薬事・食品衛生審議会
平成２５年度第１回血液事業部会安全技術調査会

議 事 次 第

日時：平成２５年７月１０日（水）
１４：００～１６：００
場所：厚生労働省１９階（専用第２３会議室）

議題：

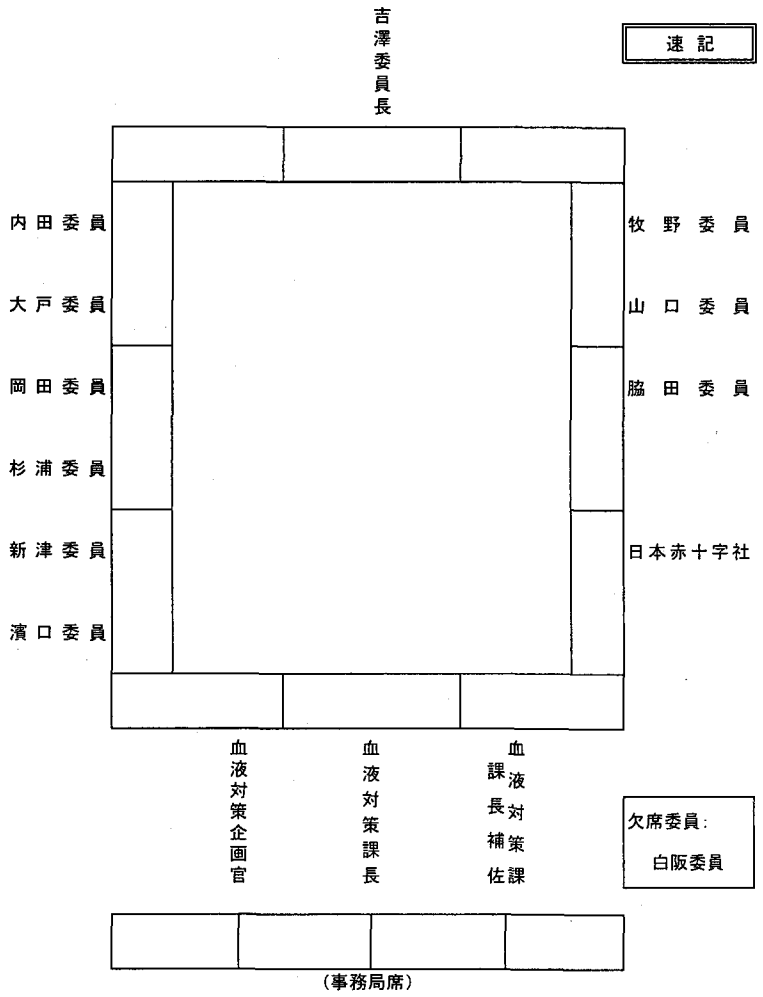
1. 日本赤十字社における平成２４年度ヘモビジランスについて
2. シャーガス病に対する安全対策の進捗状況について
3. 血小板製剤の病原体不活化技術導入に関する検討について
4. 血漿分画製剤のウイルス安全対策について（非公開）
5. その他

配付資料：
座席表
委員名簿
設置要綱

資　料　1　日本赤十字社における平成２４年度ヘモビジランスについて
（日本赤十字社提出資料）
資　料　2　シャーガス病に対する安全対策の進捗状況について
（日本赤十字社提出資料）
資　料　3　血小板製剤の病原体不活化技術導入に関する検討について
（日本赤十字社提出資料）
資　料　4　血漿分画製剤のウイルス安全対策について（E型肝炎ウイルス及びヒトパルボウイルスB19）（非公開、委員限り資料）

平成25年度第1回
薬事・食品衛生審議会薬事分科
血液事業部会安全技術調査会
座席表

平成25年7月10日(水)
厚生労働省19階
専用第23会議室
14:00～16:00



傍　聴　席

安全技術調査会　委員名簿

氏　名	ふりがな	現　職
内田　恵理子	うちだ　えりこ	国立医薬品食品衛生研究所 遺伝子細胞医薬部 第一室長
大　戸　　斉	おおと　ひとし	福島県立医科大学医学部長
岡田　義昭	おかだ　よしあき	国立感染症研究所血液・安全性研究部第一室長
白阪　琢磨	しらさか　たくま	独立行政法人国立病院機構大阪医療センターエイズ先端医療研究部長
杉　浦　　亙	すぎうら　わたる	独立行政法人国立病院機構名古屋医療センター・臨床研究センター感染・免疫研究部部長
新　津　　望	にいつ　のぞみ	埼玉医科大学国際医療センター造血器腫瘍科教授
濵　口　　功	はまぐち　いさお	国立感染症研究所血液・安全性研究部長
牧野　茂義	まきの　しげよし	国家公務員共済組合連合会虎の門病院輸血部長
山口　照英	やまぐち　てるひで	国立医薬品食品衛生研究所生物薬品部研究員
吉澤　浩司	よしざわ　ひろし	広島大学名誉教授
脇田　隆字	わきた　　たかじ	国立感染症研究所ウイルス第2部部長

（計11名，氏名五十音順）

薬事・食品衛生審議会薬事分科会
血液事業部会安全技術調査会　設置要綱

（目的）

第１条　安全な血液製剤の安定供給の確保等に関する法律（昭和31年法律第160号）において、国は、血液製剤の安全性の向上に関する基本的かつ総合的な施策を策定・実施が求められていることを踏まえ、血液製剤に関する安全性の確保対策に関する諸事項を調査・審議することを目的として、薬事分科会規程第４条に基づき、血液事業部会の下に「安全技術調査会」（以下「調査会」という。）を設置する。

（調査会の審議事項）

第２条　調査会は、以下に掲げる事項を検討する。

一　血液製剤に混入する可能性のあるウイルス等の感染性因子の検討

二　ウイルス等の感染性因子の不活化技術等に関する検討

三　血液製剤の安全性に関するガイドライン等の検討

四　その他、血液製剤の安全性の確保に関する重要事項の検討

（調査会の組織）

第３条　調査会に所属すべき委員は、部会に所属する委員、臨時委員及び専門委員（以下「委員等」という。）の中から、部会長が指名する。

２　調査審議にあたっては、議題の内容等に応じて、部会長の判断により他の委員または参考人に出席を求めることができる。

３　部会長は、第一項の規定により調査会に属すべき委員等を指名した場合は、部会においてその旨を報告しなければならない。

（座長の選任）

第４条　調査会に座長を置き、調査会に属する委員等の互選により選任する。

２　座長は、調査会の事務を掌理する。

３　座長に事故があるときは、調査会に属する委員等のうちから座長があらかじめ指名する者が、その職務を代理する。

（調査会の開催）

第５条　調査会は、年１回の開催とする。

２　前項に規定する場合のほか、委員等が必要と認めるときは調査会を開催す

ることができる。

（小委員会の設置）

第６条　座長は、必要に応じて、調査会の下に小委員会を設置することができる。

２　小委員会は、当該調査会の審議事項のうち、特に重点的な検討を要する事項の審議にあたる。

３　小委員会の委員は、調査会の委員のうちから座長が指名する。

（議決）

第７条　部会への報告の要否等、議決を行う必要がある調査会の議事は、調査会に属する委員等で会議に出席したものの過半数で決し、可否同数のときは、座長の決するところによる。

（議事の公開）

第８条　調査会は原則として公開する。ただし、公開することにより、委員の自由な発言が制限され公正かつ中立な審議に著しい支障をおよぼすおそれがある場合、又は、個人の秘密、企業の知的財産等が開示され特定の者に不当な利益又は不利益をもたらすおそれがある場合については、座長は、これを非公開とすることができる。

（雑則）

第９条　この規程に定めるもののほか、調査会の運営に関し必要な事項は、部会長が部会に諮り決定するものとする。

平成２５年７月１０日
薬事・食品衛生審議会
安全技術調査会資料

日本赤十字社

資料１

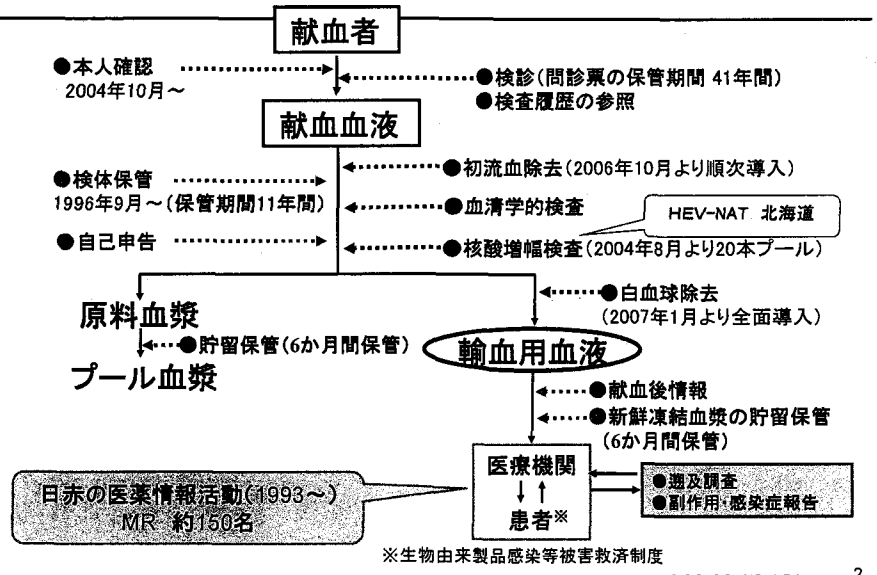
輸血後副作用・感染症報告

2012年まとめ

日本赤十字社
Japanese Red Cross Society

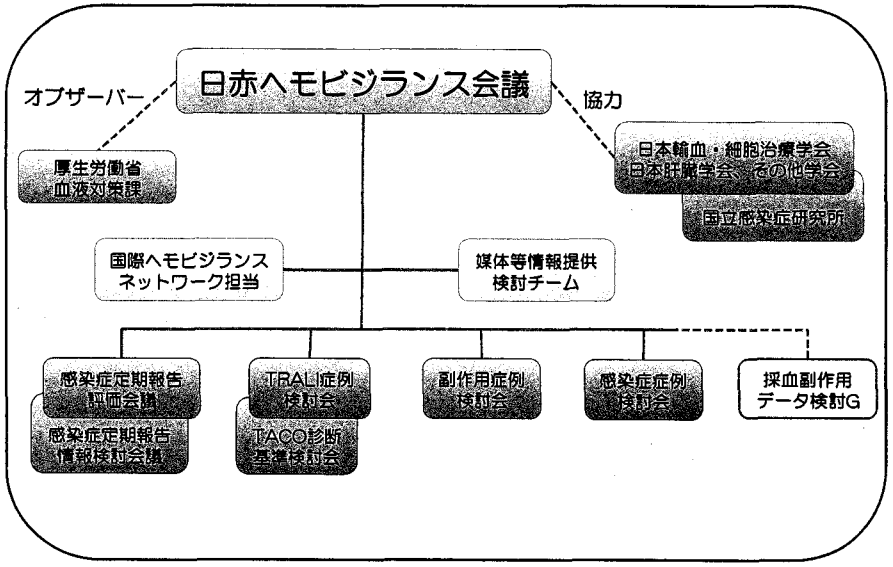
日本赤十字社

日本赤十字社の安全対策



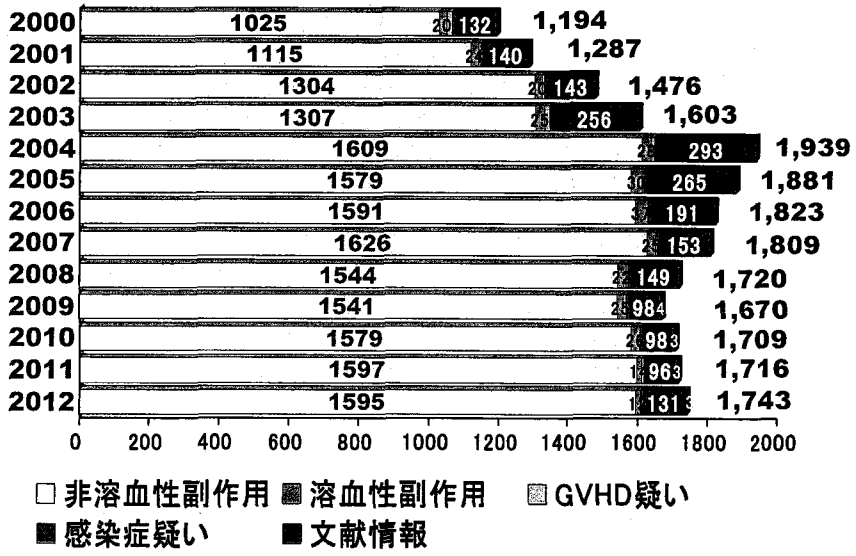
※生物由来製品感染等被害救済制度

JRCS/BSH/SV Div.



