



WP-TTID

Chair: Silvano Wendel, Brazil

Subgroup on Bacteria

Chair: Thomas Montag, Germany

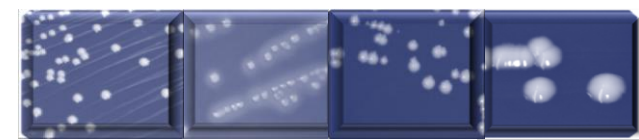
Co-Chair: Erica Wood, Australia

Enlargement of WHO Repository Transfusion Relevant Bacteria Reference Strains

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Report on experimental preparatory work and study design

Dr. Melanie Störmer



Overview

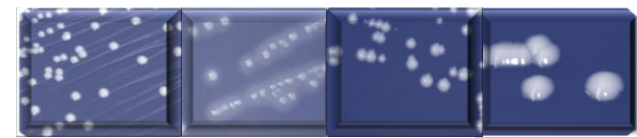
Enlargement of Repository TRBR

- **Candidates - Enlargement of Repository Transfusion-Relevant Bacteria Reference Strains**
- **Experimental Preparatory Work – Production of Defined Frozen Suspensions (DFS)**
- **Study Design - Enlargement of Repository**



Candidates Enlargement of Repository Transfusion-Relevant Bacteria Reference Strains

Extraordinary Meeting
on occasion of AABB Annual Meeting,
Baltimore MD, USA, October 9th, 2010



ISBT WP-TTID, Subgroup Bacteria, Extraordinary Meeting

On occasion of AABB Annual Meeting, Baltimore MD, USA, October 9th, 2010

Participants

No.	Name	Facility	Country
1	Morris Blajchman	McMaster University	Canada
2	Dirk de Korte	Sanquin Blood Supply Foundation, Amsterdam	The Netherlands
3	Robert Duncan	FDA, Rockville	USA
4	Ray Goodrich	Caridian BCT, Denver	USA
5	Luisa Gregori	FDA, Rockville	USA
6	Salim Haddad	FDA, Rockville	USA
7	Michael Jacobs	Case Western Reserve University, Cleveland	USA
8	Lisa Jarvis	SNBTS	Scotland
9	Susanne Marschner	Caridian BCT, Denver	USA
10	Thomas Montag	Paul-Ehrlich-Institute, Langen	Germany
11	Thomas Mueller	German Red Cross, NSTOB, Springe	Germany
12	Sandra Ramirez	Canadian Blood Service, Ottawa	Canada
13	Paolo Rebutta	Centro di Medicina Transfusionale, Terapia Cellulare e Criobiologia, Milano	Italy
14	Melanie Stoermer	Paul-Ehrlich-Institute, Langen	Germany
15	Jaroslav Vostal	FDA, Rockville	USA
16	Silvano Wendel	Hospital Sirio Libanês, São Paulo	Brazil
17	Erica Wood	Australian Red Cross Blood Service, Melbourne	Australia
18	Roslyn Yomtovian	Louis Stokes Veterans Administration Medical Center, Cleveland	USA

Enlargement of Repository

Recommendations of ISBT Bacteria Group

Candidates for Enlargement of Repository Transfusion-Relevant Bacteria Reference Strains

Species	Strain	Where available?	Remarks
Validation Study 2008 / 2009			
<i>Staphylococcus epidermidis</i>	PEI-B-06	PEI	FDA list
<i>Streptococcus pyogenes</i>	PEI-B-20	PEI	FDA list
<i>Escherichia coli</i>	PEI-B-08	PEI	FDA list
<i>Klebsiella pneumoniae</i>	PEI-B-19	PEI	
Enlargement candidates			
<i>Bacillus thuringiensis</i>	PEI-A-33	PEI	FDA list, Growth in PC
<i>Bacillus subtilis</i>	PEI-B-07	PEI	FDA list
<i>Bacillus thuringiensis spores</i>	PEI-A-33	PEI	Growth in PC
<i>Bacillus subtilis spores</i>	PEI-B-07	PEI	
<i>Candida albicans</i>	PEI-B-21	PEI	FDA list
<i>Clostridium perfringens</i>		PEI	FDA list, Growth in PC? (problems)
<i>Enterobacter cloacae</i>	PEI-A-43	PEI	FDA List, Growth in PC? (problems)
<i>Klebsiella oxytoca</i>			FDA list, <i>Kl. pneumoniae</i> ? (see above)
<i>Morganella morganii</i>			
<i>Propionibacterium acnes</i>	PEI-B-22	PEI	FDA list, Growth in PC? (problems) higher spike count
<i>Proteus mirabilis</i>			
<i>Pseudomonas aeruginosa</i>		PEI	FDA list, Growth in PC? (problems)
<i>Pseudomonas fluorescens</i>		PEI	Growth in PC?
<i>Salmonella cholerae-suis</i>			
<i>Serratia marcescens</i>		Canada	FDA list, Growth in PC?
<i>Staphylococcus aureus</i>	PEI-B-23	PEI	FDA list
<i>Staphylococcus capitis</i>			
<i>Staphylococcus lugdunensis</i>			
<i>Staphylococcus warneri</i>			
<i>Streptococcus dysgalactiae</i>		London	<i>Str. dysgalactiae</i> Growth in PC?
<i>Streptococcus viridans</i>			FDA list
<i>Streptococcus bovis</i>			
Species/Strains for Red Cell Concentrates			
<i>Yersinia enterocolitica</i>			killed in PC

