EXPERIENCE ON WHOLE BLOOD BACTERIAL CONTAMINATION

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BACKGROUND

- Awareness on bacterial contamination of blood products
- Initial study of Soeterboek et al.: 0.6 % of whole blood units contaminated, but with a large 95 % confidence interval (0.1-2.8 %)
- Possible effect of overnight storage of whole blood on bacterial contamination
- Possible reduction by removal of initial volume, containing the ‘skin plug’
Phase I

• Collection of sufficient amount of units to determine accurately the prevalence of bacterial contamination for whole blood collections under standard conditions in the Netherlands

Phase II

• Determination of the effect of diversion of initial flow
MATERIALS AND METHODS

- BacT/Alert® system (Organon Teknika), CO$_2$ production measured
BacT/Alert® system

incubator

Culture bottles
MATERIALS AND METHODS (ctd)

- **BacT/Alert**® system (Organon Teknika), CO$_2$ production measured
- **Modified Compoflex**® 4-bag system (Fresenius/NPBI) with additional sampling bag and needles
Special 5-bag system
VALIDATION OF SPECIAL 5-BAG

- Collections, equal to standard bag system
- F VIII content in plasma: no difference
- Component preparation: normal
- Storage of erythrocytes: normal
- Storage of platelets: normal

- Sample in sampling bag: representative for whole unit
7 days culture, 35°C. Positive signal: culture on blood agar plate, anaerobic and aerobic

Standardized disinfection (FDA-approved) and collection methods

Sole aseptic handling is transfer to BacT/Alert culture bottle (anaerobic and aerobic) in a laminair flow cabinet
AIMS OF PHASE I

• Reliable determination of prevalence of bacterial contamination of whole blood units (with 95% confidence interval < 0.5%)

• Testing the effect of overnight storage as whole blood:
  Group I: sampling/culture within 3 h
  Group II: sampling/culture after overnight/20°C
RESULTS PHASE I

• Group I (within 2 hours): 9219 units collected; 27 units positive (i.e. 0.29 %; 95 % confidence interval 0.19 - 0.43)

• Group II (overnight 20°C): 9038 units collected; 36 units positive (i.e. 0.39 %; 95 % confidence interval 0.28 - 0.55)

• No significant difference, overall prevalence of whole blood contamination with bacteria: 0.34 %
### Differentiation of Positive Samples

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus sp.</em></td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td><em>Propionibacterium sp.</em></td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td><em>Diphteroids, Corynebacterium sp.</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>Bacillus sp</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Micrococcus sp.</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Peptostreptococcus sp.</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Not identified</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
RESULTS PHASE I (ctd)

- Similar distribution of species in both groups
- Mainly skin-associated, not ‘pathogenic’
- *Peptostreptococcus* case: probably not intrinsic, also transient skin flora.
CONCLUSIONS PHASE I

- Prevalence of bacterial contamination in whole blood collections is 0.34% (lower than previously reported) with a small 95% confidence interval.
- Mainly skin-derived bacterial contamination: part should be preventable by improved disinfection and/or removal of first amount of blood.
- No direct effect of overnight storage as whole blood (leukocytes have to be removed for the reported effect).
• Possible reduction by removal of initial collected volume containing the ‘skin plug’
MATERIALS AND METHODS Phase II

- Modified Compoflex®4-bag system (Fresenius/NPBI) with Composampler® and additional sampling bag and needles
- other materials & methods same as Phase I
- Modified bag system was validated, like the system used in Phase I
Special 5-bag system with Composampler®
AIMS OF PHASE II

- Measurement of the prevalence of bacterial contamination in whole blood units after diversion of the first 10 ml (with the determined prevalence in phase I as base level)
- Testing the effect of diversion in two groups:
  - Group I: sampling/culture within 3 h
  - Group II: sampling/culture after overnight/20°C
## RESULTS OF PHASE II

<table>
<thead>
<tr>
<th></th>
<th>Standard whole blood collection</th>
<th>Diversion of the 1st 10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donations tested</td>
<td>18,257</td>
<td>7,115</td>
</tr>
<tr>
<td>Prevalence</td>
<td>0.34%</td>
<td>0.21%</td>
</tr>
<tr>
<td>Confidence interval</td>
<td>0.25-0.44</td>
<td>0.12-0.35</td>
</tr>
</tbody>
</table>
RESULTS PHASE II (ctd)

- After removal of the first 10-ml, the prevalence of bacteria was for both groups 0.21 %
- Group with immediate sampling: not significant
- Group with overnight sampling: significant decrease
- Total study: significant decrease: $p < 0.05$
## Differentiation of Positive Samples

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<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Propionibacterium sp.</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>Diphteroids, Corynebacterium sp.</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peptostreptococcus sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus bovis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gemella morbillorum</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>not identified</td>
<td>1</td>
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</tr>
</tbody>
</table>
RESULTS PHASE II (ctd)

• The majority of bacteria were identified as Propionibacterium species (skin flora).
• A significant decrease of the prevalence of Staphyloccoccus species (p= 0.015) was found.
DISCUSSION

• findings supported by:
  Wagner: study with in vitro model
  Bruneau: indirect evidence by measuring the contamination in the first two fractions during collection

• why only Staphylococcus sp. decreased?
  no real skin plugs but flaps?
• Even after introduction of this preventive measure, the theoretical contamination risk of random donor pooled platelet concentrates composed out of 5 single donor units is still considerable: about 1%. Additional testing required.

• First volume can be used for test purposes, provided that collection system can be assigned as “closed”.
CONCLUSIONS

- Prevalence of bacterial contamination in whole blood collections is **0.34 %** with a small 95 % confidence interval

- Prevalence of bacterial contamination in whole blood collections can be reduced significantly by removal of first amount of blood

- No gram negative bacteria cultured out of a total of 18,000 units of whole blood
Acknowledgements

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