THERAFLEX UV PLATELETS: NOTHING BUT UVC LIGHT AND STRONG AGITATION

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Purpose

Blood donations may not only be contaminated with viruses, e.g. HBV, HCV or HIV. In addition, they may contain bacteria. This is especially crucial for platelet concentrates (PCs), because they have to be stored at room temperature, at which bacteria can multiply to high levels [1-2].

Short-wave ultraviolet light (UVC, wavelength range: 200-280 nm) is germicidal, but low UV-permeability hampers its use for sterilizing PCs. A simple method was developed which overcomes this limitation.

Materials and Methods

Plasma-reduced PCs in storage medium SSP+ (volume approx. 350 mL, platelet concentration approx. 10^9/mL, plasma content 30-35%) were prepared from pools of 5 buffy coats [3]. PC volume was approx. 350 mL. The PCs were spiked with approx. 10^2-10^6 CFU/mL of different bacteria species or up to 10^7 $TCID_{50}$/mL of lipid-enveloped or nonenveloped viruses. Other PCs were spiked with $5x10^6$/mL peripheral blood mononuclear cells (PBMC). The PCs were filled into UV-transparent plastic bags and irradiated on a device (Fig.1), equipped with mercury vapour tubes emitting monochromatic UVC-light (wavelength: 254 nm). The device was equipped with an orbital agitator. Irradiation was from both sides of the bags. UVC doses applied were up to 0.6 J/cm² (approx. 90 sec). During treatment the PCs were strongly agitated. Bacteria or virus titers, PBMC viability and platelet parameters were determined before and after irradiation. Each experiment was repeated 3-6 times. Results are depicted as mean ± SD.

Results

Pathogen inactivation was enormously enhanced when the PCs were loosely placed on a quartz plate located between the two layers of UVC tubes of the irradiation device and, in addition, strongly agitated during irradiation (Fig. 2).

UVC-light at 0.3-0.4 J/cm² (irradiation time: approx. 1 min) reduced the titers of all bacteria tested by approx. 5-6 log_{10} steps. PCs spiked with approx. 100 CFU/ml of bacteria were reproducibly sterilized (Tab.1). In one experiment with *B. cereus* the PC was sterile after 3 but unsterile after 6 days storage. This was probably due to spores of *B. cereus* that are more resistant to UVC than vegetative bacteria.

UVC sensitivity of the viruses tested was not so uniform (Table 1): The small single stranded RNA viruses VSV, Sindbis and WNV were completely inactivated at approx. 0.3-0.4 J/cm². Remarkably HIV-1 (also a small single-stranded RNA virus) was only moderately inactivated at UVC doses up to 0.6 J/cm².

The small nonenveloped DNA viruses PPV and EMCV proved to be very sensitive. Complete inactivation was achieved at 0.4-0.5 J/cm².

With the exception of HIV-1, SHV-1 was more resistant than the other viruses tested. This confirms that in general large double stranded DNA viruses are not as susceptible to UVC as smaller single stranded DNA or RNA viruses.

PBMC proved to be extremely sensitive to UVC irradiation: Complete inactivation was found at less than 0.1 J/cm² (Fig. 3)

PC properties remained almost unchanged at doses up to 0.6 J/cm². The storage stability of the treated PCs for up to 6 days after treatment (8 days after blood donation) was maintained (Table 2)

Conclusions

Irradiation with UVC under strong agitation may be used to sterilize platelet concentrates at a light dose that is not harmful to the products. The UVC dose required is 0.4 J/cm². Irradiation time is not more than approx. 1 min.

Parameter	Day 1 after irradiation				Day 6 after irradiation			
	Control	UVC dose (J/cm²)			Control	UVC dose (J/cm²)		
		0.4	0.5	0.6		0.4	0.5	0.6
Pts [x10^9/mL]	10.8 ± 0.6	10.2 ± 0.6	9.8 ± 0.6	9.1 ± 0.9	10.1 ± 0.8	9.8 ± 0.6	9.3 ± 0.8	9.3 ± 0.9
pH	7.10 ± 0.04	7.04 ± 0.05	7.09 ± 0.05	7.05 ± 0.04	7.27 ± 0.15	7.09 ± 0.06	7.11 ± 0.10	6.98 ± 0.07
Lactate [mmol/L]	7.7 ± 1.0	8.0 ± 0.5	7.7 ± 0.5	8.0 ± 0.7	12.7 ± 1.0	14.9 ± 1.0	14.6 ± 1.4	16.7 ± 1.4
Glucose [mg/dL]	122 ± 9	117 ± 7	117 ± 6	115 ± 7	62 ± 11	43 ± 8	44 ± 11	29 ± 10
Swirling	ok	ok	ok	ok	ok	ok	ok	ok
HSR [%]	69 ± 5	66 ± 2	61 ± 6	62 ± 4	68 ± 2	65 ± 2	62 ± 3	56 ± 5
Collagen-induced aggregation [%]	95 ± 4	90 ± 5	88 ± 3	87 ± 2	62 ± 9	69 ± 8	67 ± 2	69 ± 5
CD62 [%]	36 ± 1	46 ± 3	47 ± 2	49 ± 1	29 ± 1	45 ± 8	50 ± 10	57 ± 8
Annexin V [%]	5 ± 1	6 ± 3	7 ± 4	7 ± 4	9 ± 5	8 ± 2	10 ± 2	12 ± 3

Tab. 3: Treatment of PCs with different UVC doses. Influence on platelet parameters and on storage stability. n=6, mean + SD

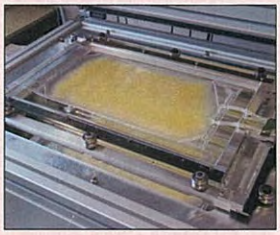
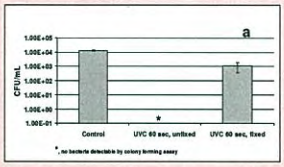