医療機関からの臨床データ、患者血液および献血者保管検体の検査結果などから評価

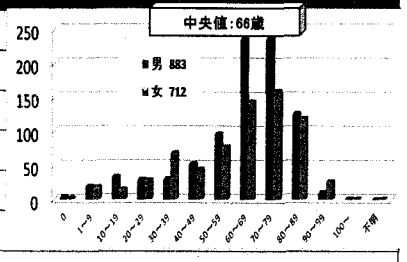
副作用・感染症報告の推移(件数)



非溶血性副作用

非溶血性副作用報告症例の概要(2012年)

2012年 報告件数 :1,595件			
患者性別	男性	883名	
	女性	712名	
患者年齢	66歳	(中央値) (0～102歳)	
非溶血性副作用の分類別内訳			
蕁麻疹等	572	件	(35.9%)
発熱反応	190	件	(11.9%)
ｱﾅﾌｨﾗｷｼｰ(様)反応	156	件	(9.8%)
ｱﾅﾌｨﾗｷｼｰ(様)ｼｮｯｸ	242	件	(15.2%)
呼吸困難	193	件	(12.1%、心原性肺水腫疑い44件を含む)
輸血関連循環過負荷	26	件	(1.6%) *2012年4月報告分よりTACO評価開始
輸血関連急性肺障害	10	件	(0.6%) : TRALI 6、p-TRALI 4
血圧低下	90	件	(5.6%)
その他	116	件	(7.3%)

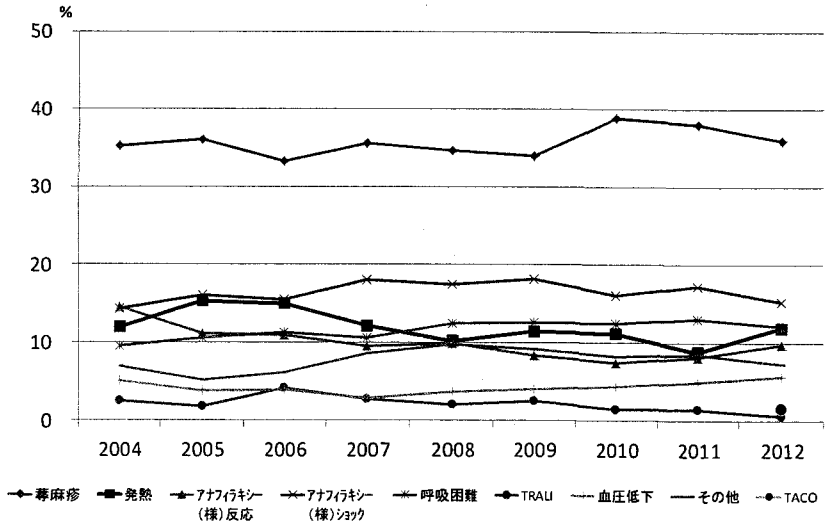


製剤供給数10,000本あたりの副作用報告頻度(2012)

	PC	FFP	RCC
蕁麻疹等	2.96	1.06	0.47
発熱反応	0.69	0.05	0.34
ｱﾅﾌｨﾗｷｼｰ(様)反応	1.17	0.24	0.08
ｱﾅﾌｨﾗｷｼｰ(様)ｼｮｯｸ	1.18	0.72	0.11
呼吸困難	0.68	0.22	0.26
TACO(輸血関連循環過負荷)	0.02	0.01	0.05
TRALI(輸血関連急性肺障害)	0.01	0.01	0.01
血圧低下	0.21	0.13	0.15
その他副作用	0.45	0.05	0.19
Total(件/供給10,000本)	7.38	2.50	1.66

呼吸困難;心原性肺水腫疑い含む

非溶血性副作用報告内訳(症状別) -2004～2012-



Transfusion-Related Acute Lung Injury
輸血関連急性肺障害(TRALI)

概念

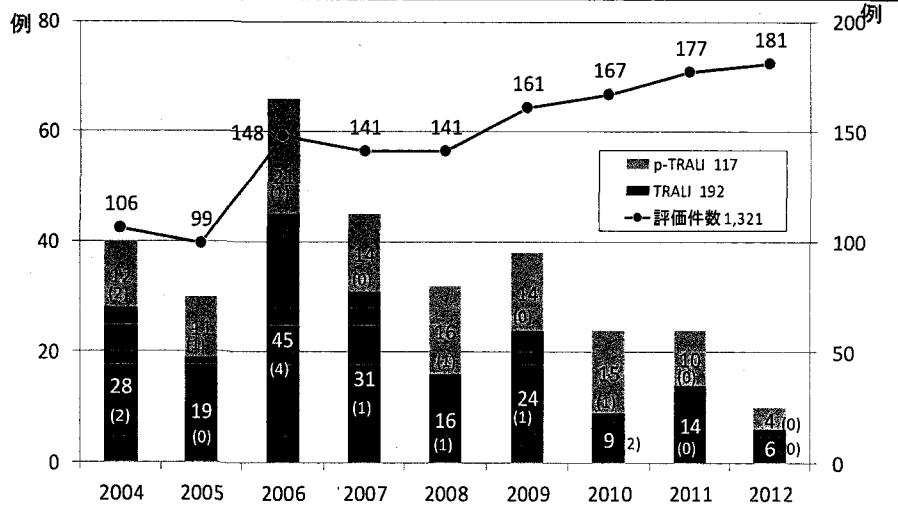
輸血後6時間以内(多くは2時間以内)に急性の非心原性肺水腫を伴う
呼吸困難を呈する重篤な非溶血性輸血副作用
適切な処置をしないと死亡する場合もある

診断基準

- ALI(急性の肺障害)
  - ・急激に発症 ・低酸素血症
  - ・胸部X線上両側肺野の浸潤影
  - ・左房圧上昇(循環過負荷)の証拠がない
- 輸血以前にALIがない
- 輸血中もしくは輸血後6時間以内に発症
- 時間的に関係のあるALIの他の危険因子がない

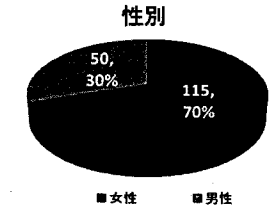
危険因子
・誤嚥、肺炎、有害物吸入、肺挫傷、溺水
・重症敗血症、ショック、多発外傷、熱傷、急性膵炎、心肺バイパス、薬物過剰投与
⇩
possible TRALI

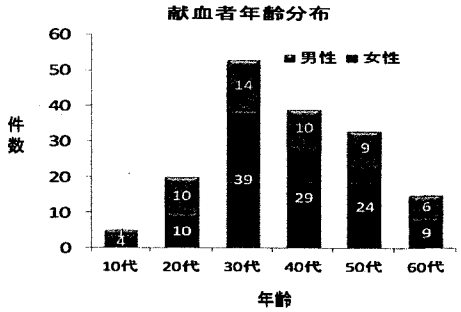
TRALIとpossible TRALI (2004-2012)

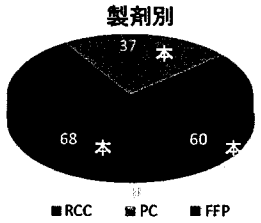


*1人の患者で2回発症(2005年)　　( )内の数字は死亡例

TRALIに係る血液製剤の抗白血球抗体陽性等の内訳







輸血関連循環過負荷TACO
(Transfusion associated circulatory overload)

輸血に伴う循環負荷による心不全であり、呼吸困難、頻脈、血圧上昇などを認める。胸部X線で肺浸潤影など心原性肺水腫の所見を認めることがある。
輸血後6時間以内の発症が多い。

TACO (Transfusion associated circulatory overload)**の暫定的診断基準**
(ISBT working party)

a. **急性呼吸不全**
b. **頻脈**
c. **血圧上昇**
d. **胸部X線上急性肺水腫もしくは肺水腫の悪化**
e. **輸液・輸血の負荷の証拠**

のうち4つを満たす。

輸血終了後6時間以内の発症。

BNPの上昇はTACOの診断の補助となる。

日赤における輸血関連循環過負荷(TACO)評価基準

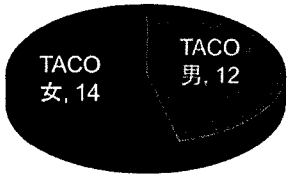
1. **急性呼吸不全 :PaO_2/FIO_2 300mmHg以下又は、SpO_2 90%以下(room air)**
2. **胸部X線上で肺浸潤影を認める。**
3. **輸液・輸血過負荷を認める。**
4. **輸血中・輸血後6時間以内に発症**
5. **血圧上昇**
6. **頻脈**
7. **BNP、NT-proBNP値を参考とする。**

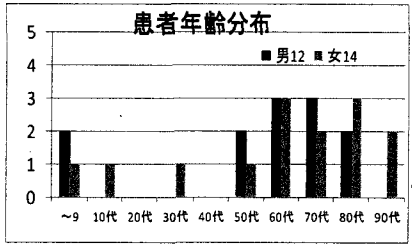
1～4は必須とする。

除外項目
- **透析中の患者**
- **人工心肺使用中・後の患者**
- **補助体外循環装置を使用中の患者**
- **現在治療をしている心不全又は慢性呼吸不全がある場合**

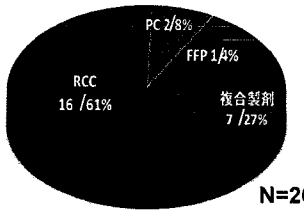
TACO評価内訳 2012

患者内訳





原因製剤



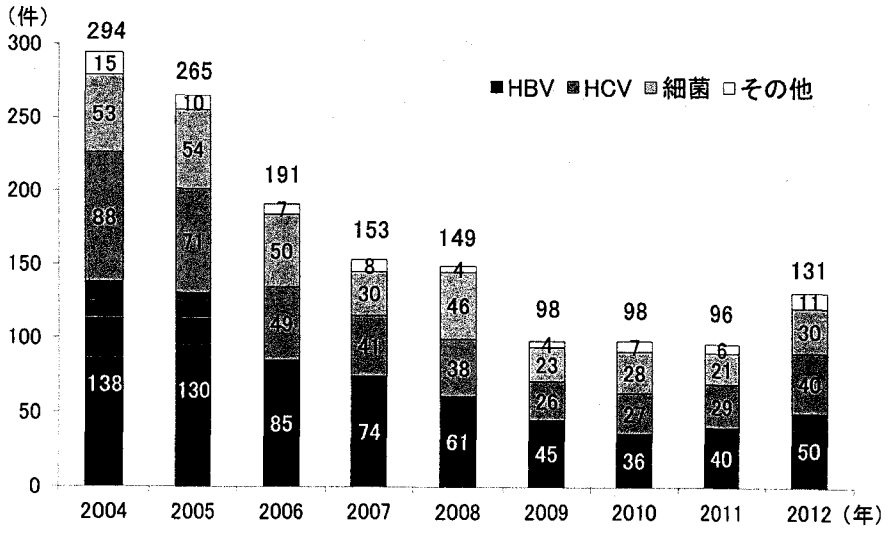
N=26

複合製剤内訳	: 7
RCC+PC	1
RCC+FFP	6

日本赤十字社

感染症

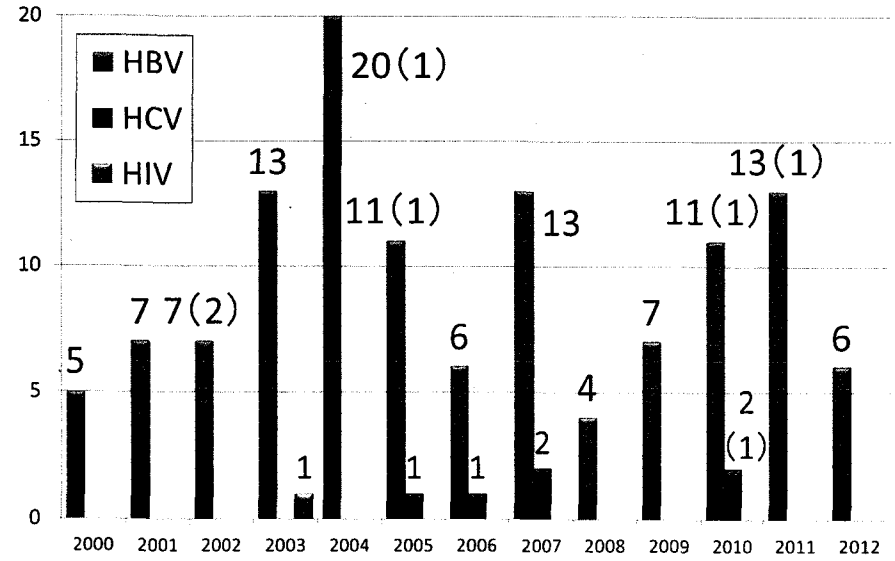
病原体別感染症報告数の推移



病原体別因果関係評価結果(2012)

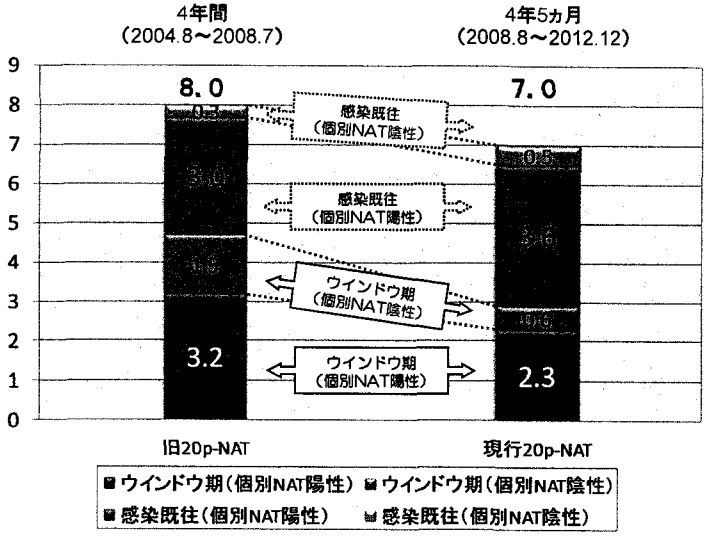
病原体	報告件数	特定
HBV	50	6
HCV	40	-
細菌	30	1
HEV	4	4
CMV	3	-
HBV&HCV	1	-
HTLV-1	1	-
VZV	1	-
真菌	1	-

輸血後感染(HBV/HCV/HIV)症例の推移(報告年)

