



**UNIKLINIK
KÖLN**

IPFA/PEI 23rd International Workshop
Surveillance and Screening of Blood Borne
Pathogens, 25.-26.05.2016 Lisbon, Portugal

Bacterial Safety of Blood Products Bacterial Detection & Reduction Technologies

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Platelet Concentrates are predominantly affected

- storage at $22 \pm 2^\circ \text{C}$ promotes growth
- represent good growth medium

Sources for bacterial contamination

- donor arm derived
- donor bacteraemia
- contaminated equipment
- during blood processing

Bacterial Species and Counts

- gram-positive organisms found on skin are the most frequent contaminants
- $>10^5$ CFU/ml cause severe transfusion reactions (STRs) (*Jacobs M.R., et al. Clin Infect Dis. 2008;46:1214-20*)



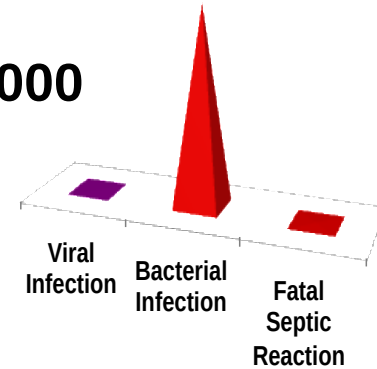
Bacterial contaminated platelet concentrate (*Klebsiella pneumoniae*)



Facts about Bacterial Contamination of Blood Products

Incidence of bacterially contaminated PCs

- Transfusion transmitted **bacterial infection**: **1:2.000 to 1:3.000**
- Fatal septic reactions due to bacterial contaminated PCs: **1:100.000 to 1:500.000**
- Transfusion transmitted viral infection: 1:1.000.000



- The incidence for bacterial contamination of PCs is unknown as a result of underdetection and underreporting!

*M. R. Jacobs. Case Western Reserve University, Cleveland, OH.
Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance.*

Hong H., et al. Blood 2016;127:496-502





- PCs were tested in 100% by aerobic culture (Bact/ALERT, eBDS) 24h after collection and at **the time of issue** by plate assay.
- 20 PCs were bacterially contaminated but none of the septic transfusion reaction (STR) was reported. In contrast, 284 other transfusion reactions were reported.

Bacterial Isolates of STRs (N=20)					
2007-2013	Total	PPC	APC	STR	Severity
<i>Staphylococcus epidermidis</i>	11	3	8	1 APC (5×10^6 cfu/ml)	moderate
<i>Staphylococcus warneri</i>	2		2	1 APC (5×10^6 cfu/ml)	fatal
<i>Staphylococcus aureus</i>	1	1		1 PPC (1×10^5 cfu/ml)	moderate
<i>Streptococcus gallolyticus</i>	2		2		
<i>Streptococcus sanguinis</i>	1		1		
<i>Streptococcus oralis</i>	2		2	2 APCs (7×10^6 cfu/ml) (1×10^6 cfu/ml)	life-threat. severe
<i>Acinetobacter baumannii</i>	1		1		





Interventions to improve bacterial safety

- optimized skin disinfection
- pre-donation sampling (diversion)
- quality control of PCs
- reduction of shelf-life
- introduction of rapid detection methods
- introduction of pathogen reduction/inactivation technologies (PRT;PIT)

Represents an unresolved problem in blood safety

- different bacterial growth properties during storage after low initial count
- spontaneous release of PCs
- implementation of detection and PRT in routine may be complicated
- additional costs