Fig. 1 : **Irradiation device for UVC treatment of PCs**



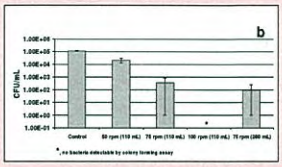


Fig. 2 : **Inactivation of *St. epidermidis* in PC aliquots (110 or 280 mL) by irradiation with UV light: fixed vs. loosely placed irradiation bags (a); dependence of bacteria inactivation in loosely placed irradiation bags on the agitation speed (b). n=3, mean ± SD**

Bacteria species	Characteristics	Gram stain	Number pf experiments	Spike (CFU/mL)	BacT/Alert result*	Remark
B. cereus	fac. anaerobic	pos	12	100-140	11 sterile 1 unsterile**	Spore former
E. coli	aerobic	neg	12	36-65	12 sterile	
K. pneumoniae	fac. anaerobic	neg	12	85-140	12 sterile	
P. acnes	anaerobic	neg	12	61-100	12 sterile	
S. aureus	fac. anaerobic	pos	22	60-110	22 sterile	
S. epidermidis	fac. anaerobic	pos	22	74-210	22 sterile	
Str. pyogenes	fac. anaerobic	pos	12	118-194	12 sterile	

*: Samples (2x10 mL each) were drawn after 3 and 6 days at 22°C
**: sterile after 3 days storage

Tab 1: **Sterilization of PCs spiked with different bacteria species by irradiation with UVC (0.4 J/cm²)**

Virus	Genome	Lipid Envelope	Model virus for	Log_{10}reduction factor
Vesicular stomatitis (VSV)	ss* RNA	X	-	≥ 6.41
Sindbis (Sindbis)	ss RNA	X	-	5.55
West Nile (WNV)	ss RNA	X	HCV	5.24
Human immunodeficiency (HIV-1)	ss RNA	X	-	1.36
Suid Herpes (SHV-1)	ds** DNA	X	HBV/CMV	3.57
Porcine Parvo (PPV)	ss DNA	-	Parvo B 19	≥ 6.42
Encephalomyocarditis (EMCV)	ss DNA	-	HAV	5.73

Tab 2: Inactivation factors of viruses by irradiation with UVC (0.4 J/cm²)

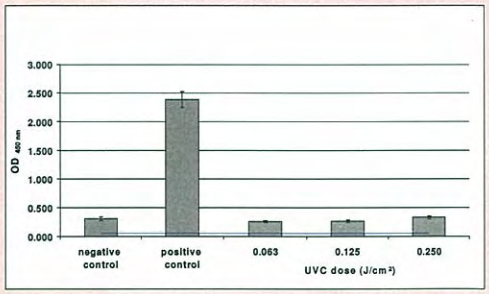


Fig. 3: Inactivation of T-lymphocytes in platelet concentrates by irradiation with UVC. Viability was assayed by mixed lymphocyte culture.