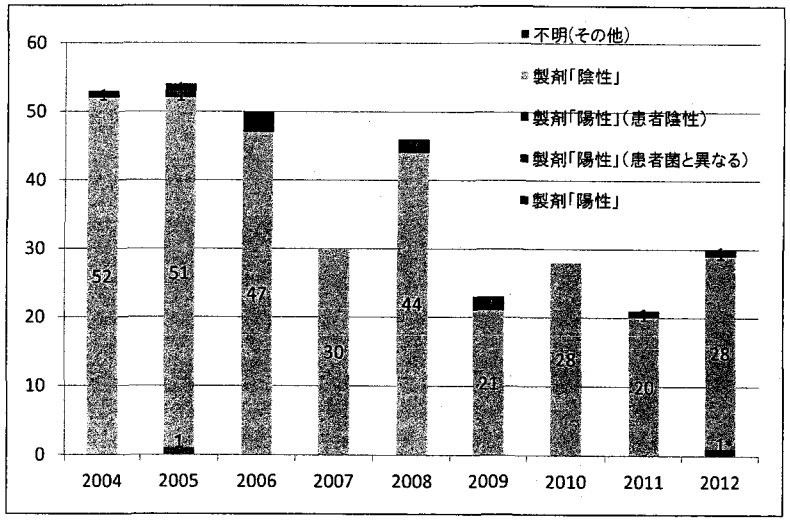


( ) : 同時製造品による感染症例

20p-NAT時期別の1年当たりHBV受血者感染症例数（献血者感染状況別分類）



細菌症例と解析結果



＊医療機関より報告取り下げ

輸血による細菌感染疑い症例

輸血との因果関係が高いと評価された症例（白血球除去製剤）

検出菌	製剤名	報告年(保存日数)
Staphylococcus aureus	PC	2006年(3)、2008年(4)
Streptococcus dysgalactiae ssp. Equisimilis（G群レンサ球菌）	PC	2008年(4)、2011年(4)
Streptococcus agalactiae	PC	2009年(3)
Streptococcus pyogenes（A群溶血性レンサ球菌）	PC	2012年(4)
Serratia marcescens	PC	2009年(4)

- 保存前白血球除去
  - ・血小板製剤 ： 2004年10月
  - ・全血採血由来輸血用血液製剤 ： 2007年1月
- 初流血除去の導入
  - ・血小板製剤 ： 2006年10月
  - ・全血採血由来輸血用血液製剤 ： 2007年3月

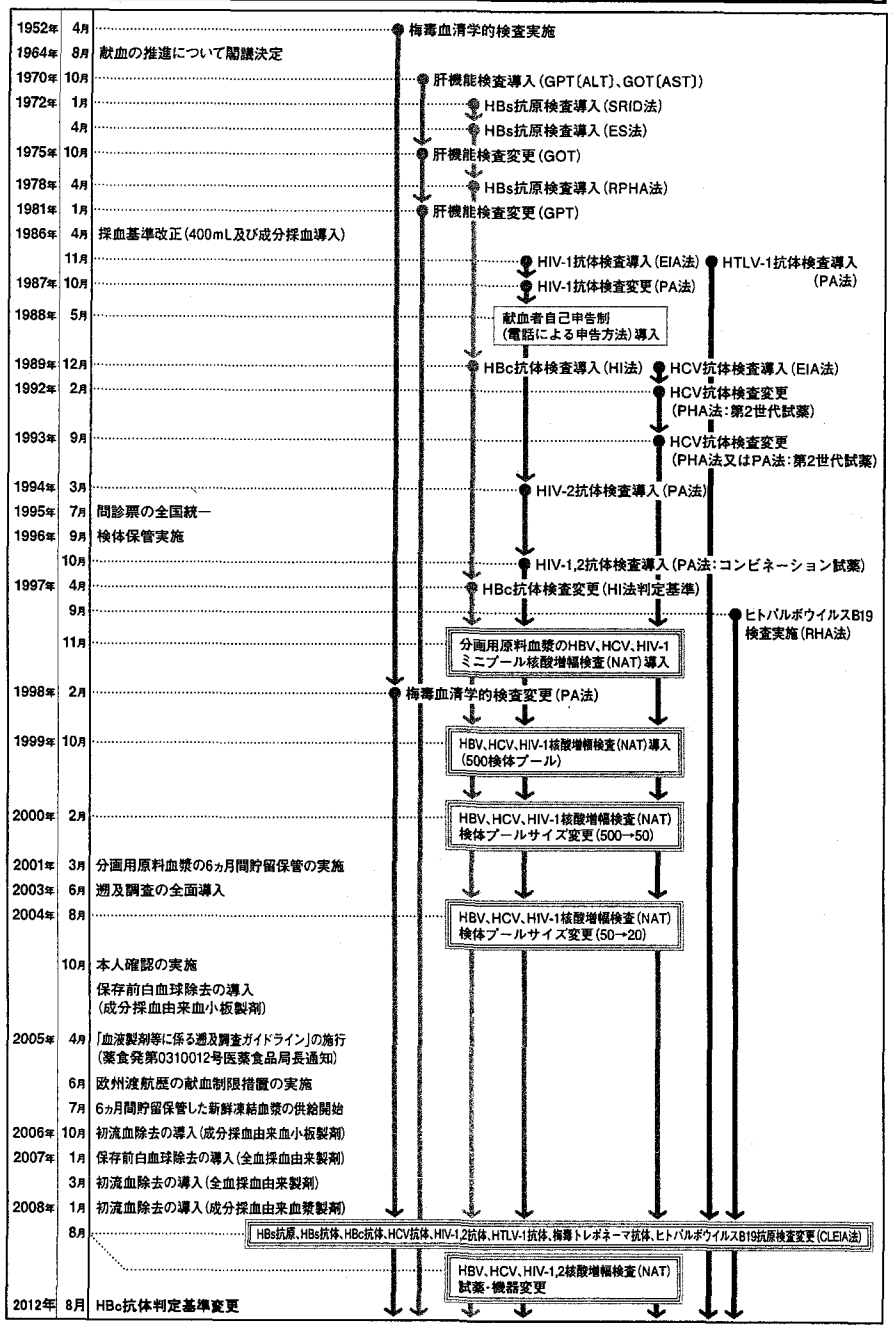
日本赤十字社

日赤ヘモビジランスのまとめ

- 輸血関連急性肺障害(TRALI)の症例は、添付文書への記載以降(2006年)、減少傾向にあり、2011年以降で死亡症例はない。
- 輸血関連循環過負荷(TACO)については、今後添付文書への記載等含めて、医療関係者に周知していく必要がある。
- 輸血後B型肝炎症例は、HBc抗体基準の厳格化により、更に減少していくと考えられるが、安全対策の評価を今後も実施していく。
- 血小板輸血による細菌感染症例は、年に1例程度発症しているが、死亡例はない。 また、保存前白血球除去導入後、赤血球製剤による細菌感染症例は確認されていない。

参考

●血液製剤の安全性確保対策の変遷



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〒105-8521 東京都港区芝大門一丁目1番3号
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医療関係者向け製品情報サイト
URL http://www.jrc.or.jp/mr/top.html
0811

平成 25 年 7 月 10 日
薬事・食品衛生審議会
安全技術調査会資料

資料2

日本赤十字社

シャーガス病に対する安全対策の進捗状況について

1. 安全対策

○平成 24 年 10 月 15 日採血分より開始

- 中南米滞在歴等確認票（別紙 1）の 1～3 のいずれかに該当する方に、献血の受付時に申告をお願いする。
- 該当献血者の血液は、血漿分画製剤用の原料血漿として利用する。（製造制限）

○実施状況

平成 25 年 6 月 4 日現在（全採血者数：3,310,073 人）

分類	調査対象者数	対採血者数比
1.中南米諸国で生まれた、又は育った。	1,328 人 1)	0.04%
2.母親が、中南米諸国で生まれた、又は育った。	188 人 2)	0.006%
3.（1.に該当しない方）で中南米諸国に通算 4 週間以上滞在した。	3,909 人	0.12%
計	5,425 人	0.16%

1）分類 1+2 に該当する人を含む。
2）分類 2+3 に該当する人を含む。

2. 疫学調査

① 実施期間：平成 25 年 1 月 8 日～平成 25 年 6 月 4 日（全採血者数：761,365 人）
　愛知・岐阜・三重・静岡県血液センター先行実施：平成 25 年 1 月 8 日開始
　全センター実施：平成 25 年 4 月 23 日開始
② 検査法：ELISA 法（Ortho 社）

分類	調査対象者	献血者数（延べ数）	調査応諾率
1.中南米諸国で生まれた、又は育った。	448 人 1)	588 人 1)	76.2%
2.母親が、中南米諸国で生まれた、又は育った。	25 人 2)	71 人 2)	35.2%
3.（1.に該当しない方）で中南米諸国に通算 4 週間以上滞在した。	779 人	1,111 人	70.1%
計 （対採血者数比）	1,252 人 (0.16%)	1,770 人	70.7%
検査実施状況	陰性：1,252 人		
	陽性：0 人		

1）分類 1+2 に該当する人を含む。
2）分類 2+3 に該当する人を含む。

3. 今後の予定

① 検査試薬の評価
　疫学調査で日本では承認されていない検査試薬を使用していることから、試薬の評価を実施する。
③ 疫学調査のまとめ
　およそ 5,000 人分のデータが集積された時点でデータを取りまとめ、今後の対応について検討する。

中南米滞在歴等確認票

以下の質問はシャーガス病の安全対策として、輸血を受けられる方の安全を守るためにうかがうものです。
質問に該当される方からいただいた血液は、血漿分画製剤用の原料血漿として利用させていただきます。
（※200mL、400mL 献血にご協力いただいた場合、赤血球成分は輸血用として利用されません。）

該当する質問番号に丸印（○）をつけてください。

1．中南米諸国で生まれた、又は育った。　（国名：　　）

2．母親が、中南米諸国で生まれた、又は育った。　（国名：　　）

3．（1．に該当しない方）で中南米諸国に通算４週間以上滞在した。
（国名：　　期間：　　）
（国名：　　期間：　　）
（国名：　　期間：　　）

＜中南米地域対象国＞

アルゼンチン	スリナム	ブラジル
ウルグアイ	チリ	ベネズエラ
エクアドル	ニカラグア	ベリーズ
エルサルバドル	パナマ	ペルー
ガイアナ	パラグアイ	ボリビア
グアテマラ	フォークランド諸島（英領）	ホンジュラス
コスタリカ	フランス領ギアナ	メキシコ
コロンビア		

代筆者名

【血液センター記入欄】

受付日：	センター名：	施設名：
献血者コード：	採血番号：	

検査：　有　・　無

資料3

平成 25 年 7 月 10 日
薬事・食品衛生審議会
安全技術調査会資料

日本赤十字社

感染性因子低減化技術ミラソルの導入検討に係る考え方

平成 24 年度第 3 回運営委員会（平成 24 年 12 月 19 日開催）において、日本赤十字社が実施したミラソルの評価データと開発メーカーが報告したデータ等の間に、感染性因子に対する低減化能に差異があるとの指摘（別紙 1）があったことから、ミラソルを選択した経緯と併せ、引き続き臨床試験開始に向けた準備を進めていくことについて考え方をまとめた。

1. ミラソル選択の経緯

- 平成 20 年 7 月 23 日に開催された、薬事・食品衛生審議会血液事業部会運営委員会・安全技術調査会 合同委員会において、次の理由によりリボフラビンを用いる感染性因子低減化技術ミラソルの導入に向けた検討について報告した。

【選定の理由】

1）導入目的及び対象製剤
輸血感染症が重篤になり易い細菌感染症対策とする。対象製剤は血小板製剤とする。スクリーニング NAT を実施している HBV、HCV、HIV の輸血感染症についても一層の予防が可能と考えられる。
また、新興・再興感染症についても低減化処理効果がある程度期待できる。

2）添加する薬剤の安全性が高い
現時点で使用可能な低減化技術は、いずれも血液に薬剤を添加する。
ビタミンであるリボフラビンを低減化剤として用いるミラソルの安全性は、他の技術と比べて高いと考えられる。

3）血液事業に導入しやすいシステム
ミラソルは、日赤の血小板製剤の採取・製造工程等を殆ど変更することなく血液事業に導入できる。また、低減化処理工程による製品供給の遅れも小さいことから、血小板製剤の安定供給に与える影響も軽微と考えられる。

- 上記の報告等に対し、血液事業部会（平成 21 年 12 月 24 日開催）において次の事項が了承された。

【血液事業部会における確認事項】

・リボフラビン（ビタミン B_2）を用いた技術を重点的に評価すること。
・当該技術について残された課題の評価を実施すること。

2. ミラソルの低減化能の評価について

感染性因子のリスク評価と低減化技術の効果について検討を行った（別紙 2）。

【検討結果の概要】

- 血小板製剤の細菌汚染に対しては、従前からの初流血除去及び白血球除去等の対策に加え、現在の有効期間を維持しながらミラソルを導入することで、より一層の感染防止効果が期待できると考える。
- 地球温暖化による国内感染も懸念されるデングウイルスに対しては、ミラソルの低減化効果がほとんどないと思われるが、そのような状況では、赤血球製剤、血漿製剤についても対応が求められる。したがって、国内のサーベイランス状況を注視しつつ、「居住地域毎の献血制限」や「NAT による地域限定的なスクリーニングの実施」等の安全対策が必要とされる。チクングニヤウイルス等の場合も同様と考えられる。

3. 結論

- 血小板製剤に対し感染性因子低減化技術を導入する主目的は細菌対策であり、細菌に対する一定の低減化効果が期待できる。
- 現時点でミラソルの検討を中断し、インターセプトに変更することになった場合、必要な機材・資材を準備し、ミラソルと同様にインターセプトも評価する必要があり、低減化技術の導入に一層の時間がかかることになる。
- ミラソルによる低減化能が期待できない感染症があるものの、それらについては、赤血球製剤や血漿製剤への対応を含めた対策を講じる必要があり、NAT 等の導入が有効と考えている。

以上より、他の技術も含め世界的な低減化技術の開発及び導入状況について、今後も情報収集を継続し、ミラソル導入に向け準備を進めていくことにしたい。

Pathogen and Leucocyte Inactivation for IBS/Theraflex®/Mirasol® Platelets

(日赤評価データ追加)

