Subgroup Bacteria

Chair: Thomas Montag, Germany
Co-Chair: Erica Wood, Australia

Annual Report 2008/9
Extraordinary organisational topics in 2008:

1. Extraordinary Meeting of Subgroup Bacteria
   Montreal, Canada (AABB October 2008)
   “Preparation of WHO ISBT International Validation Study on Blood Bacteria Standards - discussion of scientific and logistical details”
   (financed by WP-TTID, budget of Subgroup Bacteria)

2. Regular phone conferences
   - Started in April 2008
   - Erica Wood, Melanie Stoermer, Carl McDonald, Thomas Montag
   (financed by Australian Red Cross)
WP-TTI D, Subgroup Bacteria
Annual Report 2008/9

Working Schedule 2008/9

1. Preparation of an international survey on transfusion-transmitted bacterial infections.


3. WHO ISBT International Validation Study on Blood Bacteria Standards.
WP-TTD, Subgroup Bacteria
Annual Report 2008/9

Working Schedule

1. Preparation of a international survey on transfusion-transmitted bacterial infections.
   -> draft questionnaire (topics of relevance) prepared by Thomas Montag, Melanie Stoermer, Erica Wood, and Carl McDonald
   -> model questionnaire provided by Silvano Wendel (BEST, Chris Prowse)
   -> agreement with Kurt Roth to implement the inquiry on transfusion-transmitted bacterial infections into the electronic questionnaire of Subgroup Virology
   -> to be continued in 2009


3. WHO ISBT International Validation Study on Blood Bacteria Standards.
WP-TTI D, Subgroup Bacteria
Annual Report 2008/ 9

Working Schedule 2008/ 9

1. Preparation of an international survey on transfusion-transmitted bacterial infections.

   -> outline prepared by Carl McDonald, Erica Wood, Melanie Störmer and Thomas Montag
   -> installation of a writing group (several members of WP-TTI D)
   -> to be continued in 2009

3. WHO ISBT International Validation Study on Blood Bacteria Standards.
WP-TTI D, Subgroup Bacteria
Annual Report 2008/ 9

Working Schedule 2008/ 9

1. Preparation of an international survey on transfusion-transmitted bacterial infections.


3. WHO ISBT International Validation Study on Blood Bacteria Standards.
Background of WHO ISBT International Validation Study on Blood Bacteria Standards

**Why do we need a panel of transfusion-relevant bacteria?**
Infection Risk in Platelets: bacterial vs viral

According to Mike Busch, modified.
Crux in Bacterial Contamination of Blood Components

Usually, bacteria are contaminating blood donations in a very low count (10 to 100 CFU per bag corresponding to 0.03 to 0.3 CFU / ml).

Thereafter, they can grow up in platelets to $10^8$ to $10^9$ CFU / ml (in dependency on species and strain).

**Artificial contamination of platelets imitating “real life” conditions:**
- -> contamination with 0.03 CFU / ml
- -> storage at 22.5 °C under agitation
Objective validation and assessment of methods for improvement of microbial safety of cellular blood components (Screening, Pathogen Reduction) requires:

- defined (growing) strains
- "real life" conditions, i.e. bacteria multiplying in the matrix (acute spiking may produce uncertain results)
Growth of different bacteria strains in platelet concentrates

- Serratia marcescens
- Serratia rubideae Isolate
- Yersinia enterocolitica ATCC 9610
- E. coli Isolate
- E. coli ATCC 35218
- Pseudomonas aeruginosa ATCC 27853
- Enterococcus faecalis ATCC 29212
- Staphylococcus epidermidis Isolate
- Staphylococcus hominis Isolate
- Staphylococcus epidermidis ATCC 3269
- Staphylococcus epidermidis ATCC 20044
- Staphylococcus epidermidis ATCC 3270

CFU/mL

Day 0
Day 3
Day 4
The established bacterial reference strains (e.g. from ATCC) are not (automatically) applicable for validation studies regarding methods for improvement of microbial safety of cellular blood components.

