Update on the ISBT TTID study on establishment of bacterial reference strains for RBC

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Current status: overview

- Screening for candidate strains for growth in RBC concentrates
  - Compilation of a panel of 7 strains for the study
- Production of the final bacterial solutions
  - Stability testing of the strains
- Completion of the final study protocol
- Coordination of participating laboratorys worldwide
- Organization of the shipment

Putative bacterial candidates

Gram positive

- *Bacillus cereus*
- *Bacillus licheniformis*
- *Listeria monocytogenes* (2x)
- *Staphylococcus epidermidis* (2x)
- *Staphylococcus aureus* (2x)
- *Streptococcus pyogenes*

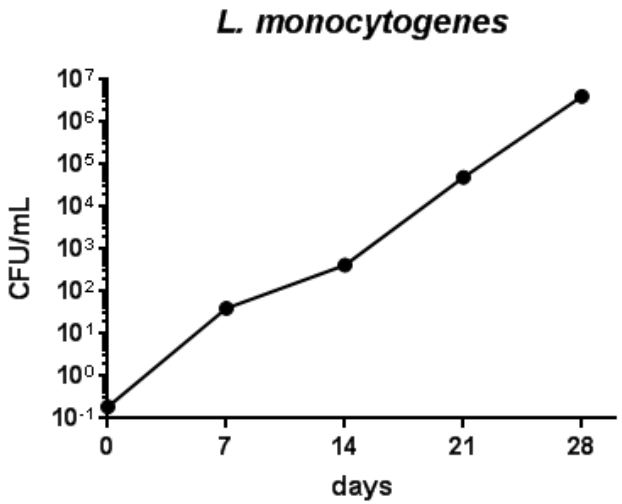
Gram negative

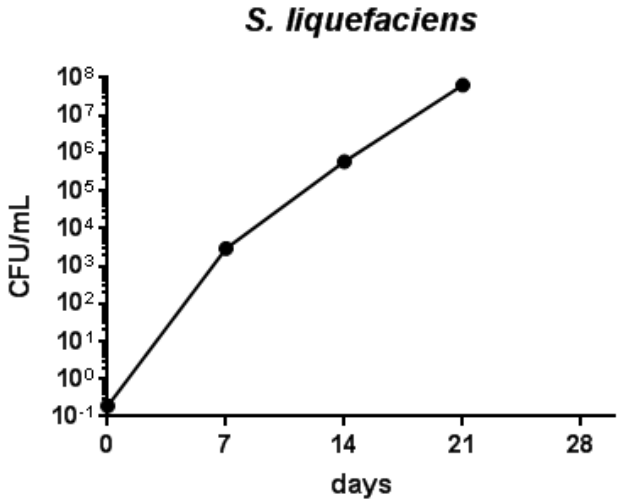
- *Serratia marcescens* (3x)
- *Serratia liquefaciens* (3x)
- *Pseudomonas aeruginosa* (3x)
- *Pseudomonas putida*
- *Aeromonas veronii*
- *Yersinia enterocolitica* (4x)
- *Escherichia coli*
- *Morganella morganii*
- *Acinetobacter junii*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Enterobacter cloacae*
- *Salmonella cholerasuis*

→ 32 strains in total

Study panel – growth behavior

strain	PEI ID	origin
B. cereus	PEI-B-P-57	1st WHO repository, enlargement
L. monocytogenes	PEI-A-199	Isolate Blood screening, England
S. marcescens	PEI-B-P-56	1st WHO repository, enlargement
S. liquefaciens	PEI-A-184	Isolate RB; C92-13-01, Roth V, et al Transfusion 2002;40(8):931-5, CDC
P. fluorescens	PEI-B-P-77	1st WHO repository, enlargement
Y. enterocolitica	PEI-A-105	Isolate RBC, Japan
Y. enterocolitica	PEI-A-176	Isolate RBC, CDC





Stability testing

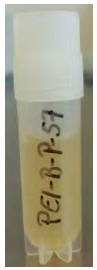
	CFU/ml		
Strain	1 week	1 month	6 month
B. cereus	1,23E+08	-	1,46E+08
L. monocytogenes	7,88E+06	8,25E+06	7,29E+06
S. marcescens	7,62E+06	8,11E+06	
S. liquefaciens	1,92E+07	2,02E+07	1,65E+07
P. fluorescens	1,14E+07	1,41E+07	1,10E+07
Y. enterocolitica	5,03E+06	6,70E+06	6,79E+06
Y. enterocolitica	7,33E+06	6,34E+06	6,20E+06