C. V. Prowse:Component pathogen inactivation:a critical review.Vox Sanguinis 2013; 104: 183-199

Viruses

Enveloped Viruses	IBS	THF	MIR	日本赤十字社
HIV-1(cell associated)	>6.1	–	4.5	–
HIV-1 (cell free)	>6.2	1.4	5.9	≧4.6
Clinical isolate HIV-1	>3.4	–	–	–
Clinical isolate HIV-2	>2.5	–	–	–
Latent proviral HIV-1	All detectable*1	–	4.5	–
Hepatitis B	>5.5	–	(2.3)	–
Hepatitis C	>4.5	–	(3.2)	–
HTLV-I	4.7	–	–	–
HTLV-II	5.1	–	–	–
Cytomegalovirus (cell-associated)	>5.9	–	–	–
Cytomegalovirus (cell-free)	All detectable*1	(3.5)	(2.1)	–
BVDV (HCV model)	>6.0	–	5.8	1.9
Duck HBV(HBV model)	>6.2	–	–	–
West Nile Virus	>6.0	5.4	>5.1	1.3
SARS-CoV	>5.8	–	–	–
Chikungunya	>6.4	–	2.1	1.7
Influenza A virus H5N1	>5.9	–	(>5)	–
Dengue	>5.0*2			0.4*2
PRV			2.5*2	2.8*2

Non-enveloped Viruses	IBS	THF	MIR	日本赤十字社

Bacteria

Gram-negative	IBS	THF	MIR	日本赤十字社
Escherichia coli	>6.4	>4.0	4.4	–
Serratia marcescens	>6.7	>4.0	4.0	4.0(平均値)
Klebsiella pneumonia	>5.6	4.8	2.8	–
Pseudomonas aeruginosa	4.5	>4.9	>4.6	–
Salmonella choleraesuis	>6.2	–	–	–
Yersinia enterocolitica	>5.9	–	3.3	–
Enterobacter cloacae	5.9	>4.3	>2.0	–
Orientia tsutsugamushi (scrub typhus)	>5.0	～	>5.0	–

Gram-positive	IBS	THF	MIR	日本赤十字社
Staphylococcus epidermidis	>6.6	4.8	4.2	3～5
Staphylococcus aureus	6.6	>4.8	4.0	☆
Streptococcus pyogenes	>6.8	–	2.2	–
Listeria monocytogenes	>6.3	–	–	–
Corynebacterium minutissimum	>6.3	–	–	–
Bacillus cereus (incl spores)	3.6	–	–	–
Bacillus cereus (vegetative)	>6.0	4.3	1.9	–
Bifidobacterium adolescentis	>6.2	–	–	–
Proprionobacterium acnes	>6.2	4.5	>2.0	–
Lactobacillus species	>6.4	–	–	–
Clostridium perfringens (vegetative)	>6.5	>4.7	–	–

Spirochaete bacteria	IBS	THF	MIR	日本赤十字社
Treponema pallidum (syphilis)	>6.8	–	–	–
Borrelia burgdorferi (Lyme disease)	>6.8	–	–	–

– : no data available

☆:day3(有効期間最終日)の状
対照群(非低減化群)‥8本中7本が10^6 CFU/mL以上
低減化群 ‥‥‥‥8本中4本が培養陰性 他の4本は10^5 CFU/mL未満

Parasites	IBS	THF	MIR	日本赤十字社
Plasmodium falciparum (malaria)	>6	–	>3.2	–
Trypanozoma cruzi (Chagas' disease)	>5.3	–	6.0	–
Leishmania mexicana (promastigote)	>5.0	–	~	–
Leishmania major Jish (amastigote)	>4.3	–	>5.0	–
Babesia microti (babesiosis)	>5.3	–	>4.0	–

Leucocyte	IBS	THF	MIR	日本赤十字社
T-cell viability (limiting dilution)	>5.4	–	>6.0	–
DNA modification (one adduct per x base pairs)	1 per 83	–	–	–
Polymerase chain reaction	Inhibited	–	–	–
Cytokine synthesis:				
No Il-8 or IL-1b synthesis	レ	–	レ	–
Murine model TA-GVHD:				
Prevents disease	レ	–	レ	–

– : no data available

レ : Tick marks show where the different study types have been undertaken for each type of component

別紙 2

感染性因子のリスク評価と低減化技術の効果

既知の感染性因子のうち次のものを評価した。

1）現在スクリーニング検査をしているが、なお輸血感染のリスクが残っていると考えられるもの
2）日本ではスクリーニングをしていないが、輸血による感染が証明されているもの
3）輸血による感染がおこることが考えられるもの

2）、3）の中には多くの感染性因子が含まれるが、その中で特に社会的影響が大きくなる可能性があり、
輸血医療界で討議・検討がなされているものを中心に選択した。

それらのリスクと Mirasol（リボフラビン法）による低減化効果を評価した上で、下記の評価基準により総合的に判定した。

総合判定

A：現状の安全対策及び導入を予定している検査法で、殆どの感染を防止することができる。
B：Mirasol の導入により、感染防止効果が期待できる。
C：Mirasol では感染防止効果が期待できない。NAT の改良もしくは他の低減化法などの安全対策の導入を考慮する必要がある。

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
細菌	● [細菌汚染の頻度] 本邦における PC の細菌汚染の潜在リスクは、4 万あまりの期限切れ PC の培養調査から、臨床的に意味のある細菌に汚染される頻度は、PC 5,400 本に 1 本とされた。これは欧米の頻度とほぼ同等または半分のレベルである。 ● [敗血症のリスク] 汚染された製剤がすべて敗血症を起こすわけではなく、細菌数が少なくとも 10^5 CFU/mL 以上に増殖した極一部の製剤が臨床的に問題となる。 (文献:Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Clin Infect Dis. 2008 Apr 15;46(8):1214-20.) 細菌の種類にもよるが、一般に 10^7 CFU/mL 以上になると症状は重篤となる。 ● [臨床の現場で同定された汚染 PC の頻度] 本邦で、この 6 年間に敗血症の原因となった汚染 PC は 7 製剤である。 原因製剤の保存期間は、採血日を day0 とした場合、day2 の製剤が 2 例、day3(有効期間最終日)の製剤が 5 例であった。 ● 2007 年以降、輸血感染による死亡例の報告はない。初流血除去、白血球除去をしていることに加え、出庫時に外観、スワーリングの有無を確認し、諸外国よりも有効期間を 1～2 日短く規定していることによると推定される。	● [スパイク実験による評価] (日赤データ) ・実際に混入すると推定される量の *S. aureus* (55～1400 CFU/バッグ)を、2 本ずつ同条件とした 8 組(合計 16 本)の PC にスパイクした実験の結果。 day3(有効期間最終日)の状態 対照群 (非低減化群) 8 本中 7 本が 10^6 CFU/mL 以上、1 本は 10^4 CFU/mL 低減化群 8 本中 4 本が培養陰性 他の 4 本は 10^5 CFU/mL 未満 ・*Serratia marcescens* および *S. epidermidis* に対しては、約 4 Log の低減化が得られている。 ● [開発企業データ] 13 種 20 株の細菌を 20～100 CFU/バッグの濃度で PC に接種し 7 日間培養した結果。 多くの文献や報告から、敗血症を起こした菌の頻度を考慮に入れると、91%の細菌感染症例に有効であると結論付けた。 (文献:A laboratory comparison of pathogen reduction technology treatment and culture of platelet products for addressing bacterial contamination concerns. Goodrich RP, Gilmour D, Hovenga N, Keil SD. Transfusion. 2009 Jun; 49(6): 1205-16.) ● Mirasol の導入により完全な不活化ができる訳ではないが、現在の有効期間内には臨床的に問題となる菌量に達しないことが予想されるので、感染防止効果が期待できる。	B

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
HBV	[理論的推計] ● HBc 抗体判定基準の見直しにより、オカルト HBV 感染による感染はほぼなくなると思われる。 ● 近い将来、現行の 20 プール NAT から新たな個別 NAT (ID-NAT) へ移行する予定であり、ウィンドウ期の献血による感染も減少することが期待される。過去 10 年間のウィンドウ期の PC による輸血感染症 (TTI) の原因製剤の 60%が ID-NAT 陽性であったことから、ヘモビジランスで日赤が把握する PC による HBV 感染確定例はこれまでの 40%以下に減少する。 ● HBV のウィンドウ期の長さ、PC 供給数などから、ID-NAT 陰性のウィンドウ期に存在しうる PC の数は 1 年間に 11.5 バッグと推定される。ID-NAT をすりぬける HBV 陽性の 1 バッグ(血漿量約 200mL)に存在する HBV は、最大で約 4,000 コピーと推定される (Roche 社の 95%平均検出感度(LOD) (18.6 コピー or 3.2IU/mL) による)。 [日赤ヘモビジランス] ● 日赤が把握している HBV 感染確定例のうち、ID-NAT 陰性の血小板を原因とするものは 0.9 症例／年である。	[リスクを有するドナーに対する効果] ● 4,000 コピー/バッグの PC に Mirasol (低減化能 (LRV) 2.3 Log) を適用すると、20 コピー/バッグとなり、ウイルスがわずかに残存する。 ● 一方、PC に含まれるウイルス数がこのウィンドウ期 25 日間に viral load の低いものから高いものまで均等に分布すると仮定すると、約 60% (年間約 6.5 バッグ) 中の HBV は完全に不活化される。 ● 残りの約 40%の PC (年間およそ 5 バッグ) において、不活化されない HBV が 1 コピー/バッグ以上残ることになる。ただし、これら少数のウイルスが残存した PC の感染性は不明である。 [ヘモビジランスへの効果] ● ID-NAT 導入後のリスクは 0.9 症例／年となるが、Mirasol によりその半数以上が防止できる。	B
HCV	[理論的推計] ● ID-NAT が施行された状態で残るリスクは、pre-ramp up phase(感染後末梢血中のウイルス量が急激に増加するまでの期間)にある血液と、そこから ID-NAT 陽性までの期間にある血液であるが、前者は感染性がほとんどないことが動物実験で示された。 (文献:Infectivity in chimpanzees (Pan troglodytes) of plasma collected before HCV RNA detectability by FDA-licensed assays: implications for transfusion safety and HCV infection outcomes. Busch MP, Murthy KK, Kleinman SH, Hirschkorn DF, Herring BL, Delwart EL, Racanelli V, Yoon JC, Rehermann B, Alter HJ. Blood. 2012 Jun 28;119(26):6326-34.) また、後者の期間は極めて短く (約 2 日)、その間にありうる献血は 0.2 例未満／年と推定される。PC だけを考えれば 0.03 例未満／年となる。 [日赤ヘモビジランス] ● 現行 20 プール NAT 導入以降、4 年間、輸血感染例はない。	● 理論的には、ID-NAT 下では 30 年に 1 例の HCV 汚染 PC が出ると推定される。 ● Mirasol による BVDV の低減化能 (LRV) は 1.9(日赤データ)であるが、ID-NAT でも検出不可の極微量のウイルスに対しては低減化効果が期待される。	A

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
HIV	[理論的推計] ● 感染から20プールNAT陽性までのウィンドウ期は約11日で、抗体検査陰性・20プールNAT陽性の献血数から計算すると、この期間内に献血が2.0例／年の割合で存在すると推定される。 ● ID-NATになるとウィンドウ期は若干短縮するが、リスクのある献血の数は大きく変化しない。このうちPCの陽性例は供給数から計算して0.3例／年ほどである。しかしID-NAT陰性の血液を原因とする感染は世界的にも報告されていない。 [日赤ヘモビジランス] ● 20プールNAT導入(2004年)以降、輸血によるHIV感染例はない。	● ID-NAT下で、3.3年に1例のHIV汚染PCが出ると理論的には推定されるが、感染例は報告されていない。 ● ID-NATでも検出不可のHIVのPC中のviral loadは最大4,000コピー／バッグである(現行ID-NATの95%LODを42IU/mLとして計算)。 ● ID-NAT下で、Mirasol（LRV≧4.6：日赤データ）により、十分に不活化される。	A
ヒトパルボウイルスB19	● 世界のどの地域においても、成人の半数以上は感染の既往がある。急性感染にある献血者もほとんどが無症状である。 ● 輸血感染を起こすと、リスクを有する患者（赤血球造血の盛んな患者、免疫抑制患者など）で一時的な骨髄無形成クリーゼ（aplastic crisis）を起こすが、後に回復する。上記の状態にない患者での輸血初感染では、軽度の貧血・発熱・発疹などの比較的穏やかな症状を認めることがある。 ● 著明なウイルス血症は通常2～3週間で終息するが、低レベルのウイルス血症は3年にも及ぶ場合がある。ウイルス血症は極期で10^{12}コピー/mLに達する。 ● 日赤では、CLEIAによる抗原スクリーニングを行っている。感度は$10^{6\sim7}$コピー/mLであり、それ以上の血液は排除されている。 ● 輸血感染を起こした血液製剤で、ウイルスレベルが判明しているもののうち最低のものは10^{3}コピー/mLレベルであった。(文献:Symptomatic parvovirus B19 infection caused by blood component transfusion. Satake M, Hoshi Y, Taira R, Momose SY, Hino S, Tadokoro K. Transfusion. 2011 Sep;51(9):1887-95)	● Mirasolによる LRVは>5 Logとされている。10^{7}コピー/mLのPCを低減化すると、10^{2}コピー/mLとなり、最低感染濃度 10^{3}コピー/mL以下となる。 ● MirasolによりヒトパルボB19に対する安全性は高まると推定される。	B