Enlargement of Repository

Recommendations of ISBT Bacteria Group

- 1 *St. aureus* and 1 Coagulase-negative *Staphylococcus* (CNS)
- 1 aerobic spore forming bacterium (isolated spores)
- no anaerobic bacteria
- 1 *Klebsiella* species
- 1 *Pseudomonas* species
- 1 *Streptococcus* species
- no fungi
- no red cells (RBC) (*Yersinia enterocolitica*)

Candidates for Enlargement of Repository Transfusion-Relevant Bacteria Reference Strains

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→ <i>Klebsiella pneumoniae</i>	PEI-B-19	PEI	
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<i>Proteus mirabilis</i>			
<i>Pseudomonas aeruginosa</i>		PEI	FDA list, Growth in PC? (problems)
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<i>Streptococcus viridans</i>			FDA list
<i>Streptococcus bovis</i>			
Species/Strains for Red Cell Concentrates			
<i>Yersinia enterocolitica</i>			killed in PC

Enlargement of WHO Repository - Candidates

Asking partners for providing candidates for enlargement!

Species
Validation Study 2008/2009
<i>Staphylococcus epidermidis</i>
<i>Streptococcus pyogenes</i>
<i>Escherichia coli</i>
<i>Klebsiella pneumoniae</i>
Enlargement candidates
<i>Bacillus thuringiensis</i> spores
<i>Enterobacter cloacae</i>
<i>Morganella morganii</i>
<i>Proteus mirabilis</i>
<i>Pseudomonas fluorescens</i>
<i>Salmonella cholerae-suis</i>
<i>Serratia marcescens</i>
<i>Staphylococcus aureus</i>
<i>Streptococcus dysgalactiae</i>

Enlargement of WHO Repository – Provided Isolates

Provided Transfusion Reaction Investigation Isolates

Partner	Strain	Source	Code	PEI-Code
Sandra Ramirez-Arcos Canadian Blood Service, Ottawa, Canada	<i>Bacillus cereus</i>	PC	CBS-09-2007	PEI-A-57
	<i>Bacillus cereus</i>	PC	CBS 12-2008	PEI-A-58
	<i>Bacillus cereus</i>	PC	CBS 03-2010	PEI-A-59
	<i>Proteus mirabilis</i>	PC	CBS 02-2009A	PEI-A-55
	<i>Serratia marcescens</i>	PC	CBS 07-2005	PEI-A-60
Carl McDonald NHS Blood and Transplant, London, United Kingdom	<i>Enterobacter cloacae</i>	RBC	PT/BAC/38/07/M	PEI-A-43
	Group G <i>Streptococcus (dysgalactiae)</i>	PPC	PT/BAC/69/08/M	PEI-A-37
	Group G <i>Streptococcus (dysgalactiae)</i>	APC	PT/BAC/44/08/H	PEI-A-42
	<i>Streptococcus bovis</i>	APC	PT/BAC/29/06/T	PEI-A-41

Enlargement of WHO Repository - Candidates

Validation Study 2008/2009

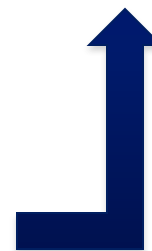
1. *Staphylococcus epidermidis*
2. *Streptococcus pyogenes*
3. *Escherichia coli*
4. *Klebsiella pneumoniae*

Enlargement Candidates

5. *Bacillus thuringiensis* spores
6. *Enterobacter cloacae*
7. *Morganella morganii*
8. *Proteus mirabilis*
9. *Pseudomonas fluorescens*
10. *Salmonella cholerae-suis*
11. *Serratia marcescens*
12. *Staphylococcus aureus*
13. *Streptococcus dysgalactiae* (G)

Buffer

14. *Bacillus cereus*
15. *Bacillus cereus*
16. *Bacillus cereus*
17. *Streptococcus bovis*
18. *Streptococcus dysgalactiae* (G)

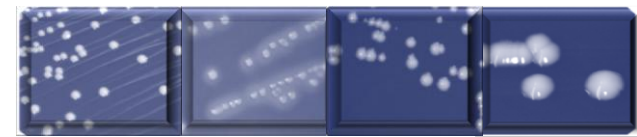


**Exclusion after
pre-characterisation
(no ability to grow in PCs)**



Experimental Preparatory Work

Production of Defined Frozen Suspensions (DFS)



