Frequency of False-negative Cultures in Screening of Platelet Concentrates for Bacterial Contamination

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INTRODUCTION

• Bacterial screening in NL since 2001
  • 500,000 units 5BC-PC tested (+ 50,000 Apheresis PC)
  • Started with 0.9% initial positive
  • After implementation pre-donation sample:
    – 0.44 % initial positive
  • Since pre-donation: 4 reported serious transfusion reactions with BacT/Alert negative TC
    – Imputability
    – Severity
INTRODUCTION

• International discussion on false-negatives
  • PASSPORT study in US
  • data ARC
  • Irish and Welsh “outdated” studies
• In these studies
  • False-negative rate similar or higher than initial positive
• Sanquin relatively high initial rate
  • Fear for high false-negative rate
  • Not to be expected based on clinical data
INTRODUCTION

• Sanquin started also “Outdated” study
  • Outdated units (>7/5 days) to Amsterdam (R&D unit)
  • Re-cultured in BacT/Alert
  • Aerobic and anaerobic bottle, 7.5 ml/bottle
  • 1 - 5 days (mean 2) after expiration
Study Rules

• Check for contamination:
  • Per day of inoculation 5 saline samples included

• Positive in “outdated” culture
  • Initial culture negative
  • Repeat “outdated” culture also positive
  • Both bottles positive (if applicable)
  • Organism derived from positive culture bottles and bag should be the same

• At least 4000 5BC-PC to be included
Results

• 4021 5BC-PC were tested
• 4 real positives (2 plasma, 2 PAS-II):
  • Both bottles positive, as well as repeated cultures
  • Same microorganism from bottles and bags
  • All Staphylococcus Coagulase Negative (S.epi)
  • Rapidly positive: 5-10 h, at least $10^6$ CFU/ml (PCR)
• 1 indeterminate:
  • One bottle positive (20 h), repeats and bag positive
    – Staphylococcus Haemolyticus
  • No signs of lower platelet quality
  • Very low contamination at day 9
## Results

<table>
<thead>
<tr>
<th>#</th>
<th>Product</th>
<th>Age of PC</th>
<th>Sequencing results</th>
<th>TTP</th>
<th>AFLP typing</th>
<th>Ct</th>
<th>Bacterial concentration (PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC in plasma</td>
<td>8 days</td>
<td><em>S. epidermidis</em></td>
<td>(I) 4.1h (II) 4.3h</td>
<td>Identical isolates</td>
<td>17.78</td>
<td>&gt; 10^7</td>
</tr>
<tr>
<td>2</td>
<td>PC in plasma</td>
<td>8 days</td>
<td><em>S. epidermidis</em></td>
<td>(I) 4.7h (II) 8.2h</td>
<td>Identical isolates</td>
<td>12.24</td>
<td>&gt; 10^8</td>
</tr>
<tr>
<td>3</td>
<td>PC in PAS II</td>
<td>7 days</td>
<td><em>S. epidermidis</em></td>
<td>(I) 11h (II) 10.6h</td>
<td>Identical isolates</td>
<td>18.83</td>
<td>&gt; 10^6</td>
</tr>
<tr>
<td>4</td>
<td>PC in plasma</td>
<td>9 days</td>
<td><em>S. haemolyticus</em></td>
<td>(I) 20.4h (II) 5.5h</td>
<td>Identical isolates</td>
<td>NA</td>
<td>Not tested in PCR</td>
</tr>
<tr>
<td>5</td>
<td>PC in PAS II</td>
<td>7 days</td>
<td><em>S. hominis</em></td>
<td>(I) 4.3h (II) 5.8h</td>
<td>Identical isolates</td>
<td>17.85</td>
<td>&gt; 10^7</td>
</tr>
</tbody>
</table>

Indeterminate: at day 9 ~ 1 CFU/ml
Results

35 false positives

• Only one bottle positive and/or late
• Repeat cultures negative
• No bug isolated from positive bottles, except 2/35
• 2 positives, appeared to be screenings positive
• Controls: about 1700, 5 false positives (early phase)
  • False positive rate (no bug isolated from positive bottle)
    – Controls: 1/400 bottles – 1/2000 bottles
    – PC screening 1/2400 bottles
    – PC outdated: 1/480 bottles
Results

with 4021 5BC-PC tested

• 4 false negatives:
  • 4/4021: 95% CI 0.03 – 0.25 (0.10%)

• Frequency similar to those reported sofar
  • Murphy (Ireland): 7/8282 = 0.08% (mainly BC-PC)
  • Dumont (PASSPORT): 4/6039 = 0.07% (apheresis)
  • Pearce (Welsh): 6/6438 = 0.09% (mainly BC-PC)
Results

• Also outdated apheresis PC were tested
  • In total > 1700 different units,
    – 261 full units
    – 1449 units spliced for pediatric use
  • 1-8 aliquots per unit (mean 3; high sensitivity)

• 1 positive sample
  • Split product, 4 aliquots, 3 tested
  • For 1 split: anaerobic bottle repeatedly positive
  • 3 different Coagulase Negative Staphylococci from bottles and unit
  • Difficult interpretation; not a false negative
Discussion

• For the Netherlands:
  Frequency of false-negatives: ~ 0.10 %
• Frequency similar to other studies, not related to initial rate of positives
Discussion

• False-negatives detected: serious contamination, but in NL not reflected in clinical problems
  • About 20% transfused after 5 days: 10,000/yr
  • Expected: 10 cases/yr
  • Haemovigilance: two cases reported last 3 years
  • During storage below critical level for patient harm?
  • Reports of CNS containing TC:
    \[10^5 - 10^8\text{ CFU/ml tolerated in most cases}\]
Possible consequences

• Reduction of shelf life
• Additional test at release if longer stored
  • PCR would be sensitive enough
  • Fast enough?
• Additional test at day 4/5 if longer stored
  • BacT/Alert culture, one bottle?
  • Negative to date?
• All skin flora (CNS)
  • More attention for skin desinfection?
• Pathogen reduction method
  • Pro’s and con’s
Thanks

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