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
CMV	● CMV感染が懸念される患者への抗体陰性血の輸血により、感染はほとんど予防されると推定される。一方、日赤では全ての製剤に保存前白血球除去を導入しており、それ単独でも抗体陰性血輸血と同程度に感染が防御されている。両者を組み合わせた場合には更に安全性が高まるといわれている。 ● ウィンドウ期の血液が感染を起こす可能性が論じられている。ただし日本ではそのような報告はない。	● Mirasolの cell associated CMVの低減化データはないが、cell freeのCMVの低減化能は2.1 Logとされている。左記の理由により残存リスクは非常に低い。	A
HEV	● ウイルス血症ドナーの頻度；北海道では約 8,500 人に一人の割合であり、*genotype 3* が 93%、*genotype 4* が 7%程度検出される。北海道以外では genotype 4 は少ないとされるが正確な数字はない。 ● Passive surveillance によれば、HEV 陽性血液が輸血された場合に感染が成立する確率は 1.1%前後と推定される（ウイルス血症ドナーは推定 675 人／年、確定された感染例は 1.2 例／年。ヘモビジランスは 6 分の 1 のみ捕捉すると仮定すると 1 年に 7.2 例感染)。 潜伏期は 3～8 週、ウイルス血症は 4～6 週持続し、genotype 4 で最大 10^{7} コピー/mL に達する。 フォローできた献血者 31 名中、ALT レベルが 45 を越えた者 52%、100 を超えた者 35%。 ● 輸血感染を起こした血液製剤で、ウイルスレベルが判明しているもののうち最低のものは 10^{3} コピー/mL レベルであった。 ● 本邦での輸血感染による死亡例は報告されていない。輸血感染例の ALT 最高値は genotype3 で 1,336IU、genotype4 で 1,665IU。 ● 文献上は、移植患者などでの慢性化、肝硬変への進行などが報告されている。	● Mirasol の低減化能は、genotype 3 について≧3Log(日赤データ)が可能（LRV の上限値はまだ不明)。 ● 過去 4 年間の HEV-NAT 陽性献血のうち、96.4%を占める 10^{5} コピー/mL 未満の製剤については、感染例をほぼ無くすことができる。	B

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
WNV	● High viremia を呈するのは鳥類である。日本でアウトブレイクが起こるには、感染した渡り鳥が日本に飛来し、それを吸血した蚊が生き延びることが条件となる。日本にいる蚊の多くが媒介蚊（イエカ、ヤブカなど）であるため、アウトブレイクが起こる可能性がある。哺乳類はウイルス血症のレベルが低いため通常、終末宿主となる。すなわち、蚊ーヒトー蚊の感染環は存在しない。 ● 感染して発症するのは15～20%、脳炎など重症に至るのは1%未満とされる。 ● 感染後5～6日で最大10^5コピー/mLレベルのウイルス血症となり、通常10日で10^1コピーレベルに下がる。 米国でNAT導入前、28例の輸血感染が報告された。米国赤十字社では、NATにより2003年から2010年までに少なくとも1,200人のウイルス血症の献血者が同定された。米国のNATは最初プールで開始されたが、その後breakthroughが見つかったため、感染の高浸淫地域・時期の時点ではID-NATを施行するようになった。 ● ヒトでのviremiaのレベルは低く、時にID-NATを必要とするレベルである。	● WNVは株の違いにより低減化効果に違いがある。 ヒト由来株； 3.0-4.0 Log (NY-99-Flamingo、USA-CDC) ≧5.1Log (Uganda1937、Bonfils lab.民間検査機関) トリ由来株； 1.3Log (New York、日赤) 1.5Log (NY-99-4122、Colorado State Univ.) ● 対策として、渡り鳥を除く感染媒体（人、蚊、野鳥）ごとに居住地域等による献血制限とNAT#を準備している。 #:NATの感度-Novartis PROCLEIX System 95% CL、8.2copies/mL ● 海外において現行ID-NAT体制下で輸血感染例は2010年の1例のみの報告である。Mirasolにより、ヒト由来株に対しては低減化効果が認められるので、感染防止効果が期待できる。	A

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
Dengue	● 自然宿主となる動物は不明。日本での媒介蚊は、通常見られるヤブカ *Aedes albopictus*（ヒトスジシマカ）であり、日本においてもアウトブレイクの可能性がある。 ● ヒトで高いviremiaを呈するので、蚊ーヒトー蚊の感染サイクルができる。オーストラリア・クイーンズランドやシンガポール国内などでアウトブレイクが起こるが地域は限定されており、現地住民による季節的な献血の中止が施行されている。 ● 輸血感染例は3事例5症例のみである（シンガポール、ホンコン、プエルト・リコ）。 ● 輸血感染が少ないと考えられている理由： 1） 蚊の唾液を介することが感染性を高める可能性がある。したがって直接的な輸血による感染性は低い。（仮説） 2）浸淫地域では受血者の多くがすでに免疫抗体を持っている。 3）同時に輸血される血液が中和抗体を持っている。 4）免疫が抑制されている患者では症状が軽い可能性がある。 ただし、浸淫地域ではデング以上に大きな問題があり、よく調査されていない。 ● 初回の感染では5～8割が無症状である。serotypeに特異的な免疫が長期間持続し、異なったserotypeのウイルスに感染し交差反応的に免疫応答が起きたときに重症化する。 ● ウイルス血症は10^5～10^9コピー/mLとされている。NAT試薬は商業ベースで開発中。米国赤十字により、プエルト・リコで試験的にNATが施行されている。	● Mirasolによる低減化は0.4 LRV（日赤データ）と低い。（オーストラリア赤十字から1.2～2.0 Logの低減化能が報告されている。2012年） ● 総じて、低減化には非常に抵抗性が高く、また力価の高いウイルス血症であるため、低減化/不活化の対象となりにくい。むしろ当該地域居住者の献血制限や、NATによるスクリーニングが適していると考えられる。	C
Chikungunya	● 家畜、鳥類、爬虫類に感染するとの情報がある。ウイルスの突然変異により、日本にもいるヒトスジシマカにも感染するようになったと言われる。 ● ウイルス血症について：無症候性患者では8×10^1～3×10^5コピー/mL(中央値3.4×10^3コピー/mL)、症状のある患者では2×10^1～2×10^8コピー/mL(中央値5.6×10^5コピー/mL)。 ● 2012年11月の時点において輸血感染例の報告はない。	● Mirasolの低減化能：日赤データで1.7LRVの低減化が可能。感染doseが不明であるが、Mirasolによる低減化だけでは安全性は保証できない。 ● 日本でも感染が広がる可能性があるが、蚊が媒介するため感染地域が一気に拡大する可能性は低く、当該地域居住者の献血制限が有効と考えられる。	C

感染性因子	リスク評価	対策及び低減化技術の効果	総合判定
Babesiosis	● 赤血球に寄生する原虫。ネズミなどが自然のリザーバーで、ベクターはダニ（マダニ）である。そのダニが直接ヒトを咬むか、またはダニがとりついたシカなどの野生動物にヒトが接触した際にヒトにとりつくと思われる。マダニは日本全土に分布する。 ● 咬傷から1～9週で発症。症状は無症候からかぜ様症状、高熱、溶血、さらに腎不全・ARDS（急性呼吸促迫症候群）・DICによる死亡まで幅広い。死亡率は3～20%。治療手段はあるが、新生児、老人、免疫抑制患者、脾摘患者でリスクが高い。 ● 赤血球製剤中では4℃保存で35日生存する。白血球除去、放射線照射は無効。米国での調査では160例以上の輸血感染が確認され、直近5年間に赤血球製剤輸血によるバベシア感染死亡例が10例報告された。血小板製剤では、その中に含まれる感染赤血球によって感染を起こしうる。寄生虫血症は数カ月から2年以上続くとされる。無症候性キャリアが輸血感染を起こしてきたものと思われ、大きな問題となっている。 ● 無症候性キャリアでの血中濃度や輸血感染を起こす最少濃度などは不明。ただし、マラリアから類推すると感染性は非常に高いと思われる。スメアによる鏡検の感度は低い。PCRの感度はスメアとほぼ同じかそれ以上である（マラリアのPCRの感度は20コピー／mL）。抗体陽性者の半数はPCR陰性である。抗体検査は、寄生虫血症ドナーと、治癒し感染性を持たないドナーを区別できない。PCR、抗体検査とも種間の交差反応性が低い。IF法は感度が高いが、処理能力は低い。現時点でライセンスを受けた検査法はない。 ● 米国では地域による発生率に大きな差があり、その地域に限定した献血制限や検査などの対応が合理的であろう。日本固有と思われるバベシア原虫は北海道を含めた日本全体に分布する。 ● ダニ咬傷や既往に関する問診は感度・特異性とも低いことが示されている。 ● 日本で発症が確認されたバベシア症例は輸血を原因とする1例のみであり、原因となった献血者血液中のDNAコピー数は、（<10^2コピー／20μL）→（5×10^3コピー／mL）であった。	● 無症候の献血者血液中のDNAコピー数を10^2コピー／20μLと仮定すると、全血400mLには2×10^6の原虫が存在することになるが、殆どが赤血球中に存在する。 ● 血小板製剤中には極僅かの赤血球しか混入しないため、血小板製剤中に存在する原虫の量は極めて少ないものと考えられる。 ● Mirasolによる低減化は>4Logとのデータが得られており、血小板製剤については感染防止効果が期待できる。	B

（別紙1－参考文献）

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Component pathogen inactivation: a critical review

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Background and Objectives Pathogen inactivation (PI)-treated plasma and platelets are increasingly becoming the products of choice, where licensed. This review summarizes the clinical evidence available for licensed component PI technologies and red cell PI under development.

Materials and Methods Available literature on licensed technologies was reviewed.

Results For the plasma and platelets technologies available, evidence for the inactivation of most pathogens is good, except for certain nonenveloped viruses. Clinical trials and haemovigilance programmes suggest the observed loss of potency is of little clinical significance, with some technology-specific exceptions. Concerns over adverse toxicological effects or neoantigen formation have not been confirmed for currently licensed products.

Conclusion While platelet PI has been adopted to reduce bacterial contamination, the ability of PI methods to replace testing for emerging bloodborne infections, or as a substitute for selective pathogen testing, gamma-irradiation or even leucodepletion, make adoption of PI for components increasingly attractive.

Keywords: clinical trial, fresh-frozen plasma, pathogen inactivation, platelet components, red cell components, review.

Introduction

Pathogen inactivation (PI) technology for fresh-frozen plasma was described in 1991 [1] with techniques for platelet and red cells (RBCs) developed more recently. The amotosalen/UVA-based INTERCEPT™ (IBS: Intercept Blood System, Cerus Corporation, Concord, CA, USA) was CE marked for platelets in 2002 and 2006 for plasma and is the most widely adopted labile component PI. Other technologies for platelet and plasma PI have since emerged [2]. This review summarizes the PI technologies for plasma and platelets, the concerns and toxicology studies associated with each, and their impact on pathogens and products, followed by the clinical trial outcomes for each product. This is followed by concluding remarks on recent events and regulatory aspects, cost-effectiveness and future prospects including RBC PI.

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The potential of pathogen inactivation for labile blood components

PI technology should ideally be inexpensive, kill a wide range of pathogens, with no toxicity or effect on product potency. Countries are increasingly adopting plasma and platelet PI because the benefits of a safer blood supply outweigh the impact on component potency and viability [3, 4]. To assess this balance, well planned *in vitro* and clinical studies are essential.

PI processing should reduce the need for additional pathogen testing, minimize residual infectious risks (bacteria, parasites, etc.) and remove the need for selective testing (e.g. CMV serology) and processing (e.g. gamma-irradiation). It should also enable proactive protection of the blood supply against emerging infections.

Technical options

Table 1 lists current PI technologies for labile blood components. Most PI technologies rely on targeting nucleic acids (NA) to kill pathogens and inactivate white cells.

Table 1 Technologies for Component PI (licensed or in clinical development)

Product	Company	Compound/Technology	Trial/Licence Status
Platelets	Cerus	Amotosalen + UVA light[a] *INTERCEPT™*	CE marked May 2002 (Class III)[b] (buffy coat & apheresis to 7d storage)
Platelets	TerumoBCT	Riboflavin & UV light[c] *Mirasol®*	CE marked Oct 2007(class IIB), (buffy coat & apheresis to 5d storage)
Platelets	Macopharma	UVC (254nm) light *Theraflex®*	CE marked 2009 (class IIB). At phase II/III trial.
Plasma	Cerus	Amotosalen + UVA light[1] *INTERCEPT™*	CE marked Nov 2006 (Class III)[d]
Plasma	TerumoBCT	Riboflavin + UV Light[c] *Mirasol®*	CE marked Aug 2008(class IIB)
Plasma	Macopharma	Methylene blue + visible light[e] *Theraflex®*	CE marked 2001 (class IIB), 2004 (class III)[e,f]
Plasma	Octapharma	Solvent Detergent[g] *OctaPlas®*	Licensed 1998 (UK) (pools)[h]
Plasma	VIPS (Colombier,Switzerland)	Solvent Detergent[g]	CE marked 2009 (Single donor or minipools)
Red Cell	Cerus	S-303[g] *INTERCEPT™*	Phase III planned (redesign after 2003)
Whole Blood	TerumoBCT	Riboflavin & UV light *Mirasol®*	Trials on whole blood planned
Whole Blood	Cerus	S-303[g]	Under development

[a]Photochemical method [5].
[b]French National authorization (AFSSAPS; now ANSM) 2003, German national authorization (PEI) 2007, Swiss national authorization (Swissmedic) 2009.
[c]Photodynamic method. MB plasma processing is also available for single donations of plasma on a contract basis via Grifols, Barcelona, Spain using the original Springe technology [12, 15].
[d]French National authorization (AFSSAPS) 2006, German national authorization (PEI) 2011, Swiss national authorization (Swissmedic) 2010.
[e]German national authorization 2011. Also produced by Grifols S.A. Barcelona, Spain
[f]Swiss national authorization (Swissmedic) 2008.
[g]Chemical method.
[h]Also produced by Bordeaux CRTS, France.
The Mirasol® 'riboflavin plus UV' technology is reported to use a range of UV wavelengths that extends from 265 to 370nm, across UVA, UVB and UVC regions [6, 7].
CE marking by the medical devices directive class IIb route just requires declaration a product conforms to a standard, to which it is inspected. Class III registration requires regulatory review of pre-clinical and clinical data to define how the product may be used.