Overview study design

Each of the following steps has to be performed in **triplicates**

- Shipment of **3 vials** of each strain

candidate strain

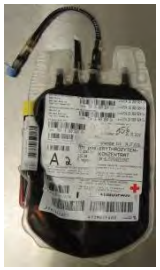


serial dilution

Inoculation

10-25 CFU

RBC bag



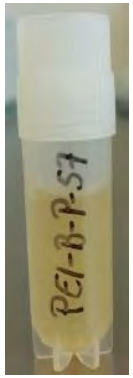
Determination of the CFU/ml over time

Storage under routine conditions (2-6 °C, 42 days)

Study design

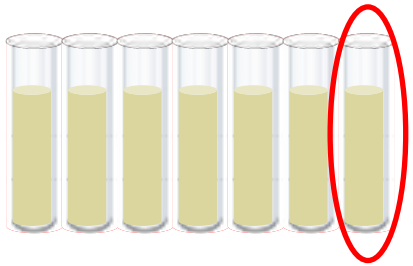
1. Preparation of the final inoculum

Bacterial solution



Known concentraion

10-fold serial dilution



Exact procedure:

Study protocol

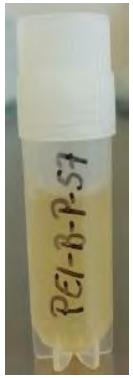
Final bacterial concentration

10-25 CFU/ml

Study design

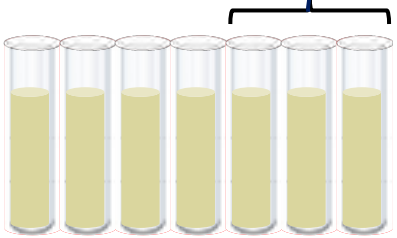
2. Verification of the final inoculum

Bacterial solution

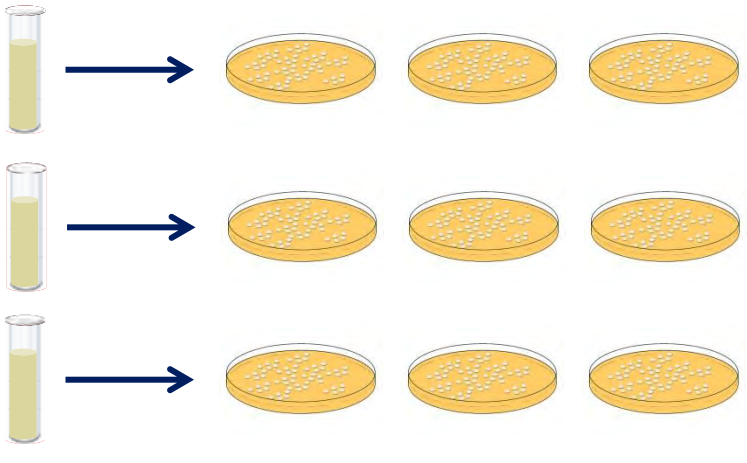


Known concentration

10-fold serial dilution



100 µl of last 3 dilutions in triplicates

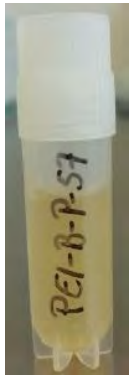


Colony counting

Study design

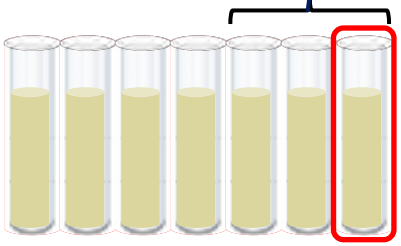
3. Spiking of red blood cell concentrate