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不活化効果試験のassay methods

Pathogen		abbreviation	assay method (after illumination)	cell or animal used on the assay	initiation date	completion date	study period (days)	report number
virus	Duck Hepatitis B virus	DHBV	in vivo assay (DNA hybridization assay)	Legarth Pekin hybrid duckling	1994/10/3	1995/2/3	124	DEL 001
bacteria	Staphylococcus epidermidis		agar plate assay and liquid broth assay		1994/9/2	1995/2/13	165	DEL 002
bacteria	Klebsiella pneumoniae		agar plate assay and liquid broth assay		1994/9/2	1995/2/13	165	DEL 002
virus	Human Immunodifficiency Virus-1 (Cell associated)	HIV-1	plaque assay	MT-2 cell	1994/9/21	1995/2/18	151	DEL 004
virus	Human Immunodifficiency Virus-1 (Cell Free)	HIV-1	plaque assay	MT-2 cell	1994/9/21	1995/2/18	151	DEL 005
virus	Bovine Viral Diarrhea Virus	BVDV	plaque assay	bovine turbinate (BT) cell	1994/11/28	1995/2/24	89	DEL 006
bacteria	Pseudomonas Aeruginoza		liquid broth assay		1995/2/27	1995/6/30	124	DEL 010
bacteria	Serratia Marcescens		agar plate assay and liquid broth assay		1995/7/21	1995/9/7	49	DEL 011
WBC	T-Cell		limiting dilution analysis (LDA)		1995/8/21	1995/12/7	109	DEL 012
bacteria	Salmonella Choleraeuis		agar plate assay		1995/10/3	1996/1/15	105	DEL 016
virus	Human Cytomegalovirus (Cell associated)	CMV	plaque assay	MRC-5 cell	1996/1/16	1996/5/13	119	DEL 021
bacteria	Staphylococcus Aureus		agar plate assay and liquid broth assay		1995/11/30	1996/4/13	136	DEL 022
bacteria	Escherichia Coli		agar plate assay and liquid broth assay		1996/1/31	1996/4/24	85	DEL 023
bacteria	Yersinia Enterocolitica		agar plate assay		1996/3/15	1996/8/21	160	DEL 025
bacteria	Enterobacter Cloacae		agar plate assay and liquid broth assay		1996/5/6	1996/8/26	113	DEL 031
bacteria	Listeria Monocytogenes		liquid broth assay		1996/7/9	1996/8/8	31	DEL 032
bacteria	Corynebactrium Minutissimum		liquid broth assay		1996/7/9	1996/9/19	73	DEL 033
bacteria	Streptococcus Pyogenes		liquid broth assay		1996/7/12	1996/9/19	70	DEL 042
番外	TA-GVHD			mice	1996/9/5	1997/2/18	167	DEL 049
bacteria	Bacillus Cereus		liquid broth assay		1997/1/31	1997/4/18	78	DEL 061
virus	Bluetongue virus (Cell Free)	BV	plaque assay	bovine turbinate (BT) cell	1998/8/27	1999/10/27	427	DEL 088
virus	Feline Conjunctivitis virus (Calicvirus) (Cell Free)		plaque assay	Crandell Feline Kidney (CrFK) cell	1998/8/31	2000/11/8	801	DEL 089
virus	Simian Adenovirus (Cell Free)	SV15	evaluated for the presence or absence of cytopathic effect with the aid of a microscope	Fetal rhesus kidney (FRhK) cell	1998/11/2	2000/11/17	747	DEL 090
virus	Hepatitis B virus	HBV	in vivio assay (PCR, biopsy etc)	Chimpanzee	1997/9/10	2000/3/16	919	IACUC173
virus	Hepatitis C virus	HCV	in vivio assay (PCR, biopsy etc)	Chimpanzee	1997/9/10	2000/3/16	919	IACUC173
virus	Clinical isolated Human Immunodifficiency Virus-1(Z84 strain)	HIV-1	Mg^{++}-dependent [^{32}P]dTTP reverse transcriptase assay and P24 assay	PBMC	1996/2/16	1996/5/16	91	CP 1472
virus	Clinical isolated Human Immunodeficiency Virus-2 (CBL20 strain)	HIV-2	Mg^{++}-dependent [^{32}P]dTTP reverse transcriptase assay and P24 assay	PBMC	1996/2/22	1996/5/16	85	CP 1473
bacteria	Lactobacillus Species		agar plate assay		2001/12/3	2002/3/29	117	DEL 224
bacteria	Bifidobacterium Adolescentis		agar plate assay		2001/12/5	2002/3/29	115	DEL 225
bacteria	Propionibacterium Acnes		agar plate assay		2002/1/17	2002/5/17	121	DEL-R 00228
bacteria	Clostridium Perfringens		agar plate assay		2002/3/25	2002/10/4	194	DEL-R 00237
parasite	Trypanosoma cruzi	T. cruzi	microscopically monitoring	3T3 cell	2000/10/24	2002/4/25	549	REL 00218
parasite	Plasmodium falciparum		microscopically monitoring (smear with staining)	RBC	2001/5/21	2002/5/6	351	REL 00220
virus	Human T-cell Lymphotropic virus type I	HTLV-I	microscopically monitoring (the staining resulting from tax protein produced as a result of infection)	BHK21pA18G indicator cell line	2000/11/17	2002/4/29	529	REEL 00225-1
virus	Human T-cell Lymphotropic virus type II	HTLV-II	microscopically mongering (the staining resulting from tax protein produced as a result of infection)	BHK21pA18G indicator cell line	2000/11/17	2002/4/29	529	REL 00225-2
spirochete	Treponema pallidum		intradermal infectivity assay (observation), intratesticular infectivity assay (VDRL and FTA-ABS assay)	New Zealand rabbit	2000/8/4	2002/4/26	631	REL-R 217

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Screening platelet concentrates for bacterial contamination: low numbers of bacteria and slow growth in contaminated units mandate an alternative approach to product safety

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Vox Sanguinis

Background and Objectives We introduced 100% screening of platelets for bacterial contamination in 2005 to reduce the risk of clinical sepsis from platelet transfusion. We test all outdating units again at expiry to assess the sensitivity of the initial test.

Materials and Methods We test all platelet concentrates prior to release for clinical use using a large volume automated culture technique on the day after manufacture. All units that expire unused are retested. Platelets still in stock on day 4 of storage may have a repeat culture performed, and are returned to stock with two extra days of shelf life.

Results Of 43 230 platelet units screened, 35 (0·08%) were positive; of 8282 expired unused, 18 (0·22%) were positive; and of 3310 day-4 retests, four (0.12%) were positive. Overall sensitivity of the initial screening test was 29·2% (95% confidence interval 19·4 to 39·1%). Thirteen of the 35 positive screening tests would have been expected to grow in both aerobic and anaerobic bottles; eight grew in aerobic culture only and five grew in anaerobic culture only, indicating that the likely number of bacteria in the contaminated platelet units at the time of sampling was less than 60 colony-forming unit per platelet unit.

Conclusions Screening platelet concentrates for bacterial contamination using the most sensitive method available has a sensitivity of less than 40% because of the low numbers of bacteria in the initial contamination. Effective resolution of this problem will require a pathogen-inactivation technique.

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Introduction

Clinical disease caused by bacterial contamination of platelet concentrates has been observed to occur in between 1 in 358 platelet transfusions [1] and 1 in 73 000 [2]. The observed rate depends on the criteria and method of data collection used, and probably varies with the method of platelet manufacture: whether pooled or not, whether made by the buffy-coat method, from platelet-rich plasma, or by apheresis, and whether an initial diversion pouch is used or not [3]. Observed contamination rates in studies where the final platelet product is routinely tested by culture for bacteria give prevalence rates of culture-positive units from 1 in 5399 [4] to 1 in 125 (P Vandekerckhove, unpublished observations), and vary with the test used, the timing of the sample and the sample volume used. Bacteria that contaminate platelet units are usually derived from the skin of either the donor or the phlebotomist, less commonly from the donor's blood during asymptomatic or symptomatic bacteraemia, and rarely from contamination of the materials used in the collection process.

The natural history of bacterial contamination of blood components is highly variable. It depends on the species and strain of the contaminating bacteria, on the size of the inoculum, the component into which the bacteria are partitioned, and on the state of the recipient. Most contaminating bacteria

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fail to grow, and perish either in the collected components or in the recipient. Some do not, and may cause a severe and sometimes fatal response in the recipient. It is possible that bacteria in a platelet transfusion cause delayed rather than acute problems in the recipient – delayed sepsis, colonization of indwelling lines, or endocarditis are all possible, particularly in immunocompromised patients. However, this has never been systematically investigated, and would be difficult, but not impossible, to verify [5].