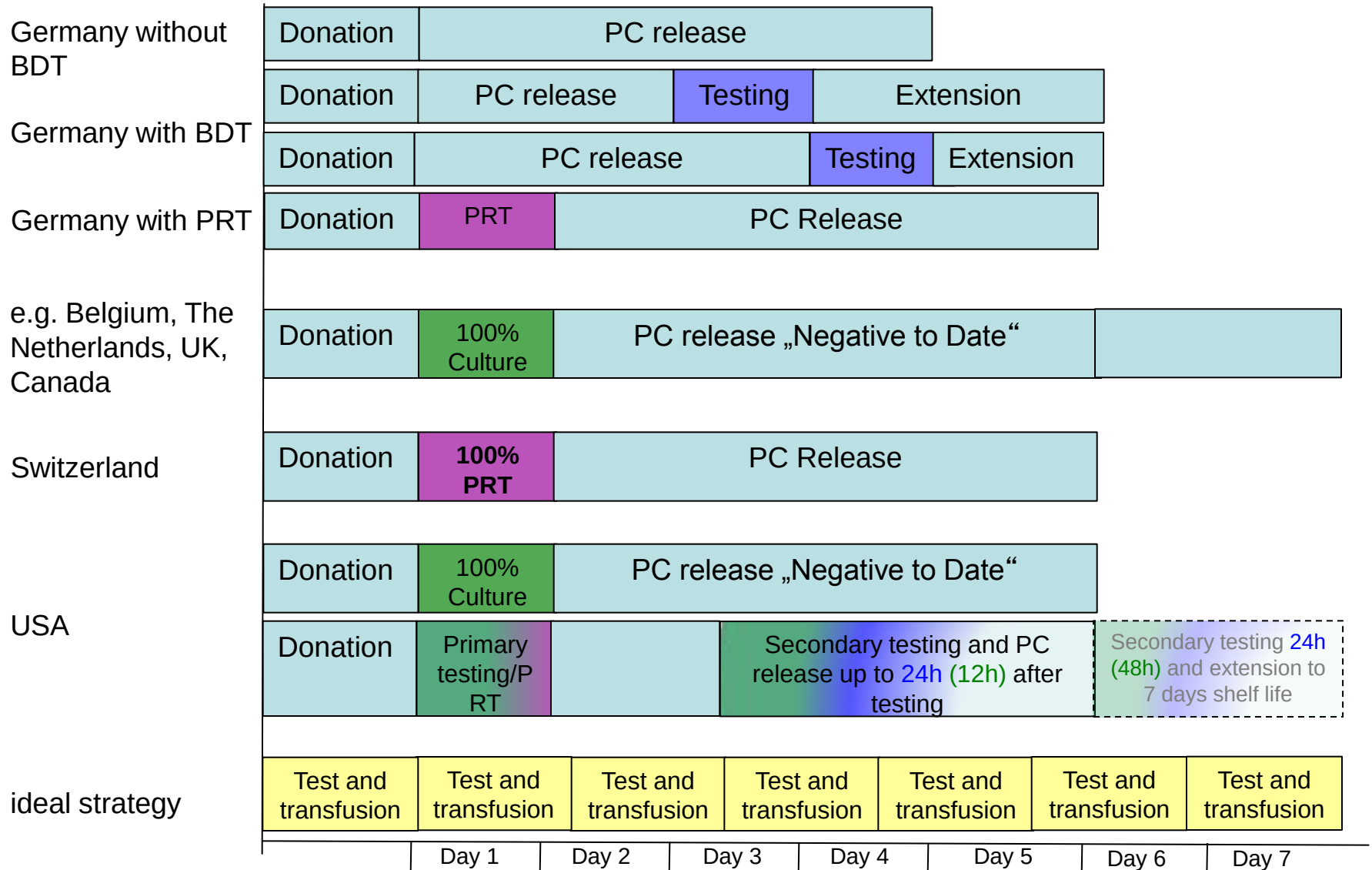
“Milestones” to reduce the risk of transmission of bacteria

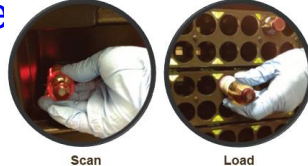
Published	Year	Content
National Advisory Committee Blood (AK-Blut) Vote 16	1997	“Minimal requirements for the sterility testing of blood components”
AABB Standard 5.1.5.1	2004	“The blood bank or transfusion service shall have methods to limit and to detect or inactivate bacteria in all platelet components”
Vote 38 Supplement V38	2008	„Specification for the Length of Thrombocyte Concentrate Shelf Life with the Aim of Reducing Life-Threatening Septic Transfusion Reactions Caused by Bacterial Contamination“; „Reduction of septicemia risk in the use of thrombocyte concentrates”
AABB Standard 5.1.5.1.1	2011	„Detection methods shall either be approved by the FDA or validated to provide sensitivity equivalent to FDA-approved methods“
FDA Center for Biologics Evaluation and Research	2016	Draft Guidance for Industry: “Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion”





