Enlargement of WHO Repository PC Transfusion Relevant Bacteria Reference Strains

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Presentation

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Definition TRBRS

Transfusion-Relevant Bacteria Reference Strains (TRBRS)

- **are deep frozen bacterial suspensions**
- **are ready to use, stable and shippable**
- **are defined in identity**
- **are defined in count [CFU/ml]**
  …allow “real life” spiking of blood components
  (i.e. artificial contamination with ~10 CFU/bag corresponding to 0.03 CFU/ml…)
- **are defined in growth characteristics in platelet concentrates**
  …grow up in PCs independent on donor properties
  …tested in PCs from at least 100 different donors

➢**TRBRS are dedicated to objective validation and assessment of both Pathogen Reduction Methods and Screening Methods.**

First WHO Int. Repository of TRBRS

Paul-Ehrlich-Institut
Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

A WHO Collaborating Centre
for Quality Assurance of Blood Products and
in vitro Diagnostic Devices

1st WHO International Repository of Platelet-Transfusion Relevant Bacterial Reference Strains
PEI code 8483/13
(Version 1.0 December 2012)

1. INTENDED USE

Bacterial contamination of platelet concentrates remains significant problem in transfusion with potential important clinical consequences, including death.

Until now, there have been no transfusion relevant bacterial reference strains available. The repository of platelet transfusion relevant bacteria is a microbiological reference material containing a precise number of viable bacterial cells. It is intended for use as a quantitative quality control sample for the standardization of validation and assessment of methods for improvement of microbial safety of platelet concentrates (PCs). The repository consists of 4 bacterial strains (*Staphylococcus epidermidis* PEI-B-P-06, *Streptococcus pyogenes* PEI-B-P-20, *Klebsiella pneumoniae* PEI-B-P-08, and *Escherichia coli* PEI-B-P-19) which were selected for their ability to replicate in PCs under routine storage conditions used in transfusion medicine. The panel members are prepared using a specially developed procedure which guarantees defined bacterial suspensions (deep frozen, ready to use, stable, shippable, defined in count of living cells). The microbiological identification of each batch of repository strains is confirmed by 16S rDNA sequencing. The panel is designed to allow objective validation of methods for Bacterial Screening in PCs under "real life" conditions, i.e. inoculating the PCs with a very low bacteria count (0.03 to 0.3 CFU/mL) followed by growth in the bag.

The repository has been evaluated in an international validation study which was organized by the International Society of Blood Transfusion (ISBT) Working Party on Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup on Bacteria. The WHO Expert Committee Biological Standardization (WHO ECBS) approved the adoption of preparations of the four bacteria strains mentioned above as a Repository for Platelet Transfusion Relevant Bacteria Reference Strains (RPTRBRS) during the annual meeting of 2010 (WHO/BS/10.2154).

2. UNITAGE

A defined unitage is assigned to the individual repository members; the details depend accessorily on the lot of the bacterial preparation. Each vial is labelled with complete information as demonstrated in Table 1.

* XX = lot number

Explanation of code:
- PEI: Paul Ehrlich Institute
- B: Blood (strain regards blood components)
- P: Platelets (strain is intended for the use in platelet concentrates)
- first number: number of bacterial strain
- second number: number of lot

(Example: PEI-B-P-06-08 stands for lot 8 of *Staphylococcus epidermidis* PEI-B-P-06)

Table 1 Unitage of bacterial suspensions / Labelling of the vials

Bacterial Strain	Lot
Staphylococcus epidermidis	PEI-B-P-06-XX*
Streptococcus pyogenes	PEI-B-P-20-XX*
Klebsiella pneumoniae	PEI-B-P-08-XX*
Escherichia coli	PEI-B-P-19-XX*

The mean value of bacterial count [CFU/mL] and the 95% confidence interval depends on the lot and will be provided with the product insert.

3. CONTENTS

Each vial closed with a screw cap contains 1.5 mL of living deep frozen bacteria suspended in tryptic soy broth and 10 % human serum albumin in saline (150 mM NaCl). The strains were characterized regarding their ability to grow up to high counts in PCs after low count spiking independent of donor's immune system.

