WHO International Repository of Red Blood Cell Transfusion-Relevant Bacterial Reference Strains PEI code 11167/16, 11180/20, 11181/20, 11182/20, 11183/20 (Version 1.0 January 2020)

1. INTENDED USE

Blood transfusion is associated with a risk of transmission of infectious diseases due to its human origin. Consequently, several measures like shelf-life reduction of blood components, first aliquot diversion or effective skin disinfection were implemented to reduce the risk of bacterial transmission. In addition, new methods to detect or eliminate potential contaminants in blood components were developed or are currently under review. The validation of these new techniques or approaches requires both blood (components) as a matrix and microorganisms that represent typical contaminants.

The repository of red blood cell transfusion-relevant bacteria consists of aliquots of microbiological reference strains containing a defined number of viable bacterial cells. They are intended as a quantitative control sample for a standardized validation and evaluation of methods for improvement of microbial safety of red blood cells (RBCs).

The repository consists of the following organisms: *Listeria monocytogenes* PEI-B-E-199, *Serratia liquefaciens* PEI-B-E-184, *Yersinia enterocolitica* PEI-B-E-105 and PEI-B-E-176, and *Pseudomonas fluorescens* PEI-B-P-77 from the Repository for Platelet Transfusion-Relevant Bacteria Reference Strains (WHO/BS/2015.2269).

The panel members are manufactured following a quality controlled protocol which guarantees the defined quantity of the bacterial suspensions. The microbiological identification of each strain is confirmed by colony morphology, Gram staining and 16S rDNA sequencing. The panel is designed to allow validation of methods for bacterial safety of RBC concentrates under “real life” conditions, i.e. inoculation of RBC units with a very low bacterial count (0.03 to 0.3 CFU/mL RBC suspension) with subsequent growth in the bag.

The strains were extensively tested for their ability to grow in RBCs under routine cold storage conditions used in transfusion medicine. The study was organized by the International Society of Blood Transfusion (ISBT) Working Party on Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup on Bacteria and the Paul-Ehrlich-Institut (PEI). The WHO Expert Committee Biological Standardization (WHO ECBS) approved the adoption of the preparations of the bacterial strains mentioned above as Repository for Red Blood Cell Transfusion-Relevant Bacteria Reference Strains during the annual meeting of 2019 (WHO/BS/2019.2377).

The panel of reference strains is listed in Table 1. All relevant information and the order form are provided for download on the PEI website:
http://www.pei.de/EN/information/license-applicants/standard-and-referencematerials/who

Table 1: Red Blood Cell Bacteria Panel composition

PEI Code number	Bacterial species	ID
11180/20	*Listeria monocytogenes*	PEI-B-E-199
11181/20	*Serratia liquefaciens*	PEI-B-E-184
11182/20	*Yersinia enterocolitica*	PEI-B-E-105
11183/20	*Yersinia enterocolitica*	PEI-B-E-176
11167/16	*Pseudomonas fluorescens*	PEI-B-P-77

2. Labelling of the vials

Each vial is labelled as followed (e.g. *L. monocytogenes*):

PEI-B-E-199-XX

Explanation of code:
PEI: Paul-Ehrlich-Institute
B: Blood (strain for blood components)
E: Erythrocyte (intended for use in red blood cell concentrates)
P: Platelet (intended for use in platelet concentrates)
digit: PEI internal number of bacterial strain
XX: lot number

For each lot, the package insert provides the mean value of the bacterial count [CFU/mL] and the 95% confidence interval, an antibiogram and the result of a growth control test.

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3. CONTENTS

Each vial is closed with a screw cap and contains 1.5 mL of viable deep frozen bacteria suspended in tryptic soy broth and 10 % human serum albumin (solved in 150 mM saline) as a cryoprotectant.

4. STORAGE

The material is supplied deep-frozen and delivered on dry ice. Upon arrival, content should be stored immediately at -75°C ±5°C. Check vials immediately after arrival. If the samples show any sign of thawing, no guarantee can be given on the predetermined live count of the bacterial strains.

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 whenever touching it. Avoid prolonged contact with the skin, as it will cause injury similar to a burn.

The preparation contains viable, potentially pathogenic bacteria that may lead to infections if not handled properly. Therefore, samples should only be operated by experienced laboratory staff qualified and trained in handling of infectious material. The samples should be used according to your laboratory's safety procedures, which should include wearing of protective clothing (gloves, lab coat, etc.) and avoiding the formation of aerosols. Please refer to national safety guidelines. Care should be exercised in opening vials because of residual liquid in the cap (do not centrifuge the vials).