Recommendations/Ideas/Strategies



Tool	Company	Principle		Approval
<p>Bact/ALERT 3D</p>  <p>BacT/ALERT VIRTUO</p>	<p>bioMérieux</p> 	<p>Colorimetric Assay:</p> <ul style="list-style-type: none"> aerobic and anaerobic incubation at 35-37° C microorganisms produce CO₂ the pH change is recognized by the sensor at the bottom change from blue to yellow 	<p>8-10ml up to 5d-7d continuous monitoring “Negative to date”</p>	<p>CE Mark FDA approved</p>
<p>Bactec</p> 	<p>Beckton Dickinson</p> 	<p>Fluorescent Assay:</p> <ul style="list-style-type: none"> aerobic and anaerobic incubation at 35-37° C microorganisms produce CO₂ CO₂ reacts with a dye in the sensor at the bottom of the bottle the sensor modulates the amount of light that is absorbed by fluorescent material 	<p>-10ml up to 5d-7d continuous monitoring “Negative to date”</p>	<p>CE Mark</p>

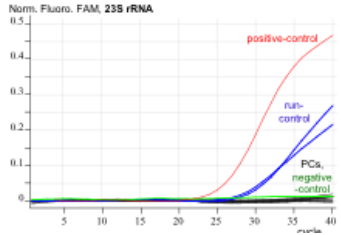
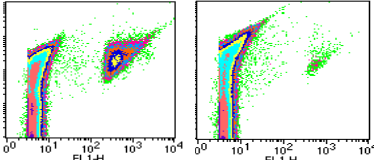
Tool	Company	Principle		Approval
VersaTREK  240 & 528 Model	TREK Diagnostics 	<ul style="list-style-type: none"> measurement of pressure changes in the headspace secondary to gas consumption/production aerobic (stirred) and anaerobic incubation at 36° C 	 0.1-10ml continuous monitoring “Negative to date”	CE Mark FDA approved
eBDS System (enhanced bacterial detection system) 	Heamonetics 	<ul style="list-style-type: none"> measurement of O₂ content in headspace sample pouch incl. two growth enhancing tablet aerobic incubation at 35° C microorganisms consume O₂ 	 3-4ml 24-30h “Endpoint Monitoring”	CE Mark FDA approved

Pros & Cons: Culture



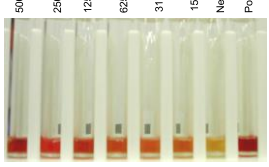
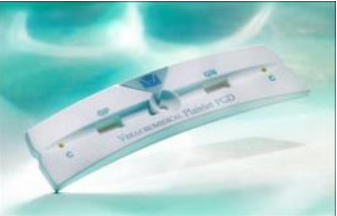
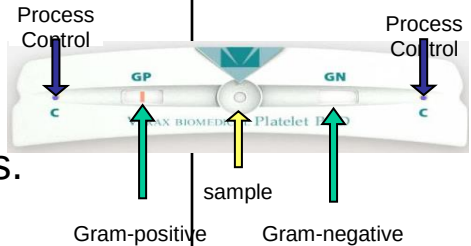
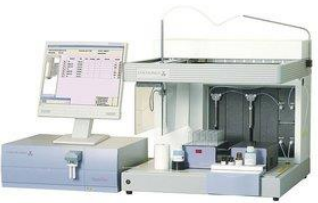
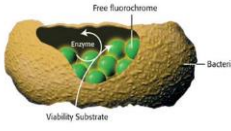
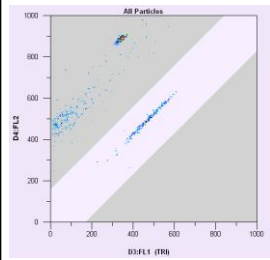
Advantages	Disadvantages
golden standard	early sampling: → sampling error
described in the guidelines	growth based: → long time to detection
high sample volume	high false positive rate → cells also produce CO ₂
negative to date principle: → no influence on product release	recalls are necessary