3.1. IDENTITY

Results of genome sequencing using the MicroSeq 16S rDNA Bacterial Identification System are shown in table 2 (Appendix)

3.2. GROWTH IN PLATELET CONCENTRATES

The figures 1 -4 (Appendix) show the growth characteristics of the bacterial strains in pooled PCs (n = 4) at + 22 °C ± 2 °C after inoculation with < 10 CFU per bag (< 0.03 CFU/mL). The kinetics may be used for experiments to calculate the bacterial count at a defined time point.

4. STORAGE

The material is supplied deep frozen on dry ice and should be stored immediately below - 70 °C ± 5 °C after arrival. Check the vials immediately after arrival. If the samples show any sign of thawing, they must be discarded.

5. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The material is supplied on dry ice. Always handle dry ice with care and wear protective cloth or leather gloves whenever touching it. Avoid prolonged contact with the skin because it will cause injury similar to a burn.
The preparation contains viable, pathogenic bacteria and may lead to infections of personnel and/or microbial contamination of material and surrounding area. Therefore the samples should only be handled by

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1st WHO International Repository of Platelet Transfusion Relevant Bacteria Strains
PEI code 8483/13
Illustration of dilution of repository strains before spiking

Procedure of dilution:

1. Label n (n = number of tubes, depends on the calculated dilution steps in order to receive a final dilution of around 10 CFU per sample) tubes for dilution of the repository strain (e.g. *Staphylococcus epidermidis* PEI-B-P-06-XX; 1 vial for Dilution 1 = D1, 1 vial for D2, 1 vial for D3.....).
2. Prepare the dilution tubes with 9 mL each of s
3. Vortex the thawed vial of the repository st seconds immediately after unfreezing (as des
4. Transfer 1 mL of the stock (vial of reposit dilution 10^{-1}).
5. Discard the tip; cap the tube and vortex for 15
6. Take a new tip and transfer 1 mL out of the tube (D2: dilution 10^{-2}).
7. Vortex the dilution D2 for 15 seconds at highe
8. Continue this procedure up to the final dilution

Series of 10-fold dilutions:

Vortex stock (vial of repository strain) for 15 s
Start: 1 mL of stock
Add: + 9 mL NaCl
} Yield: 1(

Vortex dilution above (10^{-1}) for 15 seconds at
Carry-over: 1 mL of 10^{-1} (D1)
Add: + 9 mL NaCl
} Yield: 10

Continue up to the final dilution (calculat containing around 10 CFU per sample for low spiking.

Dilution series

et cetera if necessary

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Mean value of bacterial count [CFU/mL] and the 95% confidence interval

Bacterial Reference Strain		CFU/mL	
		Mean value	95 % confidence interval
Staphylococcus epidermidis	PEI-B-P-06-02-01	9,68E+05	9,43E+05 – 9,93E+05
Streptococcus pyogenes	PEI-B-P-20-02-01	2,49E+08	2,43E+08 – 2,55E+08
Escherichia coli	PEI-B-P-19-02-01	6,47E+06	6,24E+06 – 6,71E+06
Klebsiella pneumoniae	PEI-B-P-08-02-01	1,15E+06	1,13E+06 – 1,17E+06

Result of stability testing, 2015_01_27/JB/AS

Dr. Eva Spindler-Raffel

Scope of collaborative study

- **Bacterial growth in platelet concentrates has to be demonstrated for 11 new candidate strains**
- **4 WHO strains as reference (comparability)**
- **Under real-life conditions**
  -> Low spiking directly into PC-bags: 10 to 25 cfu/bag
  (Tested in 3 PC bags per strain, 14 labs)
- **3 sampling days (2, 4, 7) -> growth kinetics**
- **Growth independent of donor influence (WHO-regions, up to 130 different donors per strain)**

Enlargement of WHO Repository: Candidates

Selected candidate bacteria for Enlargement study

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

Enlargement Candidates	
5.	***Bacillus thuringiensis* spores**
6.	***Bacillus cereus* spores**
7.	***Enterobacter cloacae***
8.	***Morganella morganii***
9.	***Proteus mirabilis***
10.	***Pseudomonas fluorescens***
11.	***Salmonella choleraesuis***
12.	***Serratia marcescens***
13.	***Staphylococcus aureus***
14.	***Streptococcus dysgalactiae***
15.	***Streptococcus bovis* (reclassified: *Streptococcus gallolyticus*)**