The residual liquid samples as well as any material that had been in contact with the bacterial suspension (e.g. vials, pipette tips, gloves, lab coat) have to be treated with appropriate methods before being discarded. I.e. to be autoclaved at least at +121°C (250°F) at 15 psi (100 kPa) above atmospheric pressure for 30 min. All work involving the use and disposal of the provided material has to be performed in accordance with all applicable laws and regulations.

6. USE OF MATERIAL

Thawing process. To assure the viability of bacteria, the cold chain must not be interrupted. Before use, transfer the vials directly from the deep freezer (-75°C ±5°C) and defrost the vials at +37°C ±2°C for 10 minutes (prevent water bath). If ice crystals are still visible, the vial should be carefully warmed up in the hand until all crystals have melted. Vortex the vial for 15 seconds to evenly distribute the bacteria in the solution.

Dilution. Normal contamination of a RBC unit is usually characterized by a low initial bacterial concentration with less than 100 bacteria per bag. In order to mimic such "real-life" scenarios, stocks of the bacterial reference strains have to be serially diluted. For this, carefully open the screw cap and dilute the bacterial suspension with sterile saline (e.g. 0.85% NaCl) up to the desired concentration. Make sure that a) the dilution series of the stock tubes is prepared immediately after thawing the stock suspension, b) the stock suspension and each dilution is intensively vortexed (highest speed) for 15 seconds and c) pipette tips are changed after each step. *Note: All dilution steps should be performed aseptically.*

Artificial contamination. Prior the spiking, please connect a blood bag with a Luer-lock connection device, e.g. a short tube using Sterile Connecting Device and remove 5 mL of the bag content using a sterile syringe, but do **not** discard it. Use a second sterile syringe and inoculate 1 mL of the previously prepared dilution (<100 CFU/ml) through the same port into the bag. Avoid any entry of air into the unit during the inoculation process. Finally, inject the previously removed 5 mL RBC sample to flush the tube segment of the bag and close the Luer-lock port. Incubate the contaminated RBC units at routine conditions following national guidelines.

Note: Close the tube by clamp whenever the Luer-lock device (e.g. before connection) is opened. The procedure described is used to overcome the "dead volume" of the tube, i.e. to bring the inoculum directly into RBCs main volume. Additionally, bacteria attached to the inner surface of the tube shall be detached by this procedure.

Sampling. In order to take a sample from the spiked RBC unit, please stick to the procedure described in the "artificial contamination" section.

Figure 1 A-E (p. 5) shows the growth behaviour of the reference strains inoculated with 10-25 CFU per RBC bag and subsequent storage at +2-6°C. The kinetics may be used as guidance for experiments to estimate the bacterial count at a certain time point. More comprehensive growth kinetics are summarized in the WHO study report (WHO/BS/2019.2377).

Note: Growth behaviour of the strains depends on the initial inoculum and the RBC unit composition and may vary from the examples shown in Fig.1.

7. STABILITY

The reference materials are stored at PEI within assured, temperature-controlled storage facilities. The reference material must be stored at -75°C ±5°C. Enumeration and stability testing is performed by PEI routinely during storage. The stability depends on the bacterial strain and batch.

8. CITATION

In any circumstance where the recipient publishes a reference to PEI materials, the title of the preparation, the PEI code number, and the name and address of PEI should be cited correctly. The intended use of the material is spiking directly after thawing. No further cultivation steps are required. If there will be further cultivation and subcultures, this has to be noted in the protocols and the publications.

9. ACKNOWLEDGEMENTS

We thank all participating laboratories and all individuals who were involved in the collaborative studies.

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10. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability, stability and use of the material provided or other aspects of our service. Please send any comments to whoccivd@pei.de.