Platelets are particularly prone to problems with bacteria, mainly because they are stored at room temperature. The standard practice in the USA where platelets are made from platelet-rich plasma separated from whole blood donations, with limited pooling times, no buffy-coat concentration and no overnight hold may exacerbate the problem, while the European practice of buffy-coat pooling with or without a prolonged hold of the blood at room temperature before removing the white cells seems to reduce it [6–8].

Testing for the presence of bacteria has been widely adopted to try to address the problem [9]. Two general approaches are employed: early testing using a culture method, or late testing using some rapid detection system close to the time of issue and transfusion. Both methods have serious drawbacks. Early testing relies on culture, which is slow, necessitating that the units are released for transfusion before slower-growing bacteria can be detected in the sample; in addition, it is now apparent that the initial inoculum in a contaminated unit is often very low – perhaps as low as 10 bacteria in the unit (or larger numbers present in a few aggregates) – which requires a delay before sampling, or a very large sample, or both, to improve the chance of detection [10]. Late testing is limited by sensitivity problems: the tests generally rely on secondary characteristics of contamination such as acid generation or glucose consumption, or microscopic detection of bacteria in the component. While culture is sensitive to below 10° per ml [10], late testing requires 10^3 bacteria or more per ml to be useful [11–13].

These sensitivity problems are exacerbated by a desire on the part of platelet producers to store platelets for longer, to improve supply and to ease logistical problems. It may be that a platelet unit that is tested by an effective culture method near the beginning of its storage time is safer at day 7 of storage than an untested platelet unit close to expiry on day 5, but this remains to be demonstrated, notwithstanding the fact that many platelet manufacturers have made that assumption.

It was not apparent to us that even a sensitive test applied early in the shelf life of a stored platelet unit provided sufficient justification for extended platelet storage to 7 days or more: we had detected a contaminant early in an initial pilot study that consistently could not be detected before day 3 of sampling; we had observed that contaminating *Staphylococcal* species were consistently and predictably reduced in number but not eradicated during component manufacture [7], making subsequent detection even more difficult; and we had calculated that a test capable of detecting bacteria growing from a very low number after manufacture to a serious problem by day 7 could not be detected with sufficient reliability until day 4, even using the most sensitive method available [10]. Our initial experience of retesting expired units that had initially screened negative subsequently confirmed these misgivings [14].

We therefore introduced a dual approach to bacterial screening of platelets: testing by large volume culture on the day after manufacture to improve the safety of platelets stored to day 5, and a retest again using a large volume culture method at day 4 of storage to allow extended storage of platelets over day 5.

Methods

Pilot study

An initial study of buffy-coat platelets was performed to validate the hypothesis that platelets testing negative by BacTAlert (BioMerieux, Durham, NC, USA) using a 10-ml sample on day 2 would not be negative on day 4 and positive on day 7. All platelet units in the study were leucodepleted during the pooling process on day 1 and sampled 14–17 h later using a 10-ml inoculum into standard BacTAlert aerobic culture bottles. Culture was repeated as above on day 4, and again on day 7.

The 134th unit tested in this protocol was positive on day 7 only and was subjected to further analysis in spiking studies to identify growth characteristics of the contaminating bacteria.

Spiking studies

The isolate from unit number 134 was inoculated into platelet concentrates ($n = 6$) immediately after pooling and leucodepletion on day 2 at a concentration of 10–100 colony-forming unit (CFU) ($n = 3$) or 1–10 CFU ($n = 3$); quantitative cultures were performed using the pour-plate method as previously described [7], on days 2, 3, 4, 5, 6 and 7 (1-ml samples). BacTAlert samples were taken on days 4 and 7 postspiking (10-ml aerobic samples).

Routine platelet screening

Apheresis platelets are stored for a minimum of 12 h at 22°C after manufacture before sampling. For pooled buffy-coat platelets, manufacture takes place on the day after collection and following overnight hold of the whole blood unit at 22°C. Sampling for bacterial culture takes place at least 14 h after manufacture is completed, that is, at least 36 h after phlebotomy. A 7·5–10-ml sample is then inoculated into an aerobic BacTAlert bottle and a similar volume into an anaerobic bottle.

For the first 10 months (15 033 units), an 8-ml inoculum in aerobic culture only was used. We changed to a two-bottle system on the basis of information from the Welsh Blood Service (G Rowe, personal communication). This blood service screens platelet concentrates prior to storage using a large sample divided between aerobic and anaerobic BacTAlert bottles. They found that a significant number of positive tests were detected in one bottle only. This suggested that our single-bottle system was probably missing a significant proportion of bacterially contaminated units. It also indicated that a two-bottle approach would provide the opportunity to quantify the size of the initial contaminating inoculum based on limiting dilution.

All units stored to expiry are retested using a 10-ml aerobic sample and a 10-ml anaerobic sample on BacTAlert. We estimate that this system can detect bacterial contamination at a concentration of 1 CFU/ml of platelet concentrate with greater than 99·5% sensitivity [10]:

- 'Confirmed positive' is a positive result on BacTAlert, a positive subculture from the BacTAlert bottle, and a further culture of the same species from the platelet unit, or, for pools, from the pool or from one other component from the donations used in the pool.
- 'False positive' is a positive signal from the bottle, but no organism detected on subculture, Gram stain and reculture of the unit.
- 'Unconfirmed positive' is a positive subculture or Gram stain from the bottle but no residual material available for culture, or all residual material negative on culture.

All platelets are leucodepleted during the manufacturing process to a residual leucocyte count of $< 1 \times 10^6$/final platelet dose, with leucocyte counting of all units before release, using flow cytometry.