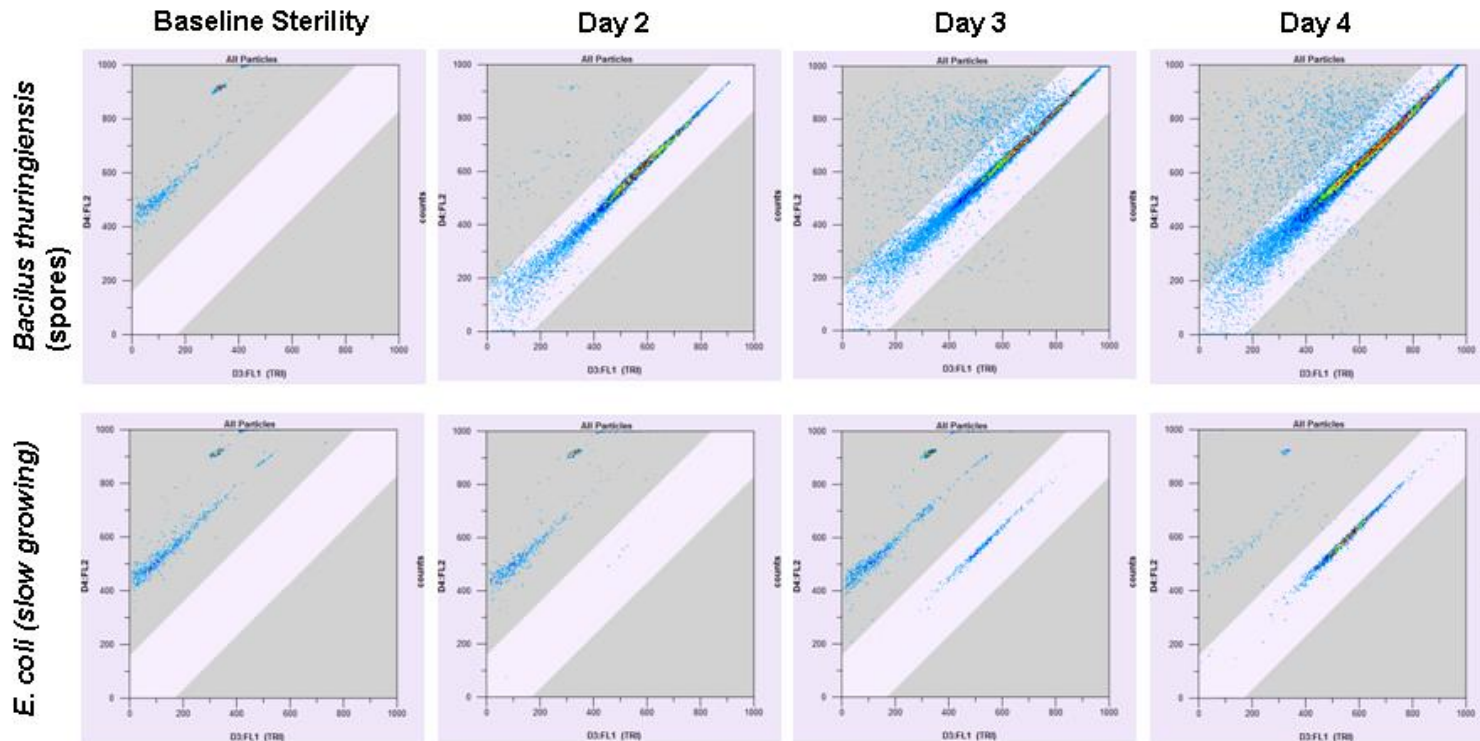
Tool	Company	Principle	Advantages/ Disadvantages	Sensitivity
NAT 	In house	1.Nucleic acid extraction (to 10ml), 2.Amplification of conserved regions of the 16S or 23S rDNA, 3.Real-Time or agarose gel electrophoresis detection.	Fast (3-4hours) and easy but high background signals cause insensitivity.	100-1000 CFU/ml
Flow Cytometry 	In house	1.Cell lysis, 2.labeling nucleic acids with thiazole orange, 3.Flow cytometric detection.	Very fast and easy but background signals cause insensitivity.	10^4 - 10^5 CFU/ml
BacDetect* 	Blood Analysis Ltd.	1.Dilution of 300µl and centrifugation, 2.Resuspension of pellet in a lysis/fluorescent dye reagent, 3.Transfer of 200µl into a 96 well plate, 4.Transfer to the autoloader and analyse by flow cytometry.	Time to result 15min to 3hours depending on sample amount.	1000 CFU/ml