Production of Defined Frozen Suspensions (DFS)

New Isolate → Cultivation



Identification of Species

(GRAM, API, 16S DNA Sequencing)



Inclusion into „Master Bacteria Bank, MBB“



Production of Mastersuspension „MBB+1“

Frozen Suspension used for production of new lots of DFS



Setting of Dilution of MBB+1

Growth characteristics (computer based impedance curves) of dilution steps → Selection of dilution growing within a workable time

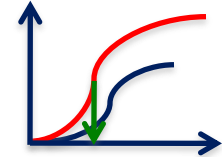


Production of Defined Frozen Suspensions

Setting Point of Harvest

Impedance curves of selected dilution

→ In parallel enumeration by plate assay: Growth curve



Production of new Lot

Harvest at the defined time point and enumeration 24 hours after freezing



Proof of Identity & Batch to Batch consistency

Gram-stain, API, Sequencing of bacterial 16S rRNA, DNA-Fingerprint



Stability Testing during storage (enumeration)



Growth ability in PCs

Growth in PCs (>100 different donors) → Growth independence on innate immunity



Bacteria Reference

Enlargement of WHO Repository - Candidates

Validation Study 2008/2009	
1.	<i>Staphylococcus epidermidis</i>
2.	<i>Streptococcus pyogenes</i>
3.	<i>Escherichia coli</i>
4.	<i>Klebsiella pneumoniae</i>

Buffer	
14.	<i>Bacillus cereus</i>
15.	<i>Bacillus cereus</i>
16.	<i>Bacillus cereus</i>
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Enlargement Candidates	
5.	<i>Bacillus thuringiensis</i> spores
6.	<i>Enterobacter cloacae</i>
7.	<i>Morganella morganii</i>
8.	<i>Proteus mirabilis</i>
9.	<i>Pseudomonas fluorescens</i>
10.	<i>Salmonella cholerae-suis</i>
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12.	<i>Staphylococcus aureus</i>
13.	<i>Streptococcus dysgalactiae</i> (G)