Day 4 retest for extended platelet shelf life

Since November 2005, we have extended the shelf life of apheresis platelets on the basis of a second test at day 4: platelets that are still in inventory on day 4 of storage and that may not be transfused within the following 36 h (i.e. may outdate) have a repeat aerobic and anaerobic culture performed using the same protocol as for the initial test, and are returned to inventory labelled with an extra 2 days of shelf life.

Estimating the number of bacteria in the initial contamination

For most of the period of the study, we used a 15-ml test volume divided into two culture bottles. Most of the organisms detected would be expected to grow with similar ease in both bottles, and the culture system is sensitive down to 1 CFU/inoculum. Therefore, an aerobic organism in a $2 \times 7{\cdot}5$ ml sample that grew in one bottle but not the other had a probable concentration of bacteria in the platelet sample of 1·386, standard error (SE) 1·414, in 15 ml by binomial distribution.

Sensitivity of the screening test

The 'false negative rate' of the initial screening test was taken as the positive rate at outdate. The 'probable total positive rate' was calculated as the sum of the observed positive rate at screening and the 95% confidence interval (CI) of the total number of false negatives based on the observed false negative rate. The sensitivity of the screening test was calculated as the number of observed positives/probable total number of positives (%).

Statistics

Confidence intervals for the observed incidence of bacterial contamination were calculated using the single Poisson data sample module of the StatXact 8 software package (Statistical Software for Exact Nonparametric Inference, Cytel Inc., Cambridge, MA, USA).

Results

Pilot study

One hundred and thirty-four consecutive platelet concentrates made from pooled buffy-coats were tested initially on the day of manufacture, and again at days 4 and 7. All were negative on day 2 and day 4. Platelet pool number 134 was positive on day 7 having been negative on day 2 and day 4. The organism was identified as *Staphylococcus capitis*.

Spiking studies

The organism that grew in the 134th platelet unit in the pilot study was spiked into fresh platelet concentrates: 3 of 3 BacTAlert cultures (10 ml aerobic samples) were positive at days 4 and 7 of storage when a 10–100 CFU inoculum was spiked on day 2; for a 1–10 CFU inoculum, 1 of 3 BacTAlert cultures was positive at day 4, and 3 of 3 BacTAlert cultures were positive at day 7. Quantitative cultures performed using 1-ml samples taken on days 2, 3, 4, 5, 6 and 7 showed no growth in any unit until day 4 in 3 units, day 5 in a fourth unit and day 7 in the remaining 2 units. Exponential growth occurred in all units after the initial positive test (Fig. 1).

Routine platelet screening (Table 1)

In total, 43 230 platelet units have been screened prior to issue; 15 033 were tested using an 8-ml aerobic sample only; 28 197 using a 15-ml sample divided equally into an aerobic

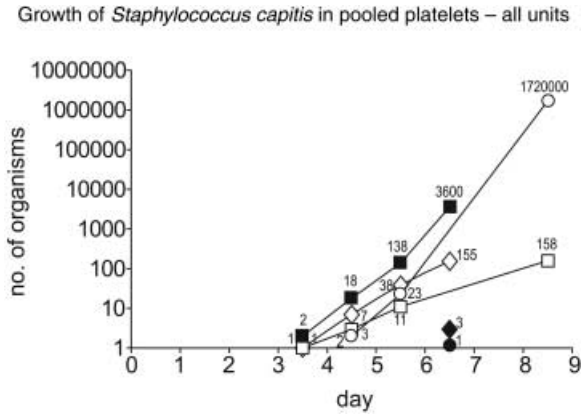

Fig. 1 An isolate of *Staphylococcus capitis* was detected from a pooled platelet concentrate during the initial pilot project. It was not detected on day 1 or day 4 of screening, but the day 7 (postexpiry) sample was positive. The isolate was inoculated at 1–10 CFU/ml (n = 3) and 10–100 CFU/ml (n = 3) into fresh platelet concentrates on day 2 of shelf life. The numbers on the growth curves indicate the concentration of bacteria (CFU/ml) in quantitative cultures from samples taken daily from day 2 onwards.

Table 1 Positive screening tests

	Confirmed positives	Unconfirmed positives	Total true positives
Apheresis platelets (n = 12 823)	4 (0·03%)	8 (0·06%)	12 (0·09%)
Pooled platelets (n = 30 407)	10 (0·03%)	13 (0·04%)	23 (0·08%)
Total platelets (n = 43 230)	14 (0·03%)	21 (0·05%)	35 (0·08%)

and an anaerobic bottle. There were 14 confirmed and 21 unconfirmed positives on the initial test, for a total positive rate of 0·08% (observed proportion = 0·001; 95% CI for the proportion: 0·001–0·001). The rate for the 8-ml sample was 11/15 033 (0·07%; observed proportion = 0·001; 95% CI for the proportion: 0·000–0·001); for the 15-ml sample it was 24/28 197 (0·09%; observed proportion = 0·001; 95% CI for the proportion: 0·001–0·001).

Twelve out of 12 823 (0·09%) were from apheresis collections; 23/30 407 (0·08%) were from pools of four buffy-coats after 22°C overnight hold of the whole blood units.

Of 43 230 platelet units, 8282 expired unused and were retested: there were 18 positives – 7 confirmed and 11 unconfirmed – for a total positive rate of 0·22% at expiry (observed proportion = 0·002; 95% CI for the proportion: 0·001–0·003).

Day 4 retest for extended platelet shelf life

Four out of 3310 (0·12%) day 4 retests for extending shelf life were positive (1 confirmed, 3 unconfirmed; observed proportion = 0·001; 95% CI for the proportion: 0·000–0·003).