International Validation Study: Participants

Austria	**Christian Gabriel, Susanne Süßner** Austrian Red Cross, Blood Centre Linz
Canada	**Dana Devine, Sandra Ramirez-Arcos** Canadian Blood Service, Ottawa
England	**Carl McDonald, Kate Aplin** NHS Blood and Transplant, London
Germany	**Erhard Seifried, Kai Hourfar** German Red Cross, Frankfurt/Main
	Birgit Gathof, Melanie Stoermer University Hospital Cologne, Transfusion Medicine
	Axel Seltsam, Bernd Lambrecht German Red Cross Blood Service NSTOB, Springe
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México	**Julieta Rojo, Dr. Gabriela Ibañez- Cervantes** Centro Nacional de la Transfusion Sanguínea
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The Netherlands	**Dirk de Korte, Jan Marcelis** Sanquin Blood Supply Foundation; Elisabeth Hospital, Tilburg
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	Roslyn Yomtovian†, Michael R. Jacobs Case Western Reserve University, Cleveland *Louis Stokes Cleveland Veterans Affairs Medical Center*
Pakistan	**Zainab Mukhtar, Shaheen Sharafat** Dow Safe Blood Transfusion Services, Dow Medical College, DUHS Karachi

Steering committee

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Richard Benjamin
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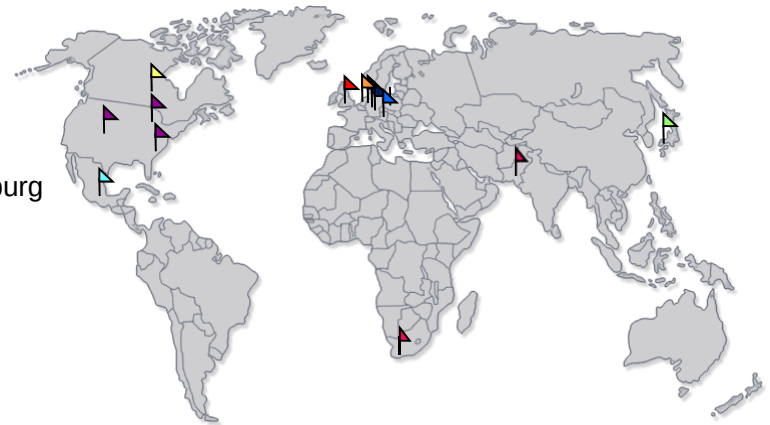
(corresponding address)