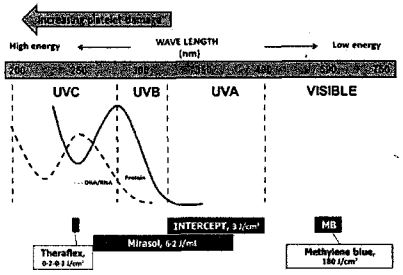

Fig. 1 Comparison of Energy and Dose for Platelet and Plasma PI Photoinactivation Technologies. Due to the absorption spectrum of proteins and nucleic acids shown, UVC radiation is directly damaging to blood components at high doses. This impact is progressively less for UVB, UVA and visible light. The range of wavelengths and dose of radiation used in Theraflex, Mirasol, IBS and MB methods is shown at the bottom as the darker boxes.

rendering the product incapable of causing graft-vs-host disease (GvHD) or of generating the cytokines causing febrile reactions. Additionally, leucocyte inactivation may reduce patient alloimmunization and complement or even replace leucofiltration. The robustness of PI technologies and their effect on blood components differ significantly. For photoinactivation, choice of dose and wavelength also determine the extent of loss of blood component functionality (Fig. 1).

IBS technology for plasma and platelets uses a synthetic psoralen, amotosalen, which after binding NA and only upon activation by UV-A light (320–400 nm; 3 J/cm^2), cross-links NA in an oxygen-independent manner (photochemical reaction) [5, 9]. Alternative photoinactivation technologies for platelet and plasma (Mirasol® 'riboflavin plus UV'; TerumoBCT,Lakewood,Colorado) [6–11] or plasma (methylene blue (MB) plus visible light, Macopharma, Tourcoing, France) [12, 13] PI depend on the generation of reactive oxygen species (ROS) for their action

(photodynamic reactions) [14, 15]. ROS have a limited life span reacting indiscriminately with adjacent molecules. MB binds NA tightly focusing generation of ROS around NAs. Mirasol® technology uses a broad-spectrum of UV (265–370 nm: 100% of UVB, 20% of UVC and 60% of UVA regions; 6·2 J/mL), the lower NA-binding of riboflavin favouring ROS generation in free solution. Theraflex® (Macopharma, Tourcoing, France) relies on narrow bandwidth UVC light (0·2 to 0.3 J/cm^2, 254 nm) to achieve PI by formation of pyrimidine dimers in NA, while limiting ROS-mediated side reactions [16, 17].

PI companies initially used proprietary additive solutions and apheresis machines. To encourage adoption, bags allowing splitting into two units [18], use of alternative platelet additives [19], harvesting on different apheresis instruments [20, 21], platelet treatment in 100% plasma [22, 23] and freezing plasma before PI [24] have been validated.

SD treatment for plasma PI inactivates lipid-enveloped pathogens by physical disruption and cannot be used on cellular products [1, 25].

Concerns about pathogen inactivation and how they have been addressed

Although cost and impact of PI on potency are issues, concern has focused on possible toxic effects and incomplete PI, particularly for large pool products, where failure could result in multiple doses of infectious product. This can be addressed by screening donations for agents for which a PI technique is ineffective, as done for Hepatitis-A and parvovirus-B19 for OctaPlas® (Octapharma AG, Lachen, Switzerland). Most PI methods cannot inactivate bacterial spores. However, Störmer demonstrated two of three bacteria tested (*B. subtilis* and *B. cereus*, but not *C. sporogenes*) emerge, after incubation in blood components, from spores into PI-susceptible vegetative forms [26]. Similarly, Schmidt *et al.* [27, 28] reported IBS technology was effective against a panel of bacteria at 100 or 1000 cfu per unit in platelets obtained as buffy coats or by apheresis, exceptions being seen for two bacteria (*K. pneumonia*, due to the high-stock titre used in the study, and *B. cereus*). Importantly, spore-forming *B. cereus* was inactivated in all units at 100 CFU and at 1000 CFU for apheresis platelets. In any case, PI would prevent the vast majority of clinically significant bacterial complications.

Toxicological concerns should be addressed preclinically using *in vitro* and *in vivo* tests [29, 30]. Table 2 shows the tests used, within ICH guidelines, to study amotosalen, S-303, riboflavin and components treated with them, and the observed safety margin where applicable. In the most relevant acute and repeat-dose models, safety margins of at least 100-fold (clinical dose vs maximum

Table 2 Toxicology Studies reported for IBS & Mirasol® PI technologies (Safety Margins) [11,18,29-35]

	Platelets [11, 29, 31, 34]		Plasma [11, 30, 34]		Red Cell [32]
Study Type	IBS	Mirasol	IBS	Mirasol	IBS
Acute Toxicology	✔ (>1120-fold)	✔	✔	✔	✔
Repeat Dose (1 month)	✔	~	✔	✔	✔
Repeat Dose (3 month)	✔ (>350-fold)	✔	✔ (>100-fold)	~	✔
General Pharmacology	✔ (>350-fold)	~	✔ (>100-fold)	~	✔
Reproductive toxicology	✔ (>350-fold)	~	✔ (>100-fold)	✔	✔
Genotoxicity	✔ (>200-fold)	✔	✔ (>50-fold)	~	~
Carcinogenicity	✔ (>350-fold)	~	✔ (>300-fold)	~	~
Phototoxicity	✔ (> 40-fold)	~	✔ (>200-fold)	~	~
Neonatal toxicity [31, 34]	✔ (>48-fold)	~	✔	✔	~
Absorption, distribution, metabolism, and excretion (ADME)	✔	~	✔	✔	✔
Occupational Safety	✔	✔	✔	✔	✔
Neoantigens [11, 33, 35]	None detected	None detected	None detected	None detected	None Detected in revised format (20mM glutathione)

Tick marks show where the different study types have been undertaken for each type of component (~ denotes the absence of published data). Safety margins are the ratio of the minimal toxic dose observed in the specified test relative the expected human exposure, using a treated blood product and are shown in brackets. In many instances, the highest exposure was defined by the volume limits for the transfusion of a treated blood product. Additional safety margins were obtained by the transfusion of the parent compound amotosalen in the same animal models. They are expressed relative to an estimated clinical dose of 1 μg amotosalen/kg for a 350 ml platelet unit or 4 μg/kg for a litre of treated plasma. For *in vitro* assays, margins are estimated based on a final concentration of 1 ng amotosalen /ml (platelet) or 12 ng/ml (plasma) in the product. For Mirasol products, doses given approximated to human doses and were nontoxic so estimation of a safety margin is not appropriate. For riboflavin itself, the safety margin has been estimated at 649-1299 [11, 34].

dose tested) were observed for amotosalen-treated blood products, with margins ten times higher for amotosalen, as it can be given at higher concentrations. In the majority of tests, no toxicity was observed at the highest doses tested. Similar safety was observed for carcinogenicity and genotoxicity [29], with lower margins (>40) in the less clinically relevant phototoxicity tests [29–31]. No evidence for the formation of novel antigens by PI was found for amotosalen-treated plasma or platelets in preclinical studies or using samples from patient trials [33]. Available data, although less rigorous (Table 2), suggests a similar absence of toxic effects and neoantigens for Mirasol® technology [11, 34, 35].

Impact of processing on pathogens and product potency

Table 3 summarizes PI for the IBS, Theraflex® and Mirasol® platelet processes [11, 16, 18, 36, 37]. Similar data are reported for amotosalen-plasma and S-303 processes [22, 34]. The extent of PI is technology-specific [38]. Generally, PI is effective against lipid-enveloped viruses, parasites and bacteria, but less so for nonlipid-enveloped viruses and bacterial spores. The observed differences in PI largely relate to:

(1) which nonlipid-enveloped viruses are susceptible to inactivation, for example SD is ineffective against these,
(2) the ability of any sensitizers or radiation used to penetrate component or cells, for example MB is ineffective on cellular pathogens due to its reduction to inactive forms within cells.

When viral testing is in place, transfusion-mediated infection only occurs during the window period where viraemia is present but testing cannot detect the infection. With low- to medium-titre pathogens (e.g. West Nile Virus) PI is well able to deal with residual risks [39, 40]. Where pathogen titres are high early after infection (e.g. parvovirus B19) PI capacity may be exceeded unless testing is also in place and enables high titre units to be discarded. No NA-targeting PI technology is effective against prion diseases. There are two areas where inactivating ***high*** titres of ***bacteria*** is important. Firstly, to cover higher loads that may arise for rapidly growing species between collection and processing (<18 h for buffy coat platelets). Second to provide better assurance that no bacteria remain after PI.

GvHD is currently prevented by gamma-irradiation of selected units to completely inactivate leucocytes. There is a relatively narrow gap between the dose of radiation to inactivate leucocytes and doses that damage RBC. PI prevents GvHD by killing leucocytes with higher margins of safety that are technology-specific [41, 42]. PI can eliminate CMV transmission [43] and reduce febrile reactions by inhibiting leucocyte cytokine production. As PI is usually adopted for all units, errors inherent in selective processing (e.g. gamma-irradiation, CMV testing) are avoided and the security and safety measures for gamma-irradiation would no longer be needed [44].

Most plasma PI methods cause 20–30% loss of coagulation factor VIII, although fibrinogen and coagulation inhibitor content are more clinically relevant for single donor plasma. Other coagulation factors are better retained [45], although there are concerns over fibrinogen levels in MB plasma [46]. The US SD plasma process gave high losses of protein S, antiplasmin and antitrypsin associated with thrombosis in liver transplant patients. Such losses are less for the OctaPlas® process [45]:

Cellular product potency is initially assessed poststorage, by autologous radiolabel studies in volunteers, to determine recovery and survival. Table 4 summarizes data for platelets, demonstrating PI methods result in some loss of cell viability. For Mirasol® and IBS platelets stored 5 days, results are close to the proposed FDA limit of 67% of fresh platelet recovery and 58% of fresh survival [47, 48]. The relationship between ***in vivo*** viability and increased glycolytic flux observed in both Mirasol® and IBS platelets remains unclear [49].

Clinical studies and pharmacovigilance

Plasma components

Most plasma trials assess coagulation factors increments in patients rather than using clinical end-points. They largely reflect factor content, although changes in factor survival, compared with literature values, were reported. Comparative kinetics after control and test transfusion in the same individuals have not been undertaken. Table 5 lists the clinical studies [50–64] for plasma. IBS plasma has the most extensive preclinical and clinical assessment programme. The TTP study had a clinical end-point (remission within 30 days) and found no difference vs standard plasma in the life-saving plasma exchange therapy, although under-powered to demonstrate noninferiority [54]. There are very few randomized clinical studies of SD or MB plasma. Table 5 summarizes these, along with some observational studies. A recent review covers these and further observational studies [45] but in general no difference was seen from standard FFP in such studies (often under-powered) other than:

(1) many European services prefer use of OctaPlas for TTP treatment given its safety record (virus transmission, TRALI) [65], validation for prion removal and competitive pricing.
(2) Three small nonrandomized studies have suggested MB plasma is less effective in TTP [62–64], despite retention of ADAMTS-13 [66].

Table 3 Pathogen and Leucocyte Inactivation for IBS/Theraflex®/Mirasol® Platelets) [11, 16, 18, 36–38]

Viruses				Bacteria				Parasites			
Enveloped Viruses	**IBS**	**THF**	**MIR**	**Gram-negative**	**IBS**	**THF**	**MIR**		**IBS**	**THF**	**MIR**
HIV-1 (cell associated)	>6·1	~	4·5	*Escherichia coli*	>6·4	>4·0	4·4	*Plasmodium falciparum* (malaria)	>6	~	>3·2
HIV-1 (cell free)	>6·2	1·4	5·9	*Serratia marsescens*	>6·7	>4·0	4·0	*Trypanozoma cruzi* (Chagas' disease)	>5·3	~	6·0
Clinical isolate HIV-1	>3·4	~	~	*Klebsiella pneumonia*	>5·6	4·8	2·8	*Leishmania mexicana* (promastigote)	>5·0	~	~
Clinical isolate HIV-2	>2·5	~	~	*Pseudomonas aeruginosa*	4·5	>4·9	>4·6	*Leishmania major Jish* (amastigote)	>4·3	~	>5.0
Latent proviral HIV-1	*All detectable**	~	4·5	*Salmonella cholerasuis*	>6·2	~	~	*Babesia microti* (babesiosis)	>5·3	~	>4·0
Hepatitis B	>5·5	~	(2·3)	*Yersinia enterocolitica*	>5·9	~	3·3				
Hepatitis C	>4·5	~	(3·2)	*Enterobacter cloacae*	5·9	>4·3	>2·0				
				Orientia tsutsugamushi (scrub typhus)	>5·0	~	>5·0				
HTLV-I	4·7	~	~								
HTLV-II	5·1	~	~	**Gram-positive**				**Leucocyte**			
Cytomegalovirus (cell-associated)	>5·9	~	~	*Staphylococcus epidermidis*	>6·6	4·8	4·2	T-cell viability (limiting dilution)	>5·4	~	>6·0
Cytomegalovirus (cell-free)	All detectable*	(3·5)	(2·1)	*Staphylococcus aureus*	6·6	>4·8	4·0	DNA modification (one adduct per x base pairs)	1 per 83	~	~
BVDV (HCV model)	>6·0	~	5·8	*Streptococcus pyogenes*	>6·8	~	2·2	Polymerase chain reaction	Inhibited	~	~
Duck HBV (HBV model)	>6·2	~	~	*Listeria monocytogenes*	>6·3	~	~	Cytokine synthesis: No Il-8 or IL-1β synthesis	✔	~	✔
West Nile Virus	>6·0	5·4	>5·1	*Corynebacterium minutissimum*	>6·3	~	~	Murine model TA-GVHD: Prevents disease	✔	~	✔
SARS-CoV	>5·8	~	~	*Bacillus cereus (incl spores)*	3·6	~	~				
Chikungunya	>6·4	~	2·1	*Bacillus cereus (vegetative)*	>6·0	4·3	1·9				
Influenza A virus H5N1	>5·9	~	(>5)	*Bifidobacterium adolescentis*	>6·2	~	~				
				Proprionobacterium acnes	>6·2	4·5	>2·0				
Non-enveloped Viruses				*Lactobacillus species*	>6·4	~	~				
Blue tongue virus type II	6·1–6·4	~	~	*Clostridium perfringens (vegetative)*	>6·5	>4·7	~				
Calicivirus	1·7–2·4	~	~								
Human adenovirus 5	>5·7	~	~	**Spirochaete bacteria**							
Parvovirus B19	3·5 to >5	(5·46)	(>5)	*Trepenoma pallidum* (syphilis)	>6·8	~	~				
hepatitis A	0	~	(1·8)	*Borrelia burgdorferi* (Lyme disease)	>6·8	~	~				

Numbers show log reduction of various pathogens or leucocytes for IBS, Theraflex® (THF) and Mirasol® (MIR) technologies. Where log reduction is shown as > X, no detectable pathogen remained after treatment. *inactivated to limit of detection. Similar data were obtained for plasma [22]. Only illustrative data for selected pathogens are shown; the cited references contain additional data. *Data in parentheses* show data for model pathogens analogous to those listed, for example animal parvovirus rather than human parvovirus B19; murine rather than human CMV. Some of the Mirasol® bacterial inactivation data were provided by R Goodrich, TerumoBCT (personal communication) but is also cited in reference 38. ~: no data available

Table 4 Recovery and Survival Data In Healthy Volunteers for Mirasol® and IBS-treated platelets after 5d storage

	Mirasol (n = 24) [47]			IBS (n = 16) [48]		
	Test	Reference	As % of Ref	Test	Reference	
Recovery (%)	50·0 ± 18·9	66·5 ± 13·4	75	42·5 ± 8·7	50·3 ± 7·7	85
Survival (d)	4·3 ± 1·01	5·9 ± 1·1	73	4·8 ± 1·3	6·0 ± 1·2	80

If it is assumed that fresh platelets have 65% recovery and a life span of 8 days then both technologies are close to the proposed FDA standard that stored platelets should have a recovery of 67% of fresh and a survival of 58% of fresh in paired healthy volunteer studies

(3) A study of MB, SD and quarantine plasmas in liver transplantation recipients found higher hepatic artery thrombosis rates for SD plasma recipients, and higher plasma use with MB [67, 68].