Screening Technologies: Rapid Methods

Methods approved and/or already in use

Tool	Company	Principle		Use
BacTx 	Immunetics 	Enzyme based colorimetric assay for detection of bacterial peptidoglycan. 1.Lysis, 2.Centrifugation, 3.Extraction, 4.Neutralization, 5.Detection reagent, 6.BacTx Reader for 30min.	1ml, 1hour  <i>S. epidermidis</i>	10^3 - 10^4 CFU/ml FDA cleared for PPCs/ APCs
PGD 	Verax Biomedical	Lateral flow immunoassay to detect lipoteichoic Acid (LTA) and lipopoly-Saccharide (LPS) antigens. Sample preparation incl. centrifugation.	 0.5ml, 30min	10^3 - 10^5 CFU/ml FDA cleared for APCs/ PPCs
BactiFlow ALS 	bioMérieux 	Flow cytometry. A non-fluorescent fluorochrome passes the cell membrane of cells with intact membrane integrity and enzymatic activity, and is cleaved by intracellular esterase.	1ml, 1hour 	300-500 CFU/ml PEI approved

Results of BactiFlow ALS validation: Low titre spiking followed by storage under routine conditions and bacterial testing on day 2, 3 and 4.



Störmer et al. ISBT 2013

- BactiFlow ALS is established in different German Blood Centers for bacterial screening on day 3 or 4 to prolong the shelf-life to 5 days.
- Interlaboratory comparison provided by RfB (Referenzinstitut für Bioanalytik, Bonn, Germany).
- Quality Control using automated culture is performed independently.



Pros & Cons: Rapid Methods



Advantages	Disadvantages
short test time	reduced sensitivity
no re-calls	release up to two days after testing → bacteria continue to grow
small sample volume	false positives → background (matrix)
automated testing possible	training technicians

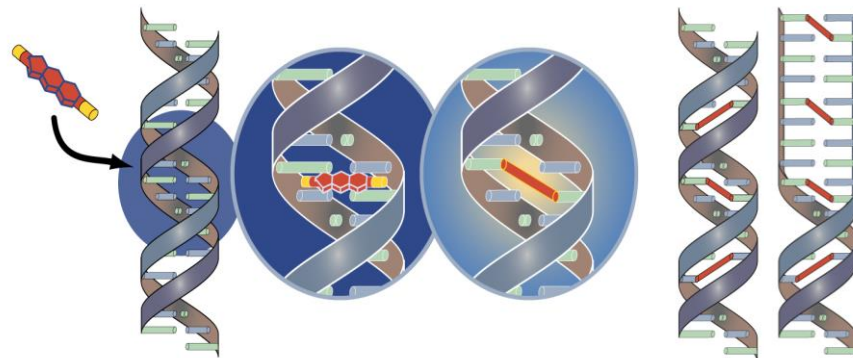




INTERCEPT Blood System, Cerus Corporation

Principle Amotosalen + UV-A	Approvals (PLT/Plasma)	Blood Products
1.Addition of Amotosalen 2.Illumination 3.CAD: compound adsorption device PLT in PAS 6 to 16h PLT in Plasma 16 to 24h 4.Storage	CE Mark (2002/2006) Afssaps (2003/2007) PEI (2007/2011) Swissmedic (2009,2007) HAS (2014) COFEPRIS (2014/2014) FDA (2014/2014) ANVISA (2015/2015)	Plasma Platelets S303: Whole Blood/ Red cells

Amotosalen docks in between the nucleic acid base pairs. Illumination results in an interstrand crosslink.