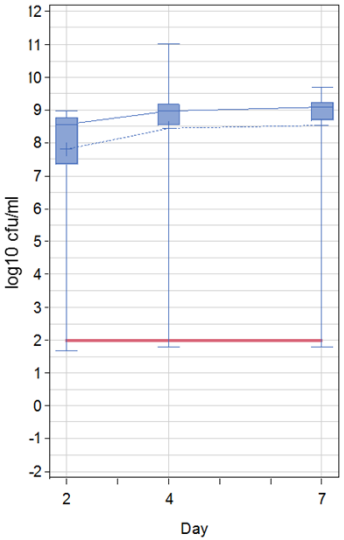


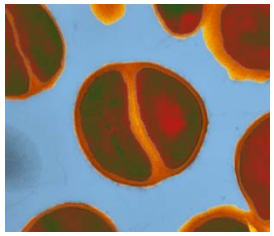
Lot of Lab-Work

Photo: Section 1/3 Microbial Safety, PEI

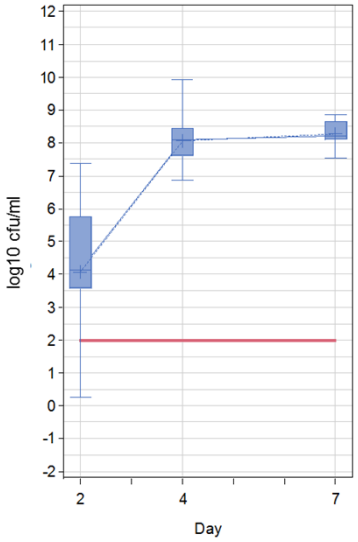
WHO strains confirmed

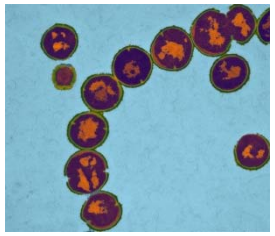
Klebsiella pneumoniae PEI-B-P-08

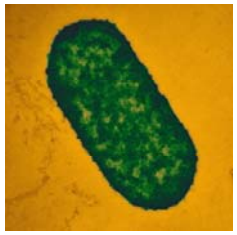




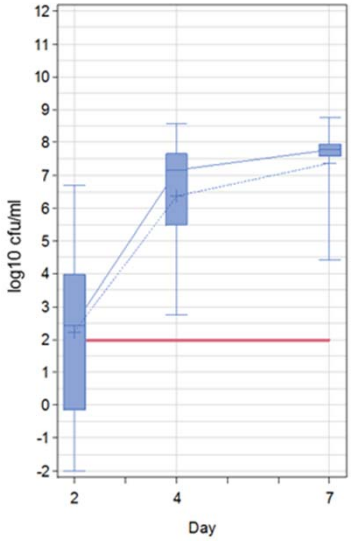
Escherichia coli PEI-B-P-19

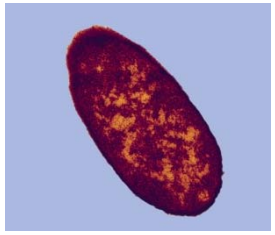




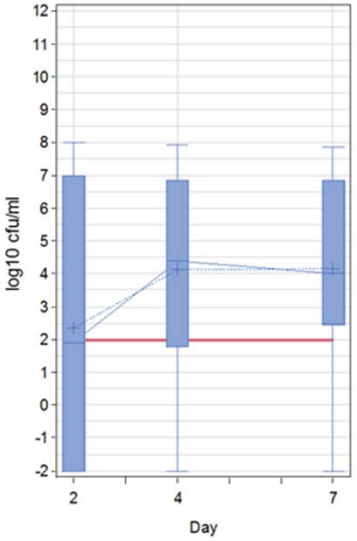


Staphylococcus epidermidis PEI-B-P- 06