Recently, AFSSAPS, due to concerns over allergic reactions to MB plasma, have replaced this product with IBS plasma in France[46]. Higher rates of allergic reactions over 4 years, as well as two severe reactions to MB plasma, requiring hospitalization due to specific immune response to MB, have been reported [69, 70].

Companies have reported over 1·9 million units of MB and over 6·5 million units of OctaPlas® transfused without major side-effects[3], despite the liver study cited earlier. Data for IBS (>500 000 units) and Mirasol® (>1000 units) plasma were less extensive, but still encouraging. A formal study of 7483 IBS plasmas reported similar adverse reactions rates to conventional plasma [71].

Platelet components

Most clinical trials of PI platelets assume products will be less effective and use a noninferiority randomized design with primary end-points of corrected count increment (CCI) or haemostatic/bleeding score (Table 6) [3, 11, 72–80]. Absolute platelet count is a poor surrogate for bleeding tendency and count increment is dependent on patient size, product dose and type, number of prior transfusions (most trials limit analysis to the first 8 transfusions, or assess single transfusions), storage medium, storage time, etc. To allow for this, longitudinal regression analysis, rather than ratio data (increments or CCIs), has been recommended [81]. Outcomes are affected by the type of reference product, older platelets and those stored in earlier platelet additive solutions performing less well. The negative impact of combining gamma-irradiation with PI also merits attention.

Of the trials listed in Table 6, four did not use a noninferiority design:

The EUROSPRITE study [72] found no difference in 1 h-CCI in 103 patients for IBS vs reference buffy coat platelets. While the 24h-CCI differed, linear regression analysis of CI at 1 or 24 h, and post-transfusion bleeding score did not. Increments for reference platelets in T-sol additive or plasma did not differ [82].

Slichter *et al.*[75] showed comparable bleeding time correction in 10 patients for reference and IBS platelets. 1h-CCI differences (10 400 vs 13 600) were not significant.

Janetzko *et al.*[74] found no difference by longitudinal regression analyses or 1h-CCI (11 600 vs 15 100) for IBS and reference platelets. The corresponding study for buffy coat platelets yielded similar data (A. Stassinopoulos, Cerus, personal communication, 2011).

Other studies in Table 6 used a noninferiority design, with margins between 7·5% and 30% for the primary end-point.

In the 645 patient, SPRINT trial [73] primary end-points of grade 2 bleeding (assuming 12·5% noninferiority margin) or grade 3 and 4 bleeding (7·5% noninferiority) confirmed noninferiority. Differences were found for 1h-CCI (11 100 vs 16 000), transfusion interval (1·9 vs 2·4 days) and number of platelet transfusions (8·4 vs 6·2), but not adverse reactions [83] or transfusion interval adjusted for dose [84]. Snyder *et al.* [83] found no difference in WHO grade 1, 2, 3 or 4 bleeding rates, although more patients in the PI arm had petechiae or faecal occult blood. Kerkhoffs noted significant differences in grade 2 *or greater* bleeding in this study, by direct comparison (no non-inferiority assumption) [85].

A pilot study [76] for 7 days stored platelets, found no difference (15% noninferiority assumption) in 1h-CCI between IBS and reference platelets (6587 vs 8935) or in haemostatic score or adverse events. The larger TESSI study [77] compared single transfusions of reference platelets in T-Sol or SSP additive with IBS platelets, after 6 or 7 days storage. The primary end-point of 1h-CCI noninferiority (30% margin) was met (8163 vs 9383, ratio 0·87) in the 201 patient study. 1h-CI, RBC use, time to next transfusion and bleeding score did not differ, whereas 24h-CCI (4589 vs 6549) and 24-CI (11·1 vs 15·2 × 10^9/l) did.

The HOVON study [78] used a 20% noninferiority design with a 1h-CCI primary end-point. It was not blinded, nor powered to detect differences in bleeding. The study included buffy coat platelets stored for up to 7 days in plasma (reference), PASIII additive or PASIII with IBS

Table 5 Clinical Trials of PI plasmas

Study	Design	Clinical Setting	Main Result
Amotosalen (S-59)			
Hambleton *et al.* [50]	Crossover volunteer *N* = 27 S-59 vs std FFP	Factor VII & PT kinetics in warfarinized volunteers	No difference PT or FVII kinetics
Hambleton *et al.* [51]	Crossover volunteer 17 protein C and 16 protein S	Kinetics in warfarinized volunteers	No difference C or S kinetics
de Alarcon *et al.* [52]	Open label Pharmacokinetic 107 Transfusions in 34 patients with congenital coagulation deficiencies	Increment (& half-life) in 31 (22): Fibrinogen: 2 (1) Prothrombin: 3 (3) Factor V: 7 (4) Factor VII: 3 (2) Factor X: 1 (1) Factor XI: 11 (6) Factor XIII: 3 (3) Protein C: 3 (3) Factor V+VIII: 0 (0)	Recovery, tolerance and efficacy OK Shorter half-life for fibrinogen, prothrombin and factor XIII vs literature values
Mintz *et al.* [53]	Randomized Controlled Trial *n* = 121. 60 S-59 and 61 std FFP	Acquired coagulopathy (Mainly liver disease, 75% had cirrhosis)	Noninferiority for changes in PT (< 4·6 sec) but not PTT (>4·8 sec). No differences in factor VII increment or component use were seen either
Mintz *et al.* [54]	Randomized Controlled Trial 17 S-59 & 18 std FFP	Thrombotic Thrombocytopenic Purpura (TTP)	No clinical difference. (20% noninferiority would require 38 patients per arm)
SD plasma[a]			
Horowitz, Pehta [55] & Pehta review [56]	Observational 396 Tx,164 patients RCT in TTP. Plus 44 liver disease (57 Tx), 10 warfarin reversal & 6 chronic TTP patients	47 hereditary deficiencies (FII, FV, FVII, FX, FXI, FXIII) 32 TTP (27 SD vs 17 standard FFP)	Prevents or arrests bleeding (87%) TTP; no different from FFP Effective in liver disease, warfarin reversal and chronic TTP
Williamson, Llewelyn, Fisher *et al.* [57]	Randomized Controlled Trial SD vs FFP (*n* = 49)	Complex coagulopathy: liver disease or transplantation	No difference clinical efficacy or haemostatic correction; 1 case B19 on standard FFP
Wieding, Rathgeber, Zenker, *et al.* [58]	Randomized Controlled Trial SD vs MB plasma (*n* = 71)	Cardiopulmonary bypass surgery	No clinical difference, Poor protein S and antiplasmin rise after SDP
Beck, Mortelsmans, Kretschmer, *et al.* [59]	Randomized Controlled Trial SD vs FFP (*n* = 40)	Severe coagulopathy	No difference clinical efficacy or haemostatic correction
Lerner, Nelson, Sorcia, *et al.* [60]	Randomized Controlled Trial 22 SD vs 23 FFP	Severe coagulopathy with PT prolonged	No difference clinical efficacy or PT correction (32% vs 26%)
McCarthy [61]	Observational 35 SD, 62 FFP, 48 cryosupernatant	TTP	90% response with SD vs 70%–75% for others
MB plasma[a]			
Wieding, Rathgeber, Zenker, *et al.* [58]	Randomized Controlled Trial SD vs MB plasma (*n* = 71)	Cardiopulmonary bypass surgery	No clinical difference, Poor protein S and antiplasmin rise after SDP
de la Rubia, Arriaga, Linares, *et al.* [62]	Observational 13 FFP vs 7 MB	Plasma exchange for TTP	MB 57% remission vs 69% FFP
Alvarez-Larren, Del Rio, Ramirez, *et al.* [63]	Retrospective 27 MB vs 29 FFP	TTP	Greater recurrence and mortality with MB
del Rio-Garma, Alvarez-Larrán, Martinez *et al.* [64]	Multicentric, observational, prospective cohort study. Compare MB (63) and quarantine FFP (39)	TTP remission by day 8 of plasma exchange	MB plasma was associated with a lower likelihood of remission on day 8 (Odds ratio 0·17). MB patients required more exchanges (11 vs 5), a larger volume of plasma (485 vs 216 ml/kg) and presented more recrudescences while on PE therapy (46% vs 21%)

[a]see also further small, observational studies summarized in Tables 6 & 7 in recent review [45].

Table 6 Phase III Platelet trials

Study	Technology (reference preparation)	Design	N (Test/Ref) Mean storage days (Test/Ref)	Primary end-point	Secondary end-points	Associated studies
EUROSPRITE van Rhenen *et al.* [72]	Amotosalen pooled BC platelets (*pooled BC platelets in T-sol or plasma*)	56d or 8 Tx observation with 28d follow-up Intention to treat RCT	52/51 (*3·4 days/ 3·5 days*)	CCI & CI at 1 h	CCI & CI at 24 h Haemostasis and bleeding score. Adverse events Tx interval	
SPRINT McCullough *et al.* [73]	Amotosalen apheresis platelets (*apheresis platelets in plasma, 99·8% gamma irradiated for test and reference*)	28-d treatment period or 8 Tx. Noninferiority RCT (12·5% or 7·5% assumption)	318/327 (*3·4 days / 3·6 days*)	Grade 2 *bleeding*	Grade 3 & 4 *bleeding.* CCI and CI at 1 and 24h. Adverse events Tx interval	[83, 84]
Unpublished	Amotosalen BC integrated sets	28d or 8 Tx	20	Logistic regression of dose vs increment	CCI	
Janetzko *et al.* [74]	Amotosalen apheresis integrated sets (*gamma irradiated apheresis platelets in plasma*)	28d or 8 Tx	22/21 (*3·1 days / 3·2 days*)	Logistic regression of dose vs increment	CCI	
Slichter *et al.* [75]	Amotosalen apheresis integrated sets (*apheresis platelets in plasma*)	Impact single transfusion on bleeding time Crossover design	32 patients but only 10 completed crossover (*2·8 days / 3·2 days*)	Bleeding time	CI and CCI at 1 h Tx interval	
Simonsen *et al.* [76]	Amotosalen BC 7 days storage *increments (pooled BC platelets in T-sol, 65% gamma irradiated*)	RCT, single Tx with noninferiority of 15% design	11/9 (*7 days / 7 days*)	CCI 1 h	Haemostatic score Adverse events Tx interval	
TESSI Lozano *et al.* [77]	Amotosalen 6 &7 days platelets (IBS) (*86% pooled BC, 14% apheresis platelets in T-Sol or SSP+ additive, 74% gamma irradiated* vs *14% for test*)	RCT; < 30% noninferiority Single transfusion	100/101 (*6·8d/6·8d*)	CCI 1 h	CI-1 h CCI and CI at 24 h Interval to next Tx Haemostatic score Red cells Transfused Acute transfusion reactions	
HOVON Kerkhoffs *et al.* [78]	Amotosalen 1–7 days *pooled BC* (plasma vs PAS vs IBS) (*pooled BC platelets in plasma or T-sol*)	42 d or 5 Tx study RCT, 20% noninferiority	97/94/85 (*4 days / 3·8 days / 4 days*)	CCI 1 h	Bleeding score CI 1 h CI and CCI 24h Tx interval Red cell and platelet usage Adverse Events	IBS arm curtailed early

Table 6 (Continued)

Study	Technology (reference preparation)	Design	N (Test/Ref) Mean storage days (Test/Ref)	Primary end-point	Secondary end-points	Associated studies?
MIRACLE [79]	Riboflavin treated apheresis platelets stored 1–5 days (*apheresis platelets in plasma*)	RCT,20% noninferiority	58/54 (*2·8 days/ 2·6 days*)	CCI-1h	CCI-24h Tx interval Red cell and platelets usage Refractoriness Infection rate WHO bleeding *score* Adverse Events	
PREPARES [3, 11, 80]	Bleeding study of riboflavin BC platelets stored in plasma in Netherlands stored 1–7 days (*apheresis platelets in plasma*)	PLANNED 42 d observation or 8 Tx Non-inferiority (< 15%)	618	Grade 2 or greater bleeding (CTCAE grade 2 bleeding complications.)	CI and CCI at 1 and 24 h. Adverse events Red cells and platelet usage Tx interval; Rate of HLA alloimmunization;	
IPTAS study THE ITALIAN PLATELET TECHNOLOGY ASSESSMENT STUDY [3, 11, 80]	Multicenter, nonrandomized, prospective controlled study involving 2 centres using Mirasol and 2 using IBS	ONGOING	420	Grade 2 or greater bleeding Prevention of alloimmunization	CCI platelet and RBC transfusions transfusion interval allo-antibody formation from Luminex assay	
Pathogen Reduction – Extended Storage Study (PRESS), Denmark [3, 11, 80]	Comparison of 2–3 days and then 7 days stored reference and Mirasol platelets	ONGOING	40	Thromboelastogram measurements and correlation with CCI	Adverse events bleeding CCI	

BC, buffy coat.

treatment. The IBS arm was curtailed early due to excess bleeding, including WHO grade 1 bleeds, often regarded as clinically insignificant. Low rates of grade 2 bleeding (6–7% vs ~60%) [84] compared with most trials [73, 77, 85–87] were observed. Differences were noted (*intention to treat* analysis) for 1h-CCI (17 100 vs 15 300, vs 11 400), the IBS data being 31% lower. However, confidence limits were within the prespecified 20% noninferiority margin.