Consequence:
What kind of reference bacteria do we need?

- Selected strains (not species!)
- Should grow/multiply in platelets
- Should grow independent on donor’s blood (plasma)
- Should enable “real life” contamination, i.e. 10 CFU per platelet bag corresponding to 0.03 CFU per millilitre in order to allow objective method validation

Consequence:
Development of Blood Bacteria Standards during the past 10 years
What are Blood Bacteria Standards?

1. Blood Bacteria Standards (References) are able to grow in PCs up to high counts (what is not automatically given in case of reference strains like ATCC strains).

2. Strains grow up in PCs independent on donor properties (tested for relevant multiplication in PCs from at least 100 different donors).

3. The standards are deep frozen, ready to use, stable, and shippable (manufactured by a special procedure).

4. They are defined in count and consist mainly of living cells (as a rule > 95% living cells).

5. The standards allow “real life” spiking of blood components, i.e. artificial contamination with ~ 10 CFU per bag corresponding to 0.03 CFU per millilitre.

6. Thus, Blood Bacteria Standards are a feasible tool for objective validation and assessment of methods for screening and pathogen reduction in blood components.
WHO-ISBT International Validation Study

Certificate
Blood Bacteria Standard

Species: *Staphylococcus epidermidis*
Code: PEI-B-06-Charge
Lot: PEI-B-06-07

store below -70°C

Developed by: Paul Ehrlich Institute
Federal Agency for Sera and Vaccines
Division Microbial Safety
Paul-Ehrlich-Strasse 51-59
63225 Langen

Generated: Date: 2005/08/28
Approved: Date: 2008/02/06

Lot: PEI-B-06-07
Species: Staphylococcus epidermidis
Isolated from: platelet concentrate (PC)
Supplied as: deep frozen in 10% Human Serum Albumin in saline (150 mM NaCl)
Volume: 1.5 mL
Bacterial load: $2.18 \pm 0.29 \times 10^8$ CFU/mL

Growth in PC: Blood Bacteria Standard *Staphylococcus epidermidis* PEI-B-06 grows donor independently in PCs.

![Graph](image.png)

**Fig.1** In the in vitro study pooled PCs (n=4) were inoculated with 0.03 CFU/mL of Blood Bacteria Standard PEI-B-06 of isolate *Staphylococcus epidermidis*. Sampling was performed during aerobic storage at 22°C and the presence of bacteria was assessed by plating culture.
Prototype Certificate of one of the Blood Bacteria Standards

2. Bacterial Strain (Blood Bacteria Standard)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Firmicutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Cocci</td>
</tr>
<tr>
<td>Order</td>
<td>Bacillales</td>
</tr>
<tr>
<td>Family</td>
<td>Staphylococcaceae</td>
</tr>
<tr>
<td>Species</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>Collection no.</td>
<td>none (isolate)</td>
</tr>
<tr>
<td>Isolated from</td>
<td>platelet concentrate</td>
</tr>
<tr>
<td>Characteristics:</td>
<td>GRAM-positive cocci (0.7 - 1.2 µm), colonies are often surrounded by a clear zone of haemolysis (beta haemolysis) due to production of haemolysins tissue invasive, produce purulent (pus-filled) lesions, nonsporeforming, facultative anaerobic, obligatory pathogenic, grows at 6.5°C to 46°C at pH 4.2 - 9.3.</td>
</tr>
</tbody>
</table>

3. Microbiological identification

- **Fig. 2**: St. epidermidis (PEI-B-06) on sheep blood agar after 24 hours incubation at 37°C

- **Fig. 3**: GRAM-stain of St. epidermidis (PEI-B-06)

| Colonies: | small, white or yellow color, approx. 1-2 mm in diameter after overnight incubation; no haemolysis |
| GRAM-stains: | GRAM-positive |

4. Molecular genetic identification (16S rDNA Sequence)

Automated microbial DNA sequencing was performed by using the MicroSeq® Microbial Identification System (Applied Biosystems).

