



**WHO International Repository of
Platelet-Transfusion Relevant Bacterial Reference
Strains**

**PEI code 8483/13, 11162/16, 11163/16, 11164/16,
11165/16, 11166/16, 11167/16, 11168/16, 11169/16,
11170/16, 11171/16
(Version 2.0 May 2016)**

1. INTENDED USE

Bacterial contamination of platelet concentrates remains an important problem in transfusion medicine, which can lead to severe infections, including lethality.

The repository of platelet transfusion-relevant bacteria consists of aliquots of microbiological reference strains containing a defined number of viable bacterial cells. It is intended for use as a quantitative quality control sample for standardized validation and evaluation of methods for improvement of microbial safety of platelet concentrates (PCs).

The first repository consisted 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). In a second step the bacterial panel was extended to 14 strains selected for their ability to replicate in PCs under routine storage conditions used in transfusion medicine. The panel members are prepared using a special procedure, which guarantees the stability of quantitatively defined bacterial suspensions (deep frozen, ready to use, stable, shippable, defined in count of viable cells). The microbiological identification of each batch of repository strains is confirmed by 16S rDNA sequencing. The panel is designed to allow quantitative validation of methods for bacterial screening in PCs under "real life" conditions, i.e. inoculating the PCs with a very low bacterial count (0.03 to 0.3 CFU/mL platelet suspension) with subsequent growth in the bag or testing of pathogen reduction technologies.

The repository has been evaluated in two international validation studies 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 Platelet Transfusion Relevant Bacteria Reference Strains (PTRBRS) during the annual meeting of 2010 (WHO/BS/10.2154) and the extended version in 2015 (WHO/BS/2015.2269).

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>

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PEI Code number	Table 1	
	Bacterial species*	
8483/13	PEI-B-P-06	<i>Staphylococcus epidermidis</i> *
	PEI-B-P-20	<i>Streptococcus pyogenes</i> *
	PEI-B-P-08	<i>Klebsiella pneumoniae</i> *
	PEI-B-P-19	<i>Escherichia coli</i> *
11162/16	PEI-B-P-57	<i>Bacillus cereus</i> (spore suspension)*
11163/16	PEI-B-P-07	<i>Bacillus thuringiensis</i> (spore suspension)*
11164/16	PEI-B-P-43	<i>Enterobacter cloacae</i> *
11165/16	PEI-B-P-91	<i>Morganella morganii</i> *
11166/16	PEI-B-P-91	<i>Proteus mirabilis</i> *
11167/16	PEI-B-P-77	<i>Pseudomonas fluorescens</i> *
11168/16	PEI-B-P-56	<i>Serratia marcescens</i> *
11169/16	PEI-B-P-63	<i>Staphylococcus aureus</i> *
11170/16	PEI-B-P-61	<i>Streptococcus bovis</i> *
11171/16	PEI-B-P-71	<i>Streptococcus dysgalactiae</i> *

* Antibiotic susceptibility testing results of each bacterial strain will be provided with the package insert.

2. Labelling of the vials

Each vial is labelled as demonstrated in Table 2.

Table 2 Labelling of the vials	
Bacterial Strain	Lot
<i>Staphylococcus epidermidis</i>	PEI-B-P-06-XX*
<i>Streptococcus pyogenes</i>	PEI-B-P-20-XX*
<i>Klebsiella pneumoniae</i>	PEI-B-P-08-XX*
<i>Escherichia coli</i>	PEI-B-P-19-XX*

* XX = lot number

Explanation of code:

- PEI: Paul Ehrlich Institute
 - B: Blood (strain for blood components)
 - P: Platelets (strain is intended for the use in platelet concentrates)
 - first digit: number of bacterial strain
 - second digit: batch number
- (Example: PEI-B-P-06-03 stands for batch 3 of *Staphylococcus epidermidis* PEI-B-P-06)

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Web: <http://www.pei.de>



The mean value of bacterial count [CFU/mL] and the 95% confidence interval depends on the batch and will be provided with the product insert as well as the result of the antibiotic susceptibility test.

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 in 150 mM saline solution. The strains were characterized regarding their ability to grow up to high counts in PCs after low count spiking independent of the donor.

3.1. IDENTITY

The microbiological species of each batch is confirmed by 16S rDNA gene sequencing and is provided in the package insert.

3.2. GROWTH IN PLATELET CONCENTRATES

The figures 1 to 4 (Appendix) show the growth characteristics of the first four bacterial strains (*Staphylococcus epidermidis* PEI-B-P-06, *Streptococcus pyogenes* PEI-B-P-20, *Klebsiella pneumoniae* PEI-B-P-08, *Escherichia coli* PEI-B-P-19) 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. The results of the enlarged panel are documented in the WHO-report WHO/BS/2015.2269.