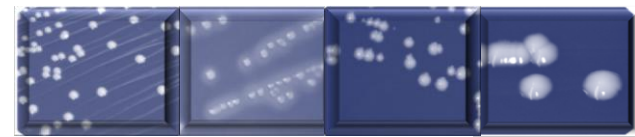


Growth ability



Study Design

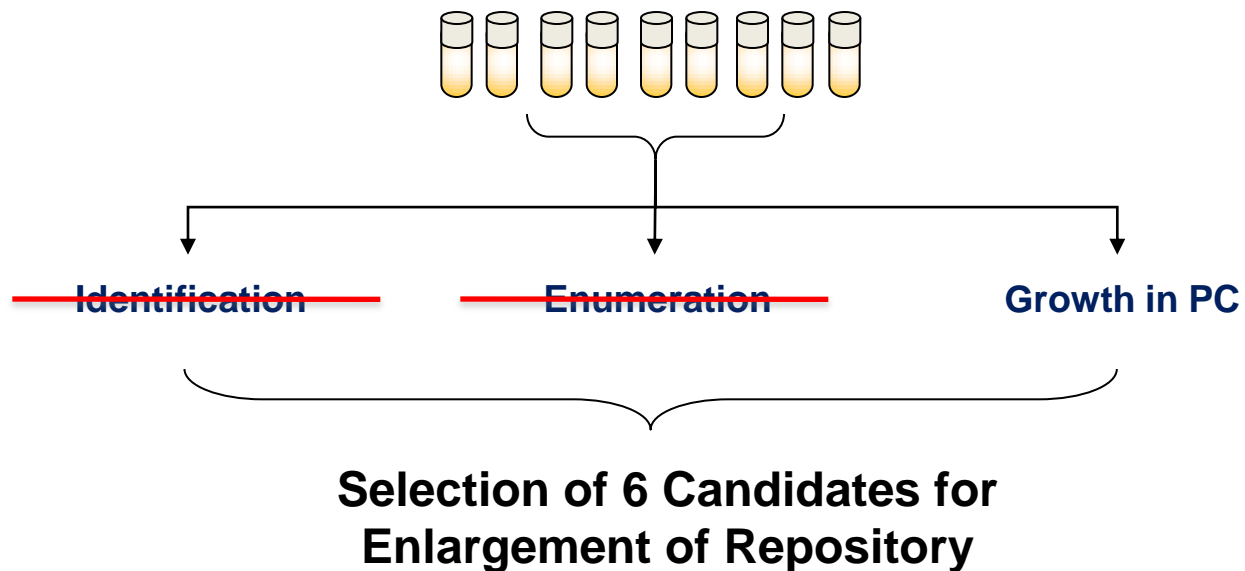
Enlargement of Repository



Enlargement: Study Design

PART 1 Pre-Characterisation

Candidates
will be sent to the participating laboratories



PART 2 Enlargement Study

Enlargement of WHO Repository

PART 1

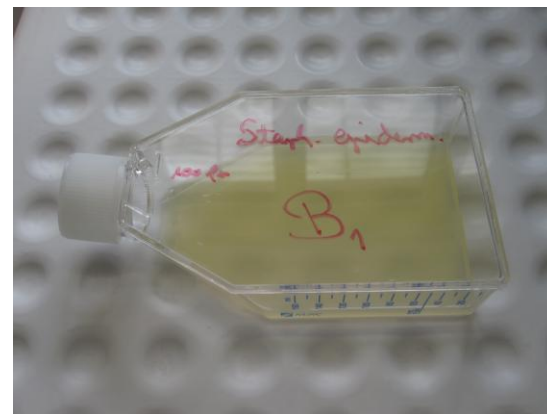
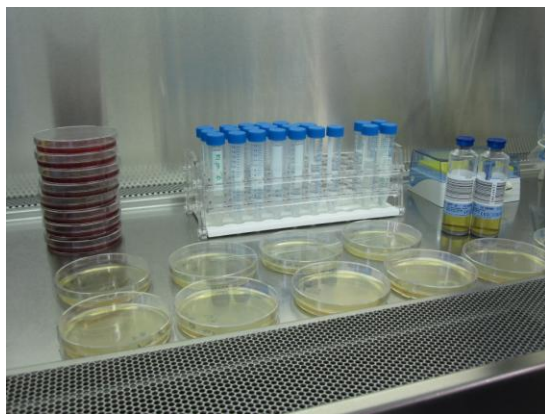
Advisement: Saving PCs

Pre-characterisation of growth ability in fresh frozen plasma instead of PCs

→ Artificial contamination of plasma (100%, 35%) in cell culture bottles (100 cfu/bottle) and incubation at 22.5°C for 4 days

→ Daily sampling for automated culture

→ Enumeration after 4 days incubation



Enlargement of WHO Repository

PART 1

Advisement: Saving PCs

Pre-characterisation of growth ability in fresh frozen plasma instead of PCs

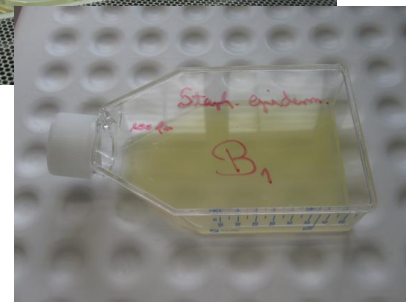
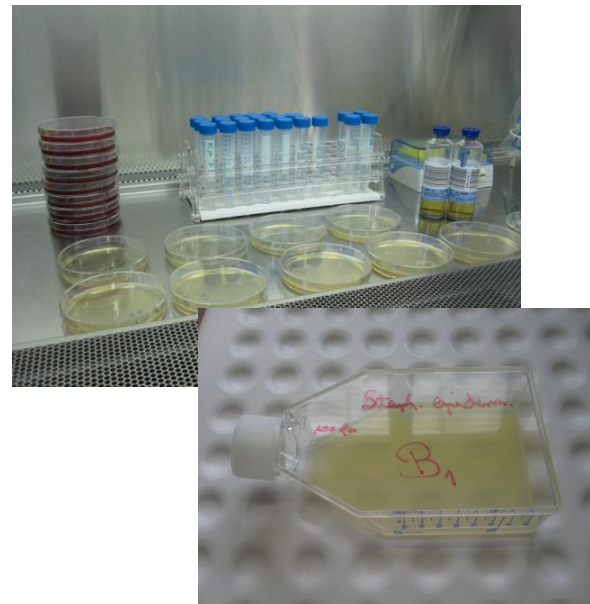
→ Artificial contamination of plasma (100%, 35%) in cell culture bottles (100 cfu/bottle) and incubation at 22.5°C for 4 days

→ Daily sampling for automated culture

→ Enumeration after 4 days incubation

Conclusion: PCs are necessary

→ Some strains did not grow in FFP



Proposal: Study Design

PART 1 Pre- characterisation

Starting as soon
as possible

Results up to May 2012

- Selection of 6 candidates
- Circulation of Study Design
Enlargement

----- Submission of Study Design to ECBS (30.06.2012) -----

PART 2 Enlargement Study

Winter 2012

Submission of results on 30.06.2013

9 Candidates
will be sent to the participating laboratories



Growth in PC
(n=5)

**Selection of 6 Candidates for
Enlargement of Repository**

Growth in PCs
(n=?)



Thank You!

**→ Discussion: Study Design
Enlargement**