<table>
<thead>
<tr>
<th>Name</th>
<th>Resultat MicroSeq Match</th>
<th>Specimen Score</th>
<th>Consensus Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEI-B-06</td>
<td>ATCC 12228</td>
<td>100 %</td>
<td>46</td>
</tr>
</tbody>
</table>

**Staphylococcus epidermidis 16S rDNA sequence**

```
GAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGACGAGGAGCTTGCTCCTCTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATATTGAACCGCATGGTTCAATAGTGAAAGACGGTTTTGCTGTCACTTATAGATGGATCCGCGCCGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGC
AACGCCGCGTGAGTGAAGGCTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAGTAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAAC
TGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAG
TACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTCTCCCTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGYAGCGAACCCCGCAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTATATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCATTGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGT
```

5. Production

5.1 Production principle

After the bacterial identification process using microbiological, biochemical (using the API Staph multittest identification system, bioMérieux) and molecular genetic methods (16S rDNA sequencing, RAPD-PCR), an impedance-monitoring system is used to characterize bacterial growth kinetics of Blood Bacteria Standard PEI-B-06 under...
Prototype Certificate of one of the Blood Bacteria Standards

defined conditions (e.g. media, temperature). Following, bacteria are removed during the logarithmic phase, enumerated and frozen in 10% Human Serum Albumin in saline (150 mM) at -80 °C. Viability control is performed 24 hours after production, while stability control is performed quarterly. The bacterial identity of each charge of Blood Bacteria Standard PEI-B-06 is confirmed by biochemical and molecular genetic methods, including 16S rDNA sequencing and DNA fingerprinting (RAPD-PCR).

5.2 Master Bank

Bacteria of Blood Bacteria Standard PEI-B-06 are cultured on appropriate agar media to a sufficient bacterial count. Under aseptic conditions bacteria are transferred to six vials of a Cryobank system to the manufacturer’s instructions and stored at -80 °C. Cryobank tubes contain a medium for suspending the bacterial culture and 25 colour-coded ceramic beads. The suspending medium comprises trypticase soy broth supplemented with glycerol and sucrose. Cryobank systems offer a reliable, convenient and versatile system for storing and preserving fastidious bacteria over long periods.

6. Batch Quality Control

6.1 Viability

To affirm the viability of the Blood Bacteria Standard PEI-B-06, vials of PEI-B-06 are thawed 24 hours after production and enumerated as described in the application section.

6.2 Stability

The stability of the Blood Bacteria Standard PEI-B-06 is confirmed quarterly by thawing and enumerating as described in the application section.

<table>
<thead>
<tr>
<th>Species</th>
<th>Charge</th>
<th>Production Date</th>
<th>Bacterial load [cfu/mL]</th>
<th>Last Stability Date</th>
<th>Bacterial load [cfu/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>PEI-B-06-07</td>
<td>28.09.2005</td>
<td>2.18 ± 0.29E+08</td>
<td>06.02.2008</td>
<td>1.56 ± 0.33E+08</td>
</tr>
</tbody>
</table>

6.3 Identity (Fingerprint)

Random amplified polymorphic DNA analysis (RAPD) was performed using different single oligonucleotide primers of arbitrary sequence. PCR products underwent electrophoresis on an agarose gel (2%) and were visualized using ethidium bromide staining.

7. Application

7.1 Storage

The vials of the Blood Bacteria Standard PEI-B-06 have to be stored immediately below 70°C after arrival. To assure the viability of bacteria of the Blood Bacteria Standard PEI-B-06 the cold chain must not be interrupted.

