tx</td>
</tr>
<tr>
<td>CoNS</td>
<td>5</td>
<td>PC, GS</td>
<td>5 x 10e6</td>
<td>Tx – septic shock***</td>
</tr>
</tbody>
</table>

BC = broth culture; PC = plate culture; GS = Gram stain
NT = Not Tested for quantity
***documented bacteremia with same organism

*BC = broth culture; PC = plate culture; GS = Gram stain
**NT = Not Tested for quantity
***documented bacteremia with same organism
# Frequency of Contaminated Units with Platelet Age

<table>
<thead>
<tr>
<th>Description</th>
<th>Platelet Age (Days)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>=2</td>
<td>3</td>
</tr>
<tr>
<td>Number Units Tested</td>
<td>4,036 (15%)</td>
<td>8,375 (30%)</td>
</tr>
<tr>
<td>True positive PGD Test</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

* Doses tested on day 5 or shortly after expiration
Results

Valid PGD test results
N=27,620

Reactive
N=151

Culture
Positive
N=9

Culture
Negative
N=142

Non-reactive
N=27,469

Culture at 3 sites
N=10,344

Culture
Positive
N=2

Culture
Negative
N=10,342
Platelet Bacterial Contamination Detection by Platelet PGD Test – false negative results

<table>
<thead>
<tr>
<th>Bacterial species isolated by culture at issue</th>
<th>Age of unit (days)</th>
<th>Confirmation method*</th>
<th>Bacterial load (CFU/ml)**</th>
<th>Transfusion status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus oralis</td>
<td>5</td>
<td>PC, GS</td>
<td>7 x 10e6</td>
<td>Septic Reaction***</td>
</tr>
<tr>
<td>CoNS</td>
<td>5</td>
<td>PC</td>
<td>4 x 10e2</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>

- Early culture negative & PGD Non-Reactive on day of transfusion
- Strep oralis (viridans group) Issued on day 5 at an outpatient location
  - LTA of some viridans group streptococci not covered by current PGD reagents
  - Patient had delayed onset of septic shock
  - Admitted to the ICU for 2 days and was discharged 5 days later
- CoNS below test limit of detection, no transfusion reaction

BC = broth culture; PC = plate culture; GS = Gram stain
***documented bacteremia with same organism
### Platelet Bacterial Contamination Identified by Passive Surveillance

<table>
<thead>
<tr>
<th>Bacterial species isolated by culture at issue</th>
<th>Age of unit (days)</th>
<th>Confirmation method*</th>
<th>Bacterial load (CFU/ml)**</th>
<th>Transfusion status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus sanguinis</td>
<td>5</td>
<td>GS -/+, BC</td>
<td>NT</td>
<td>Allergic, nonfebrile Reaction</td>
</tr>
</tbody>
</table>

- Early culture negative & PGD Non-Reactive on day of transfusion
- Initial gram stain negative, 2nd Gram Stain positive
- Patient culture negative
- Subsequent testing of isolate grown in platelets PGD reactive
- Original unit likely contaminated below PGD LoD
- 1 STR reported in 17,196 units in non-concurrent culture arm of study

*BC = broth culture; GS = Gram stain**

**NT = Not Tested for quantity
9/27,620 apheresis units detected by PGD

- Rate of detection was 1/3,069 units (95% CI 1/6,711 - 1/1,617)
- Estimated 326 contaminated units per million units (95% CI 149-618)
- Based on 1.7 million LRAP units per year in the U.S., the estimated number of breakthroughs would be expected to be 554 per year (95% CI 253-1051)

**STUDY DESIGN:**

Rate for units with bacterial loads of ≥10^5 CFU/mL (the detection limit of the PGD Test)
438 per million units (1:2,283)
Testing at issue or outdate – apheresis platelets

Incidence per million units

- **Dumont**
  - BacT/ALERT: 4/6,039

- **Jacobs**
  - Plate cul: 12/24,309

- **Yomtovian**
  - Gram stain: 0/8,761 (3 false neg)

- **Jacobs**
  - PGD Test: 9/27,620

References:
- Yomtovian R, et al. AABB 2011 abstract SP410
Prevention of transfusion of contaminated LRAP platelets using PGD based on study findings

At a detection rate of 326/million apheresis doses, 550 breakthroughs per year in 1.7 million doses, resulting in over 250 significant transfusion reactions and several deaths, could be prevented by the Platelet PGD Test.

Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets

~ 50% of contaminated units with >10^5 CFU/mL of Gram positive bacterial species cause mild, severe or life-threatening reactions.

Conclusions

- This is the most successful study of detection and interdiction of bacterially contaminated platelet doses ever performed.
- The detection rate can be extrapolated to detection of 326 contaminated units per million doses or approximately 550 contaminated units/year in the US apheresis platelet supply.
- Based on significant transfusion reactions that occurred in about 50% of recipients with transfusion of >10e5 CFU/mL of a variety of Gram positive bacteria and recent FDA fatality data, interdiction of such contaminated units has the potential to prevent over 250 significant transfusion reactions and several fatalities per year.
Regulatory change

• Following the study, the U.S. Food and Drug Administration in September 2011 cleared a new indication for the PGD test
• The PGD test was approved to be labeled and marketed as a “safety measure,” the first time the agency has used this designation for a medical device
Septic reactions from platelet transfusions can be reduced!