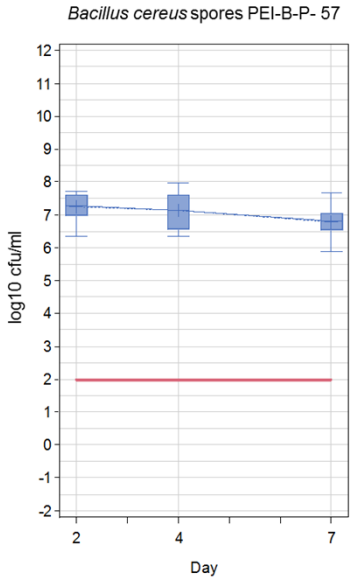


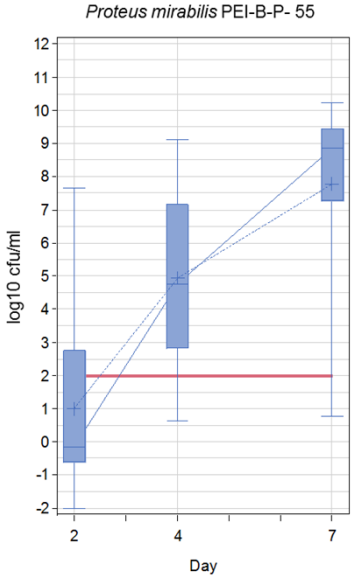
Streptococcus pyogenes PEI-B-P-20

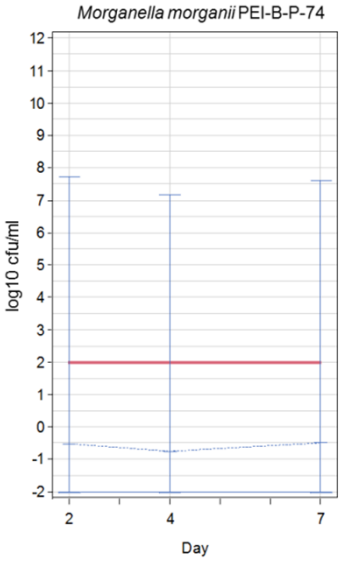


Electron microscopy: Klaus Boller, Regina Eberle, PEI

Different growth kinetics



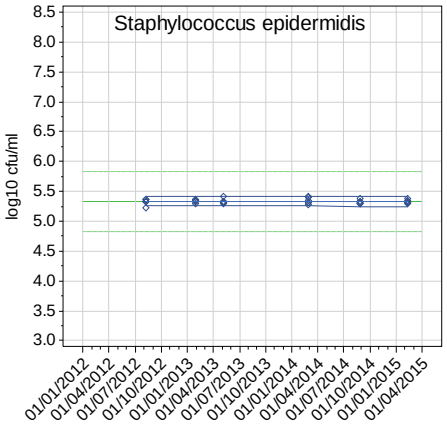


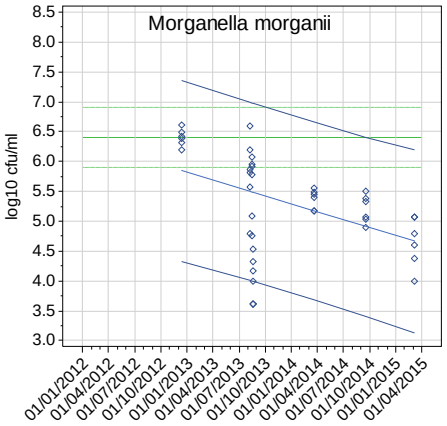


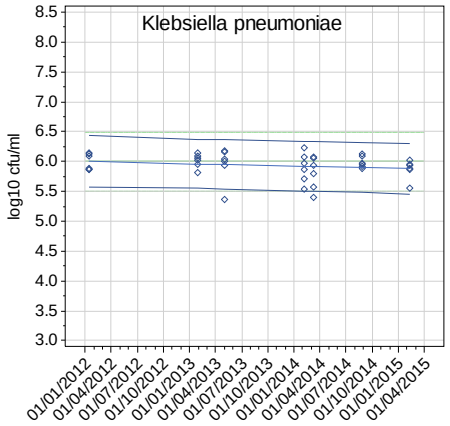
Box-and-Whisker plots for growth:
continuous line connecting the median values per day; dotted line connecting mean values