7.2 Utilization

Before use, transfer the vials of the Blood Bacteria Standard PEI-B-06 directly from the deep freezer to a dry incubator and defrost the vials at 37°C for 10 minutes. If ice crystals are still evident, the vial should be warmed in the hand until the crystals have melted. Vortex the vial for 15 seconds to be sure that all bacteria are evenly spread. Dilution steps and determination of the bacterial count have to be performed as described in the study design protocol.
Study coordinating group

Thomas Montag / Melanie Stöermer, Paul Ehrlich Institute, Germany
Carl McDonald, NBS, United Kingdom
Erica Wood, ARCBS, Australia
Ana Padilla, WHO
Cohava Gelber/ Marian McKee, American Type Culture Collection (ATCC), USA
Dirk de Korte/ Henk Reesink, Sanquin, The Netherlands

Partners

<table>
<thead>
<tr>
<th>Institution</th>
<th>Country</th>
<th>Contact</th>
<th>asked</th>
<th>confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Centre Linz</td>
<td>Austria</td>
<td>Christian Gabriel</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Canadian Blood Service, Ottawa</td>
<td>Canada</td>
<td>Sandra Ramirez-Arcos</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>CaridianBCT, Lakewood CO</td>
<td>USA</td>
<td>Ray Goodrich</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Case Western Reserve University, Cleveland</td>
<td>USA</td>
<td>Roslyn Yomtovian Michael R. Jacobs</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>German Red Cross Blood Transfusion Service Springe, Partner Lab of BD Biosciences</td>
<td>Germany</td>
<td>Thomas Mueller</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>German Red Cross Blood Transfusion Service, University of Frankfurt/Main</td>
<td>Germany</td>
<td>Michael Schmidt</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Hong Kong Red Cross Blood Transfusion Service</td>
<td>Hong Kong SAR China</td>
<td>Cheuk-Kwong Lee</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>National Blood Service, London</td>
<td>United Kingdom</td>
<td>Carl McDonald</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>National Blood Transfusion Centre,</td>
<td>México</td>
<td>Julieta Rojo</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Regional Centre for Transfusion Medicine, Bialystok</td>
<td>Poland</td>
<td>Piotr Radziwon</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>South African National Blood Service, Weltevreden Park</td>
<td>South Africa</td>
<td>Tshilidzi Mzithethi</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>St. Elisabeth Ziekenhuis, Tilburg</td>
<td>The Netherlands</td>
<td>Jan Marcelis</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>VU University Medical Centre, Amsterdam</td>
<td>The Netherlands</td>
<td>Annika Pettersson</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Walter Reed Army Medical Center, Washington DC</td>
<td>USA</td>
<td>David G. Heath</td>
<td>+</td>
<td>yes</td>
</tr>
</tbody>
</table>
Logistics

1. Shipping
   - complicated to bring living (blinded) pathogenic bacteria through the customs
   - re-decision on deadline because of delay
   - Europe and Canada: shipping by PEI
   - Hong Kong, Mexico, South Africa, USA: shipping by ATCC
   - sometimes repetition of shipping necessary
   - shipping successful with the exception of South Africa

   data provided yesterday (bacteria meeting) by Tshilidzi Muthivhi

2. Data receiving
   - successful in all cases (with exception of South Africa)
   - in one case too late for being able to perform the complete statistic calculation before Cairo meeting
Timeline of WHO ISBT International Validation Study on Blood Bacteria Standards

2008
- Organisation Study Design
- Production of BBS

2009
- Organisation Logistics
- Operating Time
  - Shipping
    - Sending inside EU
    - Sending to ATCC
  - Results
    - Resending to Mexico
    - South Africa: permission, customs, delay
- Statistics
**Phase 1** Four different blinded Blood Bacteria Standards (BBS) are sent to the partner labs

**Identification**
- cultivation
- identification

**Enumeration**
- 10-fold serial dilutions
- counting of each BBS in 5 independent replicates

**Growth in PC**
- inoculation of 2 PC units, 10 and 100 CFU/bag
- storage at 22-24°C with agitation
- counting after 4 days

Sending of results

Demonstration of results in the Workshop during the ISBT XIXth Regional Congress in Cairo, 2009