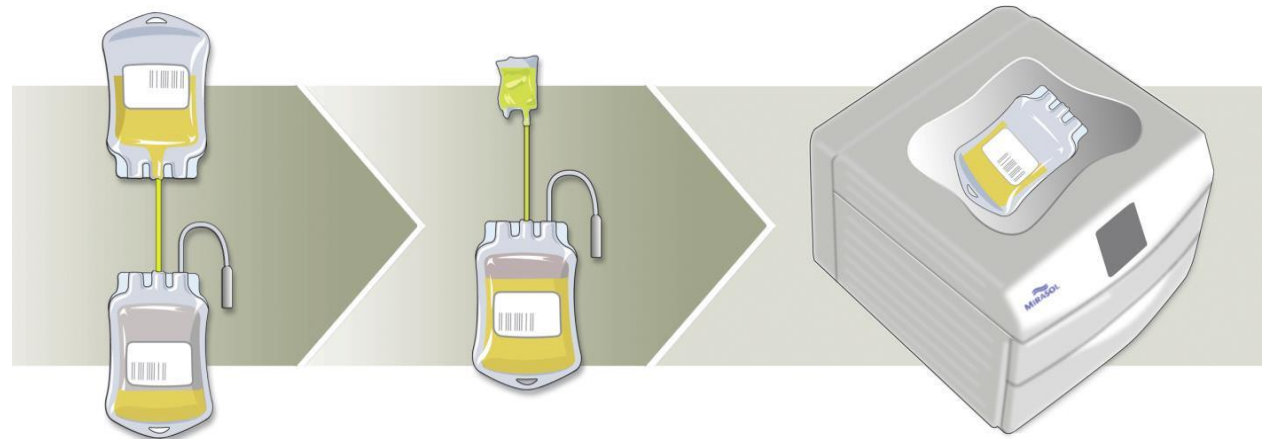




Mirasol Pathogen Reduction Technology System, TerumoBCT

Principle Riboflavin + UV	Approvals	Blood Products
1.PC Rest (2h) 2.Transfer to illumination bag 3.Addition of Riboflavin 4.Illumination (5-10min) 5.Storage	CE-Mark PC in Plasma (2007) Plasma (2008) PC in PAS (2009) WB (2015)	Plasma Platelets Whole Blood

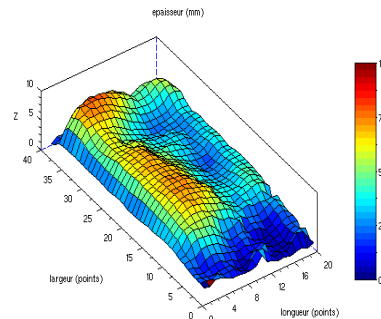
Riboflavin + UV
Riboflavin causes a
chemical alteration to
functional groups of the
nucleic acids (primarily
guanine bases).





THERAFLEX UV-Platelet System, Macopharma

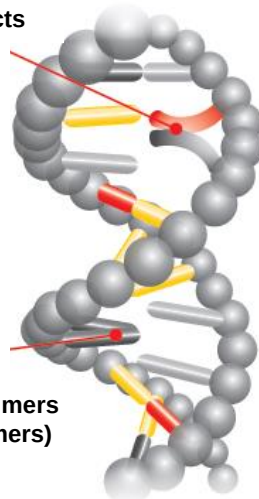
Principle UV-C + strong agitation	Approvals	Blood Products
1. Transfer to illumination bag 1. Illumination UV-C (<1min) 3. Transfer to storage bag 4. Storage	CE Mark Plasma (2011) Platelets (2009) Plasma PEI (2007)	Platelets Methylene Blue-Plasma



Formation of 6-4 photoproducts

Formation of cyclobutane pyrimidine dimers (Thymine dimers)

UV-C:
Formation of photoproducts and dimers which block the elongation of nucleic acid transcripts



Hands-on time : 5 min
Total Process time : 8 min





Species (high titre spiking)	GRAM	Reduction factor (log ₁₀)		
		INTERCEPT ^a	MIRASOL ^b (PLT in P)	THERAFLEX ^c (PLT in PAS)
<i>Bacillus cerus</i>	+	≥5.5	2.6	4.3±0.81
<i>Clostridium perfringens</i>	-	≥6.5	---	≥4.73
<i>Escherichia coli</i>	-	≥6.3	>5.4	≥4.01
<i>Klebsiella pneumoniae</i>	-	5.8	2.8	4.8±0.31
<i>Propionibacterium acnes</i>	+	≥6.5	---	4.5±1.13
<i>Pseudomonas aeruginosa</i>	+	---	4.7	≥4.92
<i>Serratia marcescens</i>	-	≥6.7	4.0	≥4.99
<i>Staphylococcus aureus</i>	+	≥5.4	4.8	≥4.99
<i>Staphylococcus epidermidis</i>	+	≥6.1	4.7	4.8±0.51
<i>Streptococcus pyogenes</i>	+	≥6.8	2.6	4.8±0.95
<i>Yersinia enterocolitica</i>	-	≥5.9	3.3	---