11. REFERENCES

(1) WHO ECBS report 2015, WHO/BS/2015.2269, Eva Spindler-Raffel, Kay-Martin Hanschmann, Thomas Montag-Lessing†, and the Collaborative Study Group. Collaborative Study to Enlarge the First WHO Repository of Platelet Transfusion-Relevant Bacterial Reference Strains. http://www.who.int/.../BS2269_Extension_Repos_Bacteria_Strains1.pdf

(2) WHO ECBS report 2010 (WHO/BS/10.2154). Thomas Montag, Kay Martin Hanschmann, Melanie Störmer: Report on the International Validation Study on Bacteria Standards (Transfusion-Relevant Bacterial Strain Panel) and Proposal for a validation study for enlargement of the transfusion-relevant bacterial strain panel" http://www.who.int/biologicals/expert_committee/WHO_BS10.2154_Bacteria_Study_2.pdf

(3) WHO ECBS report 2009. Development of WHO Biological Reference Preparations for Blood Safety-related in vitro Diagnostic Tests: Blood-borne bacteria panel, page 11. Report of the 2nd meeting with the WHO Collaborating Centers for Biological Standards and Standardization, 17-18 February 2009.

(4) Paul-Ehrlich-Institut, Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel, WHO Collaborating Centre for Quality Assurance of Blood Products and in vitro Diagnostics Devices- Annual Report 2009. http://www.pei.de/SharedDocs/Downloads/institut/who-cc/who-jahresbericht-2009,templateId=raw,property=publicationFile.pdf/who-jahresbericht-2009.pdf

(5) Montag, T. Establishment of a Transfusion-Relevant-Bacterial Strain Panel. Transfusion Today 2010; 84:8.

(6) Störmer, M., Arroyo, A., Brachert, J. , Carrero, H., Devine, D., Epstein, J. S., Gabriel, C., Gelber, C., Goodrich, R., Hanschmann, K.-M., Heath, D. G., Jacobs, M. R., Keil, S., de Korte, D., Lambrecht, B., Lee,C.-K., Marcelis, J., Marschner, S., McDonald, C., McGuane, S., McKee, M., Mueller, T., Muthivhi, T., Pettersson, A., Radziwon, p., Ramirez-Arcos, S., Reesink, H. W., Rojo, J., Rood, I., Schmidt, M., Schneider, C. K., Seifried, E., Sicker, U., Wendel, S., Wood, E. M., Yomtovian, R. A., Montag, T. International Validation Study on Blood Transfusion Bacteria Standards Relevant to Transfusion Medicine-ISBT Working Party Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup on Bacteria. Vox Sang 2011

(7) Montag, T.: ISBT International Validation Study on Transfusion-Relevant Bacterial Strain Panel. SOGAT XXI 28-29 May 2009, Brussels, Belgium.

12. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependent on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of agreement or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

13. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	liquid suspension		
Fire hazard	None		
Chemical Properties			
Stable	No	Corrosive	No
Hygroscopic	No	Oxidising	No
Flammable	No	Irritant	No
Other	CONTAINS PATHOGENIC VIABLE BACTERIA AND HUMAN SERUM ALBUMIN		
Handling	See caution, section 4		
Toxicological properties			
Effects of inhalation	Avoid – *contains pathogenic bacteria*		
Effects of ingestion	Avoid – *contains pathogenic bacteria*		
Effects of skin absorption:	Avoid – *contains pathogenic bacteria*		
Suggested First Aid			
Inhalation	Seek medical advice - *contains pathogenic bacteria*		
Ingestion	Seek medical advice - *contains pathogenic bacteria*		
Contact with eyes	Wash thoroughly with water. Seek medical advice – *contains pathogenic bacteria*		
Contact with skin	Disinfection of the affected area with a disinfectant suitable for skin. Seek medical advice – *contains pathogenic bacteria*		
Action on Spillage			
Spillage of vial contents should be absorbed with material soaked in appropriate disinfectant. Rinse area with a suitable disinfectant followed by water. Absorbent materials used to remove spillage should be treated as biological waste.			
Method of Disposal			
The residual liquid samples as well as any material that has been in contact with the bacterial suspension (e.g. vials, pipette tips, gloves, lab coat) must be autoclaved at least at +121°C (250°F) at 15 psi (100 kPa) above atmospheric pressure for 30 min before being discarded.			