Fig. 1 Principle and Subject of Validation Study Phase 1
Study Protocol

4 Standards
10 aliquots

Identification 1x

Vortex
15 seconds

Streak
Incubation
at 37°C

Titration 5x, 6 dilutions

Spread 100 µL of each dilution

Incubate at 37°C

Enumerate

Growth in 2 PC incl. titration

1 mL

According to Bernd Lambrecht, GRC, modified
Protocol for 1 of the bacteria standards in detail

4 Standards
10 aliquots

2 agar plates

150 agar plates

88 agar plates

Sum: 190 agar plates

2 PI
Identification 1x
Incubation at 37°C
Vortex 15 seconds
streak

+ gram stain, API

+1 mL 1 mL

10/100 CFU

10 CFU

100 CFU

according to Bernd Lambrecht, GRC, modified
Complete protocol (4 standards)

Sum: 4 x 190  = 760 agar plates

according to Bernd Lambrecht, GRC, modified
Results of

WHO ISBT International Validation Study on Blood Bacteria Standards
Part 1
Identification of blinded Bacteria Standards

<table>
<thead>
<tr>
<th>Code</th>
<th>Bacteria species</th>
<th>Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10  11  12  13</td>
</tr>
<tr>
<td>A</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>+  +  +  +  +  +  +  +  +  +  (+)*  +  +  +</td>
</tr>
<tr>
<td></td>
<td>(PEI-B-06-08)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td><em>Streptococcus pyogenes</em></td>
<td>+  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td></td>
<td>(PEI-B-20-06)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>+  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td></td>
<td>(PEI-B-08-10)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td><em>Escherichia coli</em></td>
<td>+  +  +  +  +  +  +  +  +  +  +  +  +  +  +</td>
</tr>
<tr>
<td></td>
<td>(PEI-B-19-06)</td>
<td></td>
</tr>
</tbody>
</table>

* In one case, determination as *Staphylococcus delphini* instead of *Staphylococcus epidermidis* (most likely to the commercial identification kit used) but identified at least as coagulase-negative Staphylococcus.
Part 2
Enumeration of Blood Bacteria Standards

Data from South Africa to be added.
Part 2
Enumeration of Blood Bacteria Standards

Data from South Africa to be added.
Part 2
Enumeration of Blood Bacteria Standards

Blood Bacteria Standard C
*Klebsiella pneumoniae*

Data from South Africa to be added.
Part 2
Enumeration of Blood Bacteria Standards

Data from South Africa to be added.
Part 2

Enumeration of Blood Bacteria Standards

Bacterial Count of BBS A, B, C, and D of all participants - single values

Blood Bacteria Standards
Part 3
Growth of Blood Bacteria Standards in PC
(summarised results)

Table does not consider all details, e.g. growth in case of contamination of PCs applying 100 CFU per bag but no growth applying 10 CFU per bag, repetitions, pooled or apheresis PCs (will be described in detail in study report).