^aprovided by R. Benjamin; ^bS. Keil, J Vis Exp 2015;102:e52820; ^cT. Müller, Transfus Med Hemother 2011;38:242-250





Advantages

inactivation of viruses and bacteria

applicable for routine use

reduction of WBCs

no sampling error

Disadvantages

reduced sensitivity against some viruses and bacteria, log reduction

only one approved technology

influence on in vitro platelet function

costs

Reasons for postponing the decision making on implementation consist of the absence of regulatory approval, and concerns on safety and increment of pathogen-reduced platelets.

Ypma PF, et al. BMJ Open 2016;6:e010156. doi:10.1136/bmjopen-2015-010156

WHO International Repository for Platelet Transfusion Relevant Bacteria Reference Strains

- deep frozen, ready to use, stable, defined in count and shippable bacterial solutions
- should be used for validation of PRT by low + high titre spiking and validation of Screening Methods by low titre spiking

→ First International Validation Study + Enlargement Study by the ISBT TTID-WP Subgroup Bacteria

(Stoermer M., et al. Vox Sang 2012;102:22-31)

Bacteria Reference Panel

We provide information on code number, type of standard, instructions for use, WHO report and other relevant documentation.



Detailed Information

Code number	8483/13
Type of standard	1 st WHO International Repository for Transfusion Relevant Bacteria Strains <ul style="list-style-type: none"> • Staphylococcus epidermidis PEI-B-P-06 • Streptococcus pyogenes PEI-B-P-20 • Escherichia coli PEI-B-P-19 • Klebsiella pneumoniae PEI-B-P-08
Special requirements	Infectious/deep frozen (dry-ice shipment)
Instructions for use	IFU 8483_13 (pdf, 161 KB, Not barrier-free file.)
International collaborative study report	Link to WHO website
Handling charge	240 EURO
Additional information	WHO Collaborating Centre: Annual Report 2009 (pdf, 522 KB, Barrier-free file)

Overview and Ordering Information:

→ [WHO Biological Reference Materials](#)

updated: 26.08.2013 created: 15.07.2013

Order:

<http://www.pei.de/EN/information/license-applicants/standard-and-referencematerials/who/4-strains-bacteria-reference-panel/referencematerial-standard-bacteria-content.html>



CONCLUSION

PATIENT SAFETY

Product Quality

Infection
Safety

Functionality
of PCs

Viruses

Product
Specifications

Bacteria



Blood Supply (Logistics)

Donor
recruitment

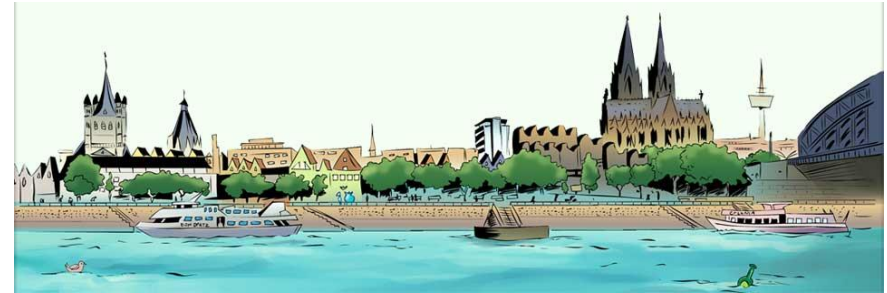
Shelf life of
PCs

Blood
Typing

Rarely
projectable
Supply

**It is not possible to find/inactivate everything.
We do have to find/inactivate what is clinically
important by using a technology that is applicable
in routine use.**





Ralf Paul, Martin Cordemann, <http://www.die-domspitzen.de/infos.html>

Cologne

Muito obrigado!
Thank YOU for Attention!

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