4. STORAGE

The material is supplied deep frozen and delivered on dry ice. Upon arrival it should be stored immediately at - 70 °C ± 5 °C. Check vials immediately after arrival. If the samples show any sign of thawing, results are invalid and 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 whenever touching it. Avoid prolonged contact with the skin as 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 contact materials and the surrounding area. Therefore, the samples should only be operated by experienced laboratory personnel 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 gloves 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 has 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

The material is supplied deep frozen and should be stored immediately at -70 °C ± 5 °C after arrival. To assure the viability of bacteria the cold chain should not be interrupted. Before use, transfer the vials directly from the deep freezer to a dry thawing approach (prevent water bath) and defrost the vials at + 37 °C ± 2 °C for 10 minutes. 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 be sure that bacteria are evenly distributed. Carefully open the screw cap.

Dilution steps should be performed in sterile saline (0.85 %) down to a final bacterial count of 10 to 25 CFU/mL. 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.

For **artificial contamination** of blood products thaw the vial as described and dilute the sample down to a final concentration of 10 to 25 CFU/mL. Combine the 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 final dilution through the same port into the bag. Avoid any entry of air into the unit during the inoculation process. Finally, add the previously removed 5 mL sample to flush the tube segment of the bag and close the Luer-lock port to incubate the contaminated units at defined conditions and use the technology for detection or reduction.

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

7. STABILITY

The reference materials are held at PEI within assured, temperature-controlled storage facilities. The reference material must be stored at - 70 °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.

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., Marcellis, 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 BACTERIA	PATHOGENIC AND HUMAN	VIABLE 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 taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat 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.	

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.	

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



15. APPENDIX

Figure 1: Growth of *Staphylococcus epidermidis* PEI-B-P-06 in pooled platelet concentrates at 22.5 °C with agitation (n=4) (PEI, data unpublished)

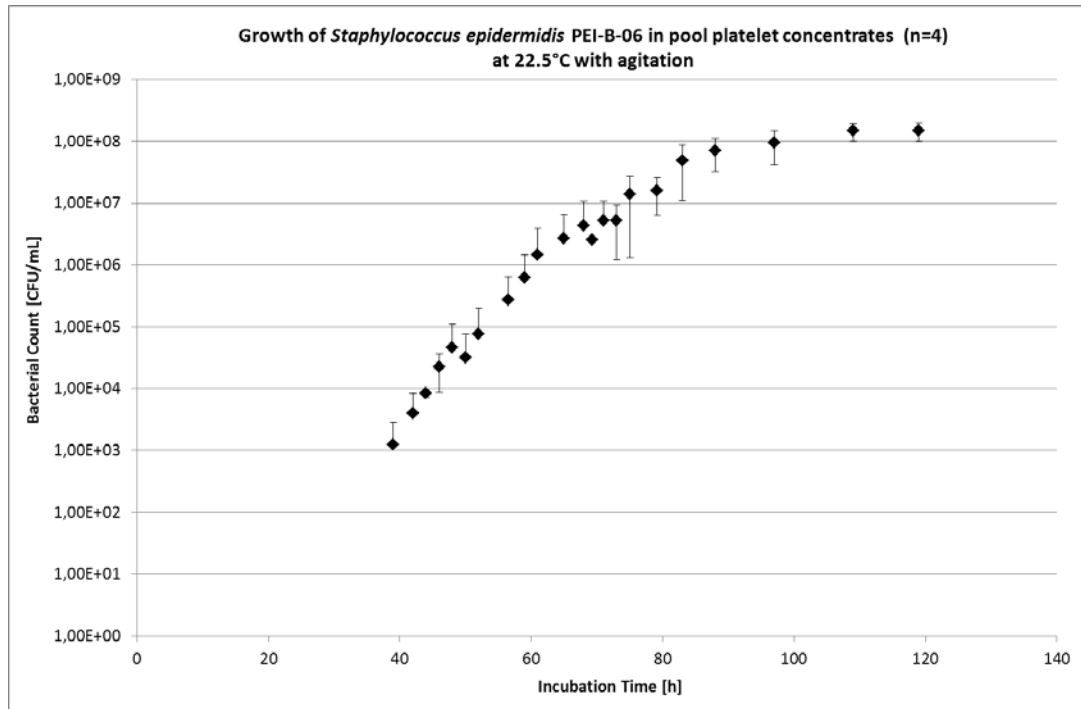


Figure 2: Growth of *Streptococcus pyogenes* PEI-B-P-20 in pooled platelet concentrates at 22.5 °C with agitation (n=4) (PEI data unpublished)