<table>
<thead>
<tr>
<th>Code</th>
<th>Groth in PC after contamination using 10 to 100 bacteria per bag corresponding to 0.03 to 0.3 CFU/ml</th>
<th>Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>Staphylococcus epidermidis (PEI-B-06-08)</td>
<td>yes</td>
</tr>
<tr>
<td>B</td>
<td>Streptococcus pyogenes (PEI-B-20-06)</td>
<td>yes</td>
</tr>
<tr>
<td>C</td>
<td>Klebsiella pneumoniae (PEI-B-08-10)</td>
<td>yes</td>
</tr>
<tr>
<td>D</td>
<td>Escherichia coli (PEI-B-19-06)</td>
<td>yes</td>
</tr>
</tbody>
</table>
Many colleagues worldwide volunteered for the study without any funding and without hesitating to invest a substantial amount of hard work.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Remarks / Details</th>
<th>Borne by:</th>
<th>Sum (circa):</th>
</tr>
</thead>
</table>
| **Shipping of deep frozen Bacteria Standards** | PEI to: Canada, Germany (2x), Poland, The Netherlands, United Kingdom  
PEI to ATCC,  
ATCC to: Hong Kong, Mexico, South Africa, USA (2x) | PEI | ~ 4,000 Euro |
| **Costs of partners** | | study partners | |
| **Materials** | Microbiology:  
~ 150 Euros per Standard, sum: ~ 600 Euros  
(600 x 13 = 7800) | study partners | ~ 7,800 Euros |
| **PCs** | at least 8 Pcs (outdated possible)  
e.g. prices in Germany:  
1 Pool PC ~ 300 Euros  
1 Apheresis PC ~ 600 Euros | | (2400 - 4800 Euros) |
| **Staff** | (calculated by Piotr Radziwon, Poland) | | 1,150 Euros |
| **Sum** | without PCs  
(in worst case with PCs) | | ~ 13,000 Euros  
(15,500 to 18,000 Euros) |
## WHO ISBT International Validation Study on Blood Bacteria Standards

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISBT Meeting 2006, Cape Town, South Africa</td>
<td>ISBT Working Party Transfusion-Transmitted Infectious Diseases (WP-TTID) decides on Validation Study</td>
</tr>
<tr>
<td>WHO CC Meeting 2007, Bethesda MD, USA</td>
<td>Official proposal to WHO to install Blood Bacteria Standards</td>
</tr>
<tr>
<td>ISBT Meeting 2007, Madrid, Spain</td>
<td>Agreement between WHO (Ana Padilla) and ISBT WP-TTID on a Collaborative Validation Study</td>
</tr>
<tr>
<td>ISBT Meeting 2008, Macao, China</td>
<td>Kick-off Meeting Validation Study, ISBT WP-TTID, Subgroup Bacteria</td>
</tr>
<tr>
<td>AABB Meeting 2008, Montreal, Canada</td>
<td>Extraordinary Meeting of Subgroup Bacteria, discussion of study details</td>
</tr>
<tr>
<td>WHO CC Meeting 2009, Langen, Germany</td>
<td>Update Blood Relevant Bacteria Panel Decision on start of formalised procedure for submission of topic to WHO Expert Committee for Biological Standardisation (ECBS)</td>
</tr>
<tr>
<td>ISBT Meeting 2009, Cairo, Egypt</td>
<td>Annual Meeting of Subgroup Bacteria, Evaluation of Study, decision on follow ups</td>
</tr>
</tbody>
</table>
Outcome of study:

WHO Collaborative Centres Meeting
2009 February 17th to 19th
Langen, Germany:

Decision on start of formalised procedure for submission of topic to WHO Expert Committee for Biological Standardisation (ECBS).
Outcome of Subgroup Bacteria meeting yesterday

1. Follow-up of study
   - report to WHO ECBS by end of June 2009
   - abstract submissions to AABB and ISBT meetings
   - manuscript submission to Vox Sanguinis

2. List of transfusion relevant bacteria/fungi
   - discussion via e-mail inside Subgroup Bacteria including the study partners
   - afterwards harmonisation (WP-TTID, ISBT, FDA ....)

3. Enlargement of panel of Blood Bacteria Standards
   - discussion of first draft in Subgroup Bacteria
## Discussion paper:
List of transfusion-relevant microbial species/strains