Estimating the number of bacteria in the initial contaminating inoculum

Twenty-four of the 35 positive day 1/day 2 tests (confirmed and unconfirmed) were identified after the addition of the anaerobic sample to the screening test. Eleven of these were *Propionibacterium* spp. or strict anaerobes; the remaining 13 would have been expected to grow in both bottles. However, none grew in both aerobic and anaerobic bottles: eight grew in aerobic culture only and five grew in anaerobic culture only. In contrast, 12 of 18 isolates from expired units would have been expected to grow in both aerobic and anaerobic cultures: six did so; three grew in aerobic and three in anaerobic bottles only. This indicates that the mean number of bacteria in the contaminated platelet units at the time of sampling for screening was 1·386 (SE: 1·414) CFU/test volume, that is, less than 60 CFU/platelet unit in most instances [mean platelet volumes in our facility are 333 ml (SD 14 ml) for pooled platelets and 244 ml (SD 4 ml) for double apheresis units].

Sensitivity of the screening test

The false negative rate of the initial screening test was taken as the positive rate (confirmed and unconfirmed) at outdate, that is, 18/8282 (0·22%). (We considered the rate at day 4 retesting to be another, comparable estimate of the false negative rate, and not an additional component of it. The later, larger sample was considered the more accurate measurement.) The sensitivity of the screening test was calculated as the number of observed (confirmed and unconfirmed) positives/probable total number of positives (%). That is (number of positives at screening) divided by [the number of positives at outdate (95% CI) × (number of platelet units tested at screening/number of platelets tested at outdate) + number of positives at screening] %; or [35/(89·4 +35)]% to [35/(180·9 +35)] %, which gives a 95% CI of 19·35 to 39·1% for the sensitivity of the screening test.

The 57 isolates detected at screening, retesting on day 4 and at outdate are shown in Table 2. There were 20 isolates of coagulase-negative *Staphylococci*; 18 of *Propionibacterium acnes*; three each of anaerobic *Streptococcus* spp., *Bacillus* spp., and *Corynebacterium* spp.; two each of *Micrococcus* spp. and *Streptococcus* spp.; one each of *Staphylococcus aureus*, *Proteus mirabilis*, *Acinetobacter baumanii*, *Bacteroides thetaiotamicron*, *Leuconostoc* spec., and *Brevibacterium* spec. The interval, in days, between inoculation of the sample into the culture bottle and the positive signal from the BacTAlert device for the 57 isolates is given in Table 2.

Twelve of the contaminated platelet units were transfused before the culture signalled positive. All patients were followed up, and none had had either an acute or delayed clinical event that could be attributed to the transfusion of contaminated

Table 2 The time, in days, between inoculation of the sample into the culture bottle and the positive signal from the BacTAlert device for bacteria isolated from platelet concentrates

Bacteria	Number of isolates (n = 57)	Detected on day 1 screening (n = 35) (time, in days, in culture bottle before detection)	Detected at day 4 retest (n = 4) (time, in days, in culture bottle before detection)	Detected at outdate (n = 18) (time, in days, in culture bottle before detection)
Coagulase-negative *Staphylococci*	20	13 (range: 0·66 to 5·8)	1 (0·75)	6 (range: 0·13 to 1·11)
Propionibacterium acnes	18	10 (range: 4·39 to 5·94)	2 (3·68, 3·51)	6 (range 4·3 to 6·57)
Anaerobic *Streptococcal* spp.	3	2 (2·05, 2·93)		1 (2·1)
Bacillus spp.	3	2 (0·88, 2.33)		1 (0·6)
Corynebacterium spp.	3	1 (1·43)		2 (4·0, 4·2)
Micrococcus spp.	2	2 (2·89, 3/03)		
Streptococcus spp.	2	2 (0·39, 0·44)		
Staphylococcus aureus	1	(3·78)		
Leuconostoc spec.	1			(0·25)
Bacteroides thetaiotamicron	1	(2·16)		
Acinetobacter baumanii	1			(0·24)
Proteus mirabilis	1	(0·85)		
Brevibacterium spec.	1		(4·8)	

platelets. The organisms cultured from these 12 platelet units were *P. acnes* (n = 6), coagulase-negative *Staphylococci* (n = 3), *Corynebacterium* spec., *P. mirabilis, Brevibacterium* spec. The *P. mirabilis* isolate was confirmed on both of the two non-transfused parts of a triple apheresis donation. No adverse reactions due to bacterial contamination of platelets were reported for the platelets tested in this period, in spite of an active, comprehensive, nationwide haemovigilance programme [15].

Discussion

Testing platelets for bacterial contamination has been widely adopted by blood transfusion services to try to prevent sepsis caused by transfusing such units. Bacterial contamination of blood components is inevitable from time to time – it is not possible to render the venepuncture site sterile, and in addition occasional asymptomatic bacteraemia in blood donors will result in septic collections. Cooling of red cell units before transfusion reduces the incidence of sepsis from transfusions of red cell units to a very low level. Sepsis in red cell transfusions is usually caused by psychrophilic bacteria such as *Yersinia enterocolitica*, *Klebsiella pneumoniae* or *Pseudomonas* spp. Because platelets are stored at 22°C many other species of bacteria that enter the blood collection may thrive and cause serious clinical adverse events in the recipient of the unit. Testing at day 1 or 2 of shelf life will detect many, possibly most, but not all [4] serious infections, particularly those from blood-borne bacteria that are not in lag phase, and other organisms that will grow rapidly during early storage. The longer the delay between phlebotomy and testing, the more likely the test will detect a growing, and therefore clinically relevant, contaminant. Larger volume sampling will also increase the detection capability of a test. It is difficult to determine the true rate of contamination, or to compare the rates between centres that vary in timing of sample, sample volume and detection method. Rates of approximately 1 in 5000 quoted for American studies of apheresis platelets [4] reflect the timing and the sample volume used; rates of up to 0·8% from Belgium (P Vandekerckhove, unpublished observations) may similarly reflect timing and sample size differences; the overall rate of 0·1–0·3% that we observed by combining the outdate rate with the initial screening rate after manufacture probably reflects the true rate of contamination with organisms that survive initial conditions in platelets in our system, which includes isopropyl alcohol and 0·5% chlorhexidine skin preparation, diversion of the first 40 ml collected, leucodepletion, and, for pooled platelets, overnight hold of the whole blood unit at 22°C, and buffy-coat pooling prior to leucodepletion.