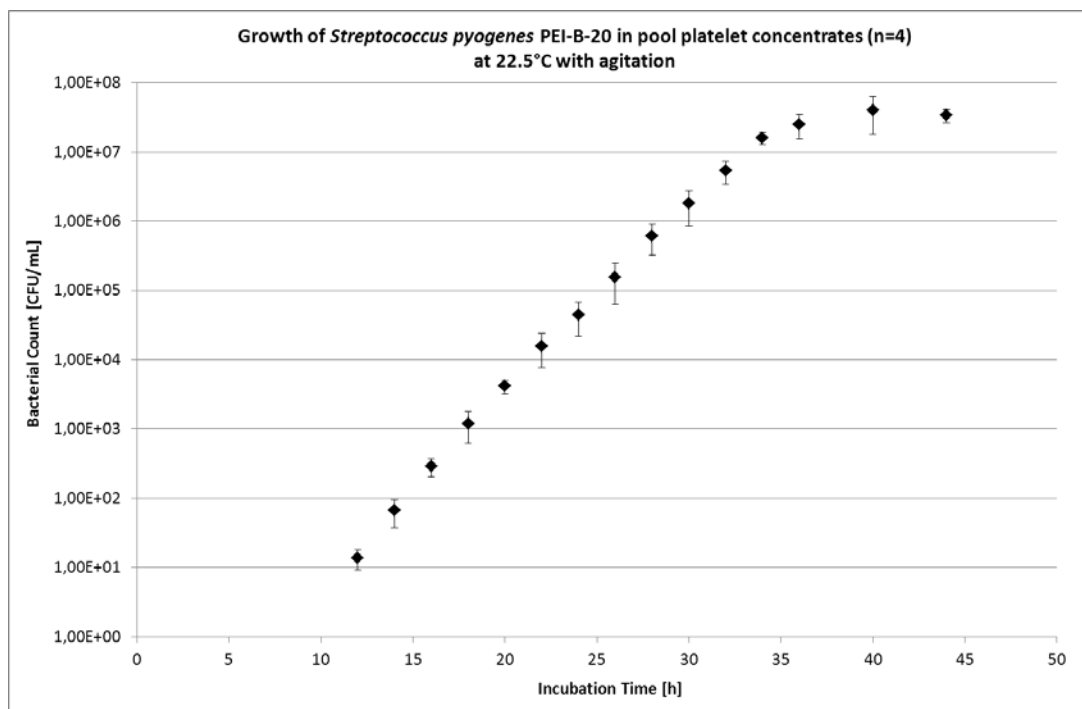




Figure 3: Growth of *Klebsiella pneumoniae* PEI-B-P-08 in pooled platelet concentrates at 22.5 °C with agitation (n=4), (PEI data unpublished)

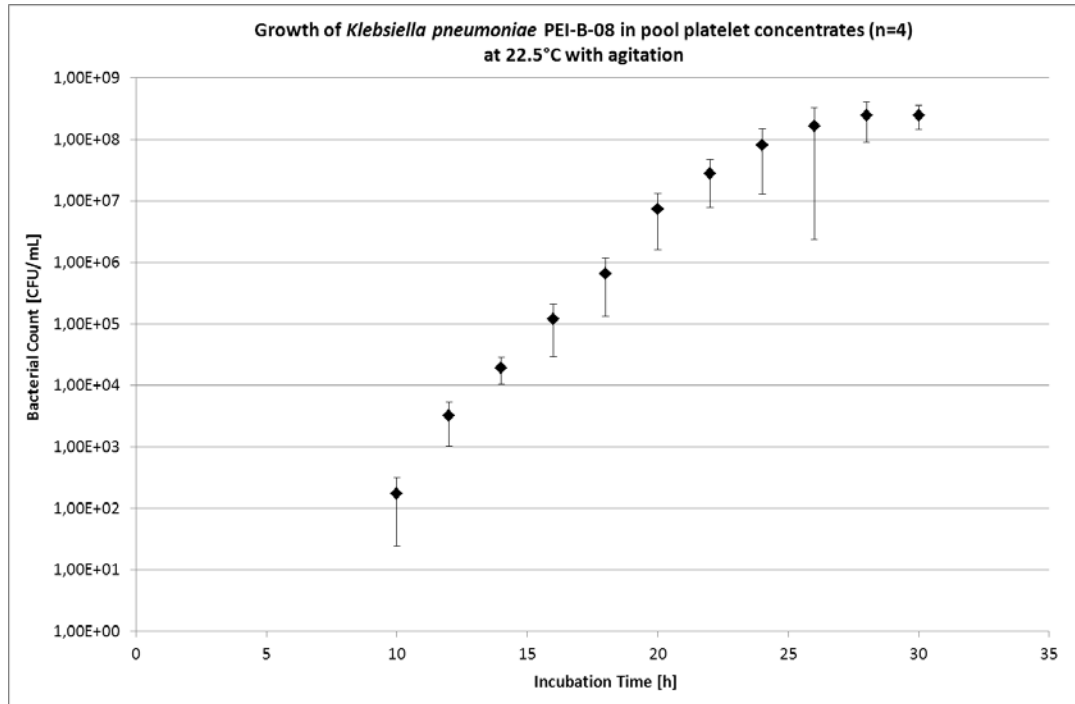


Figure 4: Growth of *Escherichia coli* PEI-B-P-19 in pooled platelet concentrates at 22.5 °C with agitation (n=4), (PEI data unpublished)