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Strain</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Gram-positive Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>PEI-B-06</td>
<td>FDA</td>
</tr>
<tr>
<td>2.</td>
<td><em>Staphylococcus aureus</em></td>
<td>PEI-B-23</td>
<td>FDA</td>
</tr>
<tr>
<td>3.</td>
<td><em>Streptococcus pyogenes</em></td>
<td>PEI-B-20</td>
<td>FDA</td>
</tr>
<tr>
<td>4.</td>
<td><em>Streptococcus viridans</em></td>
<td></td>
<td>Str. bovis (NBS) FDA</td>
</tr>
<tr>
<td>5.</td>
<td><em>Streptococcus faecalis</em></td>
<td></td>
<td>??</td>
</tr>
<tr>
<td>6.</td>
<td><em>Streptococcus agalactiae</em></td>
<td></td>
<td>fatal case</td>
</tr>
<tr>
<td>7.</td>
<td><em>Propionobacterium acnes</em></td>
<td>PEI-B-22</td>
<td>FDA</td>
</tr>
<tr>
<td>8.</td>
<td><em>Corynebacterium species</em></td>
<td></td>
<td>FDA, ??</td>
</tr>
<tr>
<td>9.</td>
<td><em>Bacillus cereus</em></td>
<td>PEI-B-07</td>
<td>FDA</td>
</tr>
<tr>
<td>10.</td>
<td><em>Bacillus cereus, spores</em></td>
<td>PEI-B-07-S</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td><em>Bacillus subtilis</em></td>
<td></td>
<td>FDA</td>
</tr>
<tr>
<td>12.</td>
<td><em>Bacillus subtilis, spores</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td><em>Clostridium perfringens</em></td>
<td>PEI-B-25</td>
<td>FDA</td>
</tr>
<tr>
<td>14.</td>
<td><em>Clostridium perfringens, spores</em></td>
<td>PEI-B-25-S</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Gram-negative Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>PEI-B-08</td>
<td>FDA</td>
</tr>
<tr>
<td>2.</td>
<td><em>Klebsiella oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>Escherichia coli</em></td>
<td>PEI-B-19</td>
<td>FDA</td>
</tr>
<tr>
<td>4.</td>
<td><em>Serratia marcescens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Yersinia enterocolitica</em></td>
<td>DSM 11502</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td><em>Salmonella cholerae suis</em></td>
<td></td>
<td>fatal case</td>
</tr>
<tr>
<td>7.</td>
<td><em>Proteus vulgaris</em></td>
<td></td>
<td>??</td>
</tr>
<tr>
<td>8.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td>FDA</td>
</tr>
<tr>
<td>9.</td>
<td><em>Pseudomonas fluorescence</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Candida albicans</em></td>
<td>PEI-B-21</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus species</em></td>
<td></td>
<td>??, species?</td>
</tr>
</tbody>
</table>
Outcome of Subgroup Bacteria meeting yesterday

1. Follow-up of study
   - report to WHO ECBS by end of June 2009
   - abstract submissions to AABB and ISBT meetings
   - manuscript submission to Vox Sanguinis

2. List of transfusion relevant bacteria/ fungi
   - discussion via e-mail inside Subgroup Bacteria including the study partners
   - afterwards harmonisation (WP-TTID, ISBT, FDA ....)

3. Enlargement of panel of Blood Bacteria Standards
   - discussion of first draft in Subgroup Bacteria
   - will be discussed and completed by e-mail

4. Continuation:
   - preparation of survey
   - review on microbial safety of blood components
Relevant remark:

The Blood Bacteria Standards are applicable for the novel Advanced Therapy Medicinal Products (ATMPs), e.g. Cell Based Medicinal Products.
Additional outcome of study:

The Blood Bacteria Standards are applicable for the novel Advanced Therapy Medicinal Products (ATMPs), e.g. Cell Based Medicinal Products.

Thus, Subgroup Bacteria will have a meeting here in Cairo with colleagues from BEST in order to discuss a draft design of a study on sterility testing of stem cells. It is intended to start the study in the next time applying a panel of Blood Bacteria Standards.
I have to say “Thank you very much!” to:

PEI
Melanie Stoermer
Utta Schurig
Sven-Boris Nicol
Julia Brachert
Ute Sicker
Rekia Beshir
Christian Schneider

The study partners
The study coordinating group
WHO
Ana Padilla
Gabriele Unger
Michael Chudy
Heiner Scheiblauer

and to you for attention