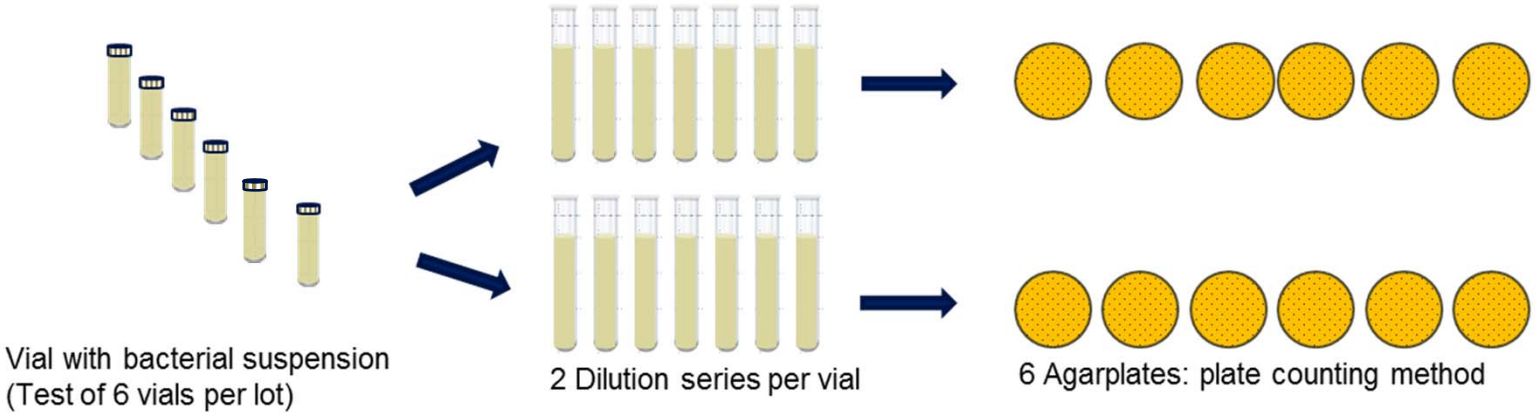
Poster Presentation P-421 and P-432

Test of stability

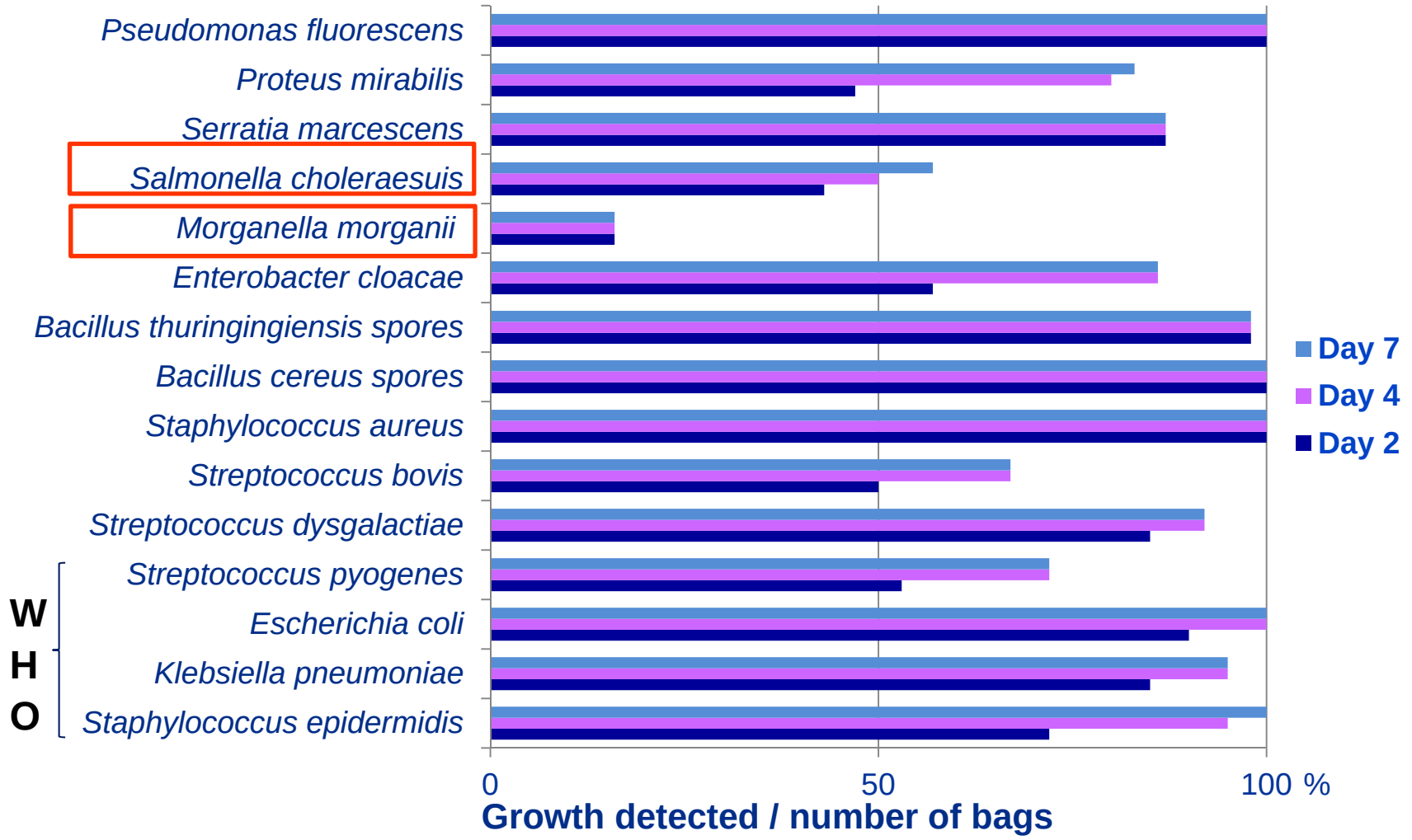








Growth rates per Sampling Day



Summary and Outlook

- All participants received the deep frozen bacteria strains in good condition without any complaint. As in the first study deep frozen, pathogenic bacteria strains could be shipped worldwide without any difficulties.
- The tested inocula proliferated well and were successfully used for spiking. The bacterial identification performed by the study partners complied with the ID of PEI. The results of bacteria counting of all participants are homogenous since the measured divergence factors represent an acceptable value in the estimation of high bacteria cell counts.
- The results of the four strains of the existing WHO Repository are equivalent to the first study. (spiking of 10 to 25 CFU per PC unit)
- Growth for *Salmonella choleraesuis* was lower than for other strains and showed a high variability among participants
- Morganella morganii failed to grow beyond that amount of bacteria in the initial inoculation.

Next steps:

- Final report and proposal for strain selection to WHO
- Paper in Vox sang

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Thank you very much for your attention !

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