Bacterial solution



Known concentration

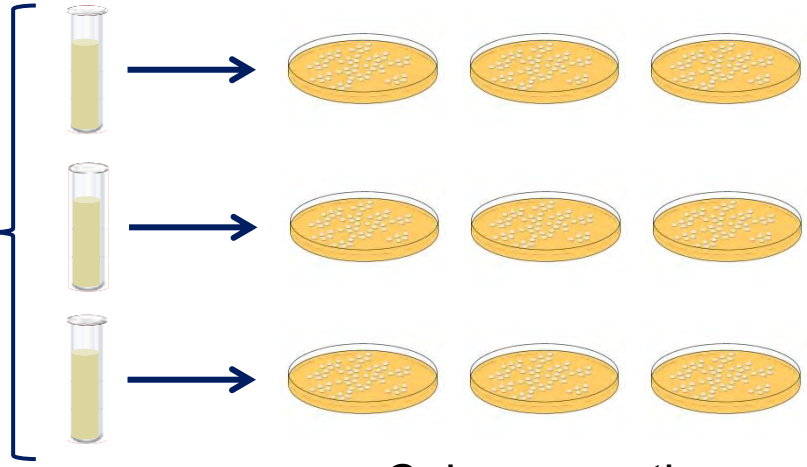
10-fold serial dilution



10-25 CFU/ml

Storage under routine conditions 2-6 °C, 42 days

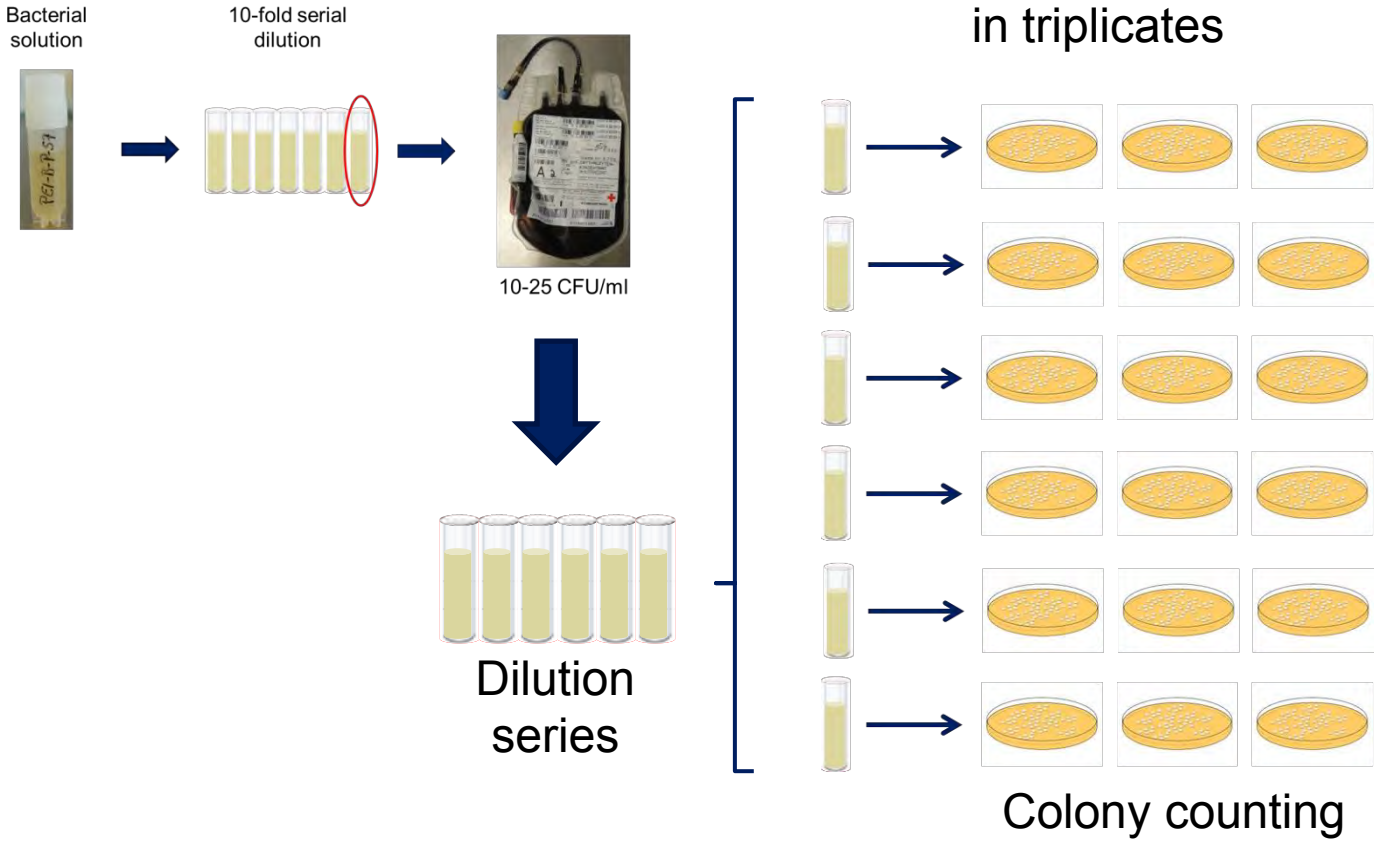
100 µl of last 3 dilutions in triplicates