The only Mirasol® trial (Miracle) [79] compared 1h-CCI in 110 patients given reference or treated platelets stored up to 5 days (*20% noninferiority limit*). The study failed the noninferiority end-point (mean 1h-CCI reduced by 24% from 16 939 to 11 725) and, although it was a small study, bleeding scores (any WHO bleed grade from 1 to 4) were different: 59% of Mirasol product recipients bled vs 43% for reference. Neither this, nor RBC or platelet usage, were significantly different for the two arms. Further studies of Mirasol platelets are planned in the Netherlands, Italy and Denmark [3, 11, 80].

About 600 000 IBS platelets have been transfused vs 10 000 Mirasol® units [3, 11]. Safety surveillance reports for IBS product include use in Belgium, France, Spain, Norway and Italy [88–90]. Comparison of component usage, including RBC transfusions, during introduction of IBS platelets into routine use has revealed no significant change in either Belgium or France [91, 92]. Reports from Germany and Switzerland are also encouraging [93, 94]. French annual haemovigilance reports include adverse events (mainly allergic) for plasma and platelets. These are lower for IBS platelets stored in additive solution than for platelets in plasma (10–15 per 100 000), while MB plasma (~7 per 100 000) rates were higher than IBS or SD plasmas [95].

The observations of reduced CI for PI platelets with little impact on haemostasis [73] are consistent with the PLADO trial which reported no difference in haemostasis between high, medium and low dose groups of platelet recipients [87]. In contrast, a meta-analysis of PI platelet trials [96] reported reduced increments *and* haemostatic efficacy. The increment data is consistent with most individual trials [97], current study designs suggesting a 20% reduction in increment is acceptable. The increased bleeding tendency reported [96] has not been observed in IBS trials with this primary end-point (the major one, SPRINT, having a noninferiority design) or in a subsequent meta-analysis [98]. That point is partially conceded in a later report by the same author, no excess bleeding being found for IBS products [99]. Inclusion of grade 1 bleeds, possibly predictive of more severe bleeding, and period of observation are potential confounders [98, 100]. The absence of change in platelet, RBC or plasma use in services adopting PI, suggests no gross changes in haemostatic efficacy [91, 92].

Outcomes from recent meetings on pathogen inactivation and its implementation

The 2007 Canadian PI consensus conference concluded PI was worth pursuing, particularly to safeguard against emerging infections [101]. The IBS system allowed maintenance of platelet supply in Réunion during a Chikungunya virus outbreak [102]. A 2010 meeting in Strasbourg of PI companies, blood services and regulatory agencies [3] emphasized the increasing adoption of PI in Europe. Sixteen countries have either partially or completely adopted plasma PI; 13 platelet PI. MB and SD plasmas are the only PI components licensed in Canada, with evaluations ongoing in Japan. The UK SABTO committee recommended that PI platelets not be introduced in 2010 [103]. The AABB published a 2010 monograph on available technologies and implementation experience [104].

Regulatory aspects

Despite sales exceeding 1 000 000 IBS kits, no component PI technologies are licensed by the FDA. There is a continuing discussion about the design of pivotal platelet PI trials with the FDA. A number of PI technologies are CE marked (marketing authorization) in Europe, where Octa-Plas® is licensed as a pharmaceutical. PI companies tended to gain CE marking using the medical devices directive class IIb route but there is now a strong trend towards class III registration, which requires regulatory review of preclinical and clinical data (Table 1).

Individual European countries require marketing authorization at national (France, Switzerland) or regional (Germany) level. Approval by insurance (Belgium) or government funders (UK) may also be required.

Methods, including chromatographic assay of amotosalen photodegradation products [105], use of UV sensitive labels [106] and PCR inhibition assays [107], have been established to confirm completion of processing on individual units.

Cost-effectiveness

Table 7 summarizes published data on the cost-effectiveness of SD plasma. These do not take account of reducing bacterial or emerging infections. They do illustrate the significant impact of reduced TRALI risk (due to dilution of causative antibodies in the large plasma pools used [45]) on cost-effectiveness for this product [111], although male-only plasma provides an alternative. The TRALI benefit would not apply to single donor products and it not yet clear if it does for minipools (6–10 donations), as used in the IBS-treated, plasma minipool product PLASMIX [112, 113].

Cerus developed costing models, including bacterial and emerging infection risks, and applied these in Japan, Belgium, Netherlands and USA [114–117]. Independent assessments have also been published. Custer *et al.* [118] considered whole blood, as well as plasma and platelet, PI approaches and assessed infections, TA-GVH, febrile reactions and transfusion induced immune modulation, concluding the cost per Quality Adjusted Life Year remained above $1 million for most patient groups, well above usually accepted limits. However, at the 2011 AABB conference, he included emerging infections

Table 7 Solvent Detergent Plasma Cost-Effectiveness studies

Study	Mean Cost per QALY (US$) (Range)	Comment
Aubuchon & Birkmeyer [108]	$289 300 (All > $55 300)	1 unit prolongs life 35 min
Jackson *et al.* [109]	$9 743 000 (All>$2 800 000)	
Pereira [110]	$2 156 398 ($710 000-$7 600 000)	1 unit prolongs life 71 min
Reidler *et al.* [111]	$90 000 (approx £50 000:) (£12 335–£99 005)	Includes TRALI avoidance (1 in 3500 with 10% mortality)

modelled on HIV or West Nile virus, concluding: *'Pathogen Reduction Technology would be "a bargain". For an acute agent the cost-effectiveness would be very favourable within the blood safety context. Meanwhile, for a chronic agent of similar prevalence there would be substantial cost savings based on avoided health-care (and litigation) costs'* [119].

Future directions

Development of RBC PI

Photoinactivation methods for optically dense RBC requires high doses of UV light, dilution or use of thin layers of product. Cerus have developed an alternative purely chemical approach. Their S-303 compound (Fig. 2) is a FRangible Anchor-Linker-Effector(FRALE) compound [120] designed to react quickly at neutral pH after binding NA but also to decompose, through hydrolysis of a strategic Linker bond. This separates the NA-reactive part from the NA-binding group and generates a compound with reduced affinity for NA. Initial clinical experience demonstrated efficacy in patients undergoing cardiovascular surgery, but antibodies to treated RBC formed in two multitransfused patients, without clinical consequence. The process has now been revised, using the same compounds but increased levels of protectant glutathione (going from 2 to 20 mM) to minimize side reactions and is restarting efficacy Phase III patient trials [121]. Preclinical toxicology (Table 2) [32] is acceptable and inactivation of pathogens is reported as similar to those shown in Table 3 [122].

TerumoBCT's Mirasol® technology is being developed for the treatment of *whole blood* through adjustment of the dose from 6 J/mL_{PLASMA} for platelets and plasma to 80 J/mL_{RBC} for whole blood [123–125].

Table 8 summarizes PI RBC studies in man [126–130]. The 2003 finding of antibodies to treated RBC in multitransfused patients curtailed all clinical studies on PI RBC at that time. The Cerus revised, second-generation S-303 process demonstrated equivalent 24-h recovery to conventional RBC after 35 days of storage, *in vitro* characteristics meeting EU and US RBC standards and will be used in Phase 3 patient efficacy studies [127].

Recovery and survival studies of RBC *from whole blood (WB)* processed using Mirasol® technology found significant loss of viability associated with UV doses required to achieve PI [128]. For existing RBC PI processes, which have yet to be finalized or licensed, shelf life may be reduced to 35 days (S-303) or possibly even <28 days (extrapolated from Mirasol® data: Table 8) [126–128].

Studies on plasma and platelets from treated WB have yet to be reported.

Use of such an approach in forward military settings may be less restricted, with continued military interest in this field (*R Goodrich*, TerumoBCT *BCT*, personal communication). The US Department of Defence has also funded WB PI studies with S-303 since 2002 [122].

Other directions

The 2009 National Heart, Lung and Blood Institute workshop on research opportunities in blood component

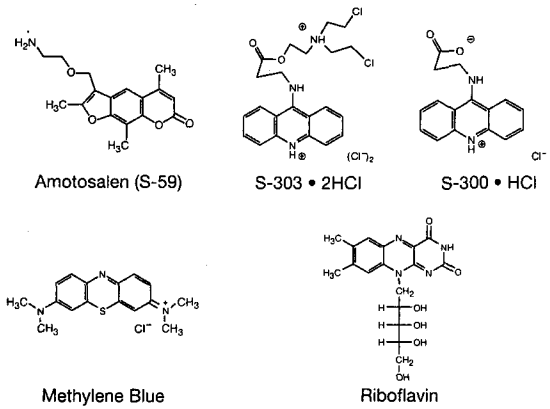

Fig. 2 Structures of Amotosalen, S-303 and its degradation product S-300, Methylene Blue and Riboflavin.

Table 8 Red Cell Studies

Study type	Study design	Patients		Reference
24-h Recovery (version 1 S-303)	Two period, cross over study	42		Rios *et al.* [126]
24-h Recovery (version 1 S-303)	Single arm, with some subjects having prior exposure to S-303 treated RBCs	28		
Phase 1 safety and tolerability (version 1 S-303)	Single-arm safety evaluation of full unit transfusions	10		
Recovery and Life span (version 1 S-303)	Two period crossover study design	29	Test 82% vs 85% 24 h recovery at 35 days (half-life 33 days for test and 40 days reference)	
Phase 3 Acute anaemia (version 1 S-303)	Efficacy and safety of allogeneic transfusions. Noninferiority design.	200 but stopped at 148 (74 + 74) Primary end-point met		Benjamin *et al.* [129]
Phase 3 Chronic anaemia (version 1 S-303)	Efficacy and safety of allogeneic transfusions. Two period crossover study. Noninferiority design.	50 but stopped at 26		Conlan *et al.* [130]
Recovery and Life Span (version 2, S-303)	Two period crossover study	27	88% vs 90% 24 h recovery at 35 days (half-life 37 days for test and reference)	Cancelas *et al.* [127]
Recovery and Life span whole blood treated with Riboflavin & UV[a]	Recovery and Life span. Dose ranging	11	50–73% 24 h recovery at 42 days (half-life 10–35 days)	Cancelas *et al.* [128]

[a]Results reported for 4 volunteers each at 22 and 33 J/ml $_{RBC}$, and 3 at 44 J/ml $_{RBC}$.
Latest reports for whole blood cite using a dose of 80 J/ml $_{RBC}$ [123–125]

PI identified two fundamental questions, among others [131]:

First, could PI be applied to whole blood, making it more affordable and easier to implement? Based on preliminary data [126–128], this remains challenging and may be limited to whole blood use in military applications with minimal storage (see section Development of RBC PI).

Second, can PI render components 'virtually leucodepleted', with savings and improvements from filters facilitating PI introduction? Data from the TRAP trial, which found *equivalent reductions* in alloimmunization for leucodepleted and UVB-treated platelets, are promising [132]. Encouraging data from Belgium show alloimmunization with IBS platelets was reduced, despite use of non-PI leucofiltered RBC [133].

Since 1990, significant progress has been made in PI technology. In routine use, the anticipated benefits of PI become tangible, offering hope for an even safer blood supply. For example, French haemovigilance reports found no cases of sepsis associated with use of IBS platelets (0/104 118 between 2006 and 2011, whereas 33, including 5 deaths, were observed in conventional platelets recipients (33/1 466 478), corresponding to 0 and 22·5 cases per million [95].

Where IBS plasma and platelet PI has been adopted, there has been little change in component usage, and the PI process has secured microbiological safety from viruses and bacteria. Concerns about adverse toxicological and immunological effects seem unfounded. Elsewhere cost of PI remains a concern, but PI inactivation of leucocytes can substitute for gamma-irradiation and leucodepletion. Together with the security against emerging pathogens, these considerations increasingly make PI technologies good value.

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MAJOR ARTICLE

Relationship between Bacterial Load, Species Virulence, and Transfusion Reaction with Transfusion of Bacterially Contaminated Platelets

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Background. Bacterial contamination is currently the major infectious hazard of platelet transfusion, but associations between bacterial species and quantity and transfusion reactions have not been characterized.

Methods. Patients receiving platelets from July 1991 through December 2006 were observed using active surveillance by quantitative culture of platelets at the time of issue or passive surveillance by investigation of clinical reactions in patients and culture of implicated units. Patient reactions were classified by type and severity and were correlated with bacterial species and number. Endotoxin content of gram-negative contaminants was determined by limulus lysate assay.

Results. Fifty-two bacterially contaminated platelet units were detected (50 by active and 2 by passive surveillance). Rates of bacterial contamination and septic transfusion reactions were 32.0-fold and 10.6-fold higher, respectively, as determined by active