14. DRY ICE MATERIAL SAFETY SHEET

Physical properties	
Physical appearance	Solid
Fire hazard	None
Chemical Properties	
Chemical Name	Carbon Dioxide
Stable	YES
Conditions To Avoid	Moisture
Materials To Avoid	Carbonic acid/salt/corrosive chemicals
Hazardous polymerization Occurrence	NO
Handling	See caution, section 4
Toxicological properties	
Effects of inhalation	Avoid - *causes hyperventilation, headaches, dyspnoea*
Effects of ingestion	Avoid – *may cause cryogenic burns*
Effects of skin absorption:	Avoid – *may cause cryogenic burns*
Suggested First Aid	
Inhalation	Remove to fresh air - *Seek medical advice to supplement oxygen if not breathing.*
Ingestion	Seek medical advice - *wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel.*
Contact with skin	Seek medical advice - *may cause cryogenic burns.*
Contact with eyes	Seek medical advice - *remove any contact lenses. Immediately flush eyes with plenty of water.*
Action on Spillage and Method of Disposal	
Ventilate indoor areas well to avoid hazardous CO_2 concentrations. Ventilate area well and avoid contact. CO_2 is heavy gas and will remain in low spots. Spilled frozen tissues should be flooded with tepid water. Do not use hot water. Do not dispose of residual CO_2 in compressed gas cylinders.	

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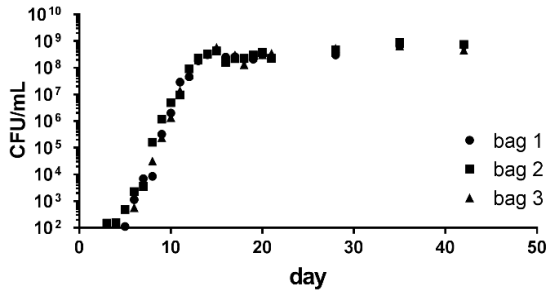
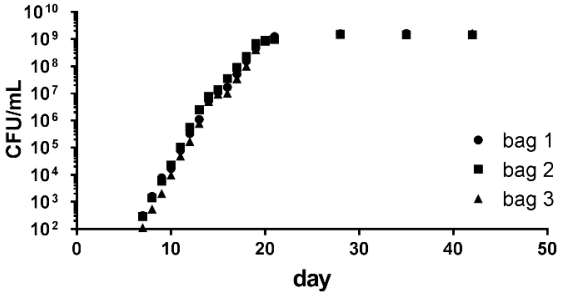
Figure 1: Growth kinetics of the reference strains spiked in three RBC units each. Bags were inoculated with 10-25 CFU/bag and stored at +2-6°C. Samples were taken in a daily interval upon reaching stationary phase.[1]

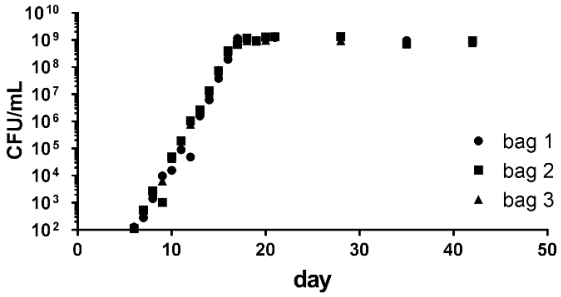
A) *Pseudomonas fluorescens* PEI-B-P-77



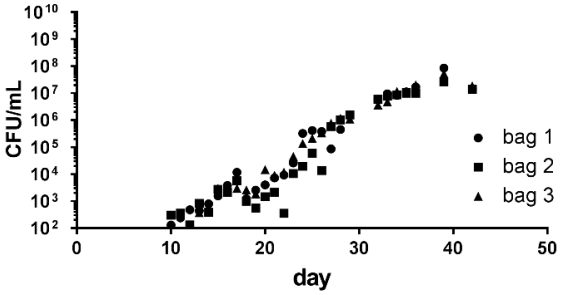
D) *Yersinia enterocolitica* PEI-B-E-176



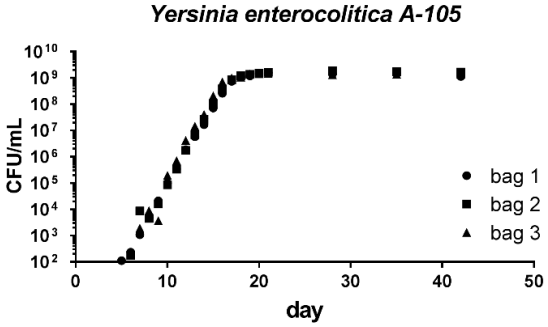
B) *Serratia liquefaciens* PEI-B-E-184



E) *Listeria monocytogenes* PEI-B-E-199



C) *Yersinia enterocolitica* PEI-B-E-105



1) Ramirez-Arcos, Sandra, et al. "Bacterial safety of blood components–a congress review of the ISBT transfusion-transmitted infectious diseases working party, bacterial subgroup." ISBT Science Series 14.2 (2019): 239-247.

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