Colony counting

Study design

4. Sampling on days 7, 14, 21, 28, 35, 42 (?)



Documentation

1. CFU counting

Bacterial strain ID	Dilution 100µl of…	Plate 1	Plate 2	Plate 3	Mean value	Bacterial count [CFU/mL]	Mean Value Vial C1 [CFU/mL]
Bag 2	Dilution 1				#DIV/0!	N/A	#DIV/0!
	Dilution 2				#DIV/0!	N/A	
	Dilution 3				#DIV/0!	N/A	
	Dilution 4				#DIV/0!	N/A	
	Dilution 5				#DIV/0!	N/A	
	Dilution 6				#DIV/0!	N/A	

2. Identification of bacteria

Strain:
Identification (number) of sample:
Growth after day:
Macroscopic view Colony morphology:
Microscopic view: (shape: rod, coccus)
Result of Gram-staining:
Description of identification Method (down to species level, i.e. API, PCR) (Identification panel)

3. Lab protocol

Test strain:

RBC Concentrates: Exp. Day:
Volume
Blood type /rh

Control Inoculum (Dilution of stock):_____CFU/ml (mean value)

(CFU plate 1:_____CFU plate 2:_____CFU plate 3:_____)

Result of enumeration of inoculum control:_____CFU/ml

Bacterial growth after storage	Sampling after	Yes (Growth)	no
	7 days		
	14 days		
	21 days		
	28 days		
	35 days		
	42 days		

4. Questionnaire

Please complete this questionnaire and return with the first set of completed results to allow accurate assessment.

Study Partner: Contact

Name:

Contact details:
(Postal address, fax, phone, e-mail)

Were you a participant of the first WHO-ISBT International Validation Study on Blood Bacteria Standards? **Yes / No**

Lab equipment used:

Microbiological Safety Cabinet (Class II) / Laminar flow hood: **Yes / No**
If **yes**, please give details: (Make, model)

Study partners (preliminary)

Sandra Ramirez-Arcos
Canadian Blood Service, Ottawa, Canada

Carl McDonald, Kate Aplin, Anjana Roy, NHS Blood and Transplant, London, England

Axel Seltsam, Bernd Lambrecht, Michael Schmidt, Erhard Seifried
German Red Cross Blood Service NSTOB, Springe, Germany

Birgit Gathof, Melanie Störmer, University Hospital Cologne, Transfusion Medicine, Germany

Susanne Marschner, Shawn Keil
Terumo BCT Biotechnologies, Lakewood, USA

Susanne Süßner, Austrian Red Cross, Blood centre Linz, Austria

Stephen J. Wagner, Holland Laboratory, American Red Cross, Blood Component Dep. Rockville, USA

Masahiro Satake, Japanese Red Cross, Tokyo, Japan

Julieta Rojo, Centro Nacional de la Transfusión Sanguínea Mexico

José Eduardo Levi, Fundação pró-Sangue/Hemocentro de São Paulo, Brasil

Ute Jentsch, Truscha Niekerk, Xoliswa Mpumlwana
South African National Blood Service, Weltevreden Park, SA

Rita Lo, Lily Tang, Cheuk-kwong Lee, Hong Kong Red Cross BTS, Hong Kong

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Masahiro Satake, Japanese Red Cross, Tokyo

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Thank you very much for your attention

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