Screening platelets for bacteria prior to issue, using relatively large sample volumes to increase detection rates, has a mean sensitivity in our hands of 33%. This limits the overall benefit of testing prior to release, and calls into question whether platelet shelf life can be extended to 7 days based on an initial screening test.

It is one thing to test platelets for bacteria in an attempt to reduce the incidence and severity of a serious and relatively frequent complication of platelet therapy; it is quite another to compromise the safety of patients by extending platelet

shelf life on the basis of a test that is ineffective at detecting low-grade contamination that may have the potential to cause clinically relevant adverse events after prolonged storage. We could not convince ourselves that prolonged platelet storage could be justified on the basis of a sensitive test for bacteria test earlier than day 4, based on theoretical calculations [14], observations of the recovery of spiked *Staphylococci* over the manufacturing process [10] and the early detection of an isolate in a pilot study that repeatedly escaped detection by testing before day 4.

Using a day 4 retest prior to extending platelet shelf life is a practicable approach in our hands to the problem of low initial sensitivity of bacterial screening. Day 4 testing is relatively straightforward, and need only be performed on platelet units that are likely to expire on day 5, and whose preservation makes logistical sense based on current inventory, so that there is high return on the costs involved. Because the platelets are labelled for 7-day storage before they outdate on day 5, computer safety blocks on allowing outdated products back into inventory are avoided. The repeat sample from the platelet unit will have a 36-h minimum incubation period in the culture system before the original day 5 expiry is reached. This can be expected to detect all contaminating bacteria in the life-extended platelets, with the probable exception of *P. acnes*. *Propionibacterium acnes* is not reliably detected before transfusion by any available testing algorithm. There are no clinical reports of adverse events from the tens of thousands of *P. acnes*-contaminated platelets that have undoubtedly been transfused. However, *P. acnes* is not wholly benign [16,17], and in addition is a sentinel marker of persistent problems with bacterial testing of platelets.

A detection system with a quicker read-out and a lower sensitivity could be used at day 5 instead of the BacTAlert system at day 4 with comparable safety profile, and is worthy of further study. Nevertheless, the low utility of bacterial testing in eliminating the problem of contamination in platelet units, even using the large sample volumes that we do (which in turn indicates a high comparative sensitivity of our testing system), indicates that a pathogen-inactivation system may be needed to solve the problem.

In the studies reported here, apheresis platelets are not significantly more likely to be contaminated after collection than platelets manufactured from pooled buffy-coats made from whole blood units stored overnight at 22°C, in spite of the fewer numbers of venepunctures involved in a plateletpheresis collection. This is compelling evidence for a protective effect from overnight storage of buffy-coats at 22°C prior to platelet manufacture, as has been previous reported by ourselves and others [6–8]. It is in marked contrast to the observations by investigators who have used platelets manufactured by the platelet-rich plasma method [2], but consistent with the observations from the Serious Hazards of Transfusion programme in the UK [18] where, although the numbers are necessarily small, severe reactions attributed to apheresis platelets are over-represented compared to the relatively more frequently transfused pooled buffy-coat-derived platelets, even though the UK does not include the step of overnight warm hold of whole blood prior to making the platelets.

Twelve of the 35 contaminated platelet units were transfused. This reduced the practical effectiveness of the screening programme by a further 34%. We have not observed any cases of bacterial sepsis ascribed to platelet transfusions since the introduction of this programme, in spite of a comprehensive national haemovigilance programme [15]; this suggests that bacteria escaping detection in this system are of limited clinical significance. However, the numbers are relatively small and the natural history of low-grade contamination in platelet transfusion has not been examined in detail, especially in immunocompromised patients or in patients with indwelling lines. In addition, Eder *et al.* [4] have demonstrated, in a study of over 1 million platelet units, that contaminated platelet units escaping detection by their protocol, using a similar approach to the one reported here, could cause severe septic complications in patients, including death.

These studies indicate that the initial contamination levels in platelet concentrates are extremely low – even after a minimum of 12 h following venepuncture or leucodepletion most bacteria detected were at a concentration in the order of 1 CFU per 15 ml, that is, less than 60 CFU/platelet unit. This is consistent with earlier laboratory studies [7,10] and indicates that the sensitivity required to detect all contaminating bacteria cannot be achieved by any sampling protocol unless almost the entire unit is sampled. It will not be possible to provide complete protection from bacterial growth in platelet units by any screening test currently available. Photochemical treatment of platelets after manufacture using a sufficiently effective method will be necessary to achieve this. Such an approach will provide the added benefit of rendering all platelet units cytomegalovirus-safe regardless of serological status, graft-versus-host disease-safe without irradiation, and will also provide protection from emerging viruses, of which there are many [19].

It is generally accepted that bacterial contamination of platelets for transfusion is a serious problem requiring effective preventative action. Possible approaches include bacterial detection at the manufacturing centre (before the product is released for clinical use), bacterial screening at the clinic, shortly before the product is transfused, or treatment of the product during the manufacturing process to remove contaminating bacteria. Each of these approaches (or any combination of these) has limitations: poor sensitivity of early screening methods, and to a lesser extent limited sensitivity of near-patient tests for slow-growing, low-concentration organisms; safety and cost concerns for photochemical or other eradication methods. Platelets constitute a large-volume intravenous medicine, often given to immunocompromised

patients, and often to patients with indwelling lines that form a prime site for colonization. It is unthinkable that a manufacturer of other intravenous medications could eschew reasonable methods to eradicate possible contamination on the basis that only organisms of questionable clinical significance persisted in the preparations infused. It is also unthinkable that end users of intravenous agents would be asked to check sterility before use, provided a reasonable alternative was available to the manufacturer. It is apparent to us that bacterial testing, whether early or late, lacks sufficient robustness of design [20] to persist as the method of choice once a method of eradication of adequate proven safety and utility is available.

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平成20年5月23日
血液事業部会運営委員会・安全技術調査会合同委員会提出資料

文献4,5

資料2

委員からの質問に対する各社からの回答

○不活化技術に関するヒアリング概要

（参考）

社名	マコファルマ社 株式会社アムコ	BCT Japan株式会社 （旧 ガンブロ株式会社）	バイオワン株式会社 シーラス社	シーラス社
①不活化法の種類	メチレンブルー	リボフラビン	アモトサレン（S-59）	S303
②化合物添加	要	要	要	要
③光照射	要	要	要	不要
④薬剤除去工程	要	不要	要	不要
⑤適応製剤	FFP（実用化済み）	PC （2007年実用化済み） FFP （2008年7-9月予定）	PC（実用化済み） FFP（実用化済み）	RBC （2008年5月時点で第Ⅰ相試験まで終了）
⑥不活化効果	**ウイルス**：可（HIV-1, WNV, BVDV, Influenza, Duck HBV, 等） **細菌**：不可	**ウイルス**： HIV, active 5.9,latent 4.5, HCVmodel V. 3.2, HBV 2.0-3.0, WNV 5.2, HumanB19model v .> 5.0 **細菌**： S.aureus 3.6-4.8, S.epidermidis 4.2, E.coli > 4.4, B.cereus 1.9-2.7, P. aeruginosa 4.5Parasites測定限界値まで(>3 to>6) 生体外検定法（TCID 50)による。 単位：HBV除きログ/ml, HBVはgEq/mL	**ウイルス**：HIV-1>6.2, HBV>5.5, HCV>4.5, WNV>5.5, SARS HCoV>5.8 ParvovirusB19 3.5->5.0, HTLV,CMV等可 （単位ログ/mL） **細菌**：S.epidermidis>6.6, S.aureus>6.6, E.coli>6.4, S.pyogenes>6.8, B.cereus >5.5, P.aeruginosa 4.5等, **白血球**>5.3, **原虫等** T.cruzi>5.3	**ウイルス**：HIV、ウシのウイルス性下痢ウイルス、アデノウイルス，水泡性口内炎 **細菌**：黄色ブドウ球菌、表皮ブドウ球菌、エルシニア・エンテロコリチカ、セラチア・マルセセンス、大腸菌
⑦製剤への影響				
1）容量変化	処理する血漿製剤から約15ml減少（キット内及びキットに含まれるフィルター2個に残留するボリュームとして）	血小板製剤250mLに対し35mL(500μM)のリボフラビン溶液を加える。従って35mLの容量増加となる。	**血小板**：血漿65%を保存液インターソルで置換した血小板溶液約300mLにS-59溶液15mLを加えるが、処理ロス約7%があるので約5mL減少する。 **血漿**：血漿(385-635mL)にS-59溶液15mLを加えるが、処理ロス約7%あるので約20mL減少する。	
2）凝固因子活性低下	Fibrinogen=-14.3%/ FII=-3.4%/ FV=-21.4%/ FVII=-4.2%/FVIII=-15.4%/ FIX=-7.4%/FX=-13.3%/ FXI=-16.2%/ FXII=-11.8%/FXIII= -2.8%/vWF=-0.7%	血小板の凝固機能：5日保存でp－selectinは通常の輸血時同様程度の若干の上昇（17.9±7.0→57.8%±14.8）。GP1bα、GP11b－11a,GpⅣ,vWF等処理前と比較して機能性異常なし。FFP：52週保存で凝固因子Ⅷc活性0.8±0.1IU/mL,Fibrinogen活性。69週保存211±33.5mg/dl等すべて欧州ガイドラインを満たす。（資料MirasoPRTシステム参照）	**血漿**：Fibrinogen=-21%/ FII=-11%/ FV=-5%/ FVII=-18%/FVIII=-23%/ FIX=-11%/FX=-11%/ FXI=-10%/ **血小板**：**In vitro:**5日保存 pH 6.8(6.94), HSR 58.8% (58.5), Pセレクチン 51.7%(31.0), pO2 24.2mmHg(29.9), 乳酸 11.3mM(10.5) **In vivo:**輸血後のｳｻｷﾞ耳出血時間 259秒(274) ( )内は未処理	
3）血小板回収率		98% 程度（この処理により約2%の血小板回収率低下が想定される。	92-93%（容量減少分を含む）	（赤血球）24時間回収率＞75%
⑧安全性について	すでに臨床で広く使用されており、メチレンブルー処理された血漿製剤輸血の臨床例は累計400万件以上に上るが副作用報告は無い。現在同社製品は年間30万例以上の臨床使用が続けられている。	リボフラビンは生理物質(ビタミンB2)で、FDAにより食品着色用に安全物質（GRAS)として認知されている。輸血用血液に新たな化学合成物質が加えられることはなく、広範な生体内外の毒性試験で毒性および変異性がないことは実証されている。	ICHガイドラインに沿って全ての毒性試験を実施し安全性はEU,FDAより認められている。一般毒性は臨床量の100倍遺伝毒性は1万倍の量で安全である。臨床試験、10万例以上の臨床使用で問題は報告されていない。	安全性の実証：発がん性 遺伝毒性 安全性薬理試験 生殖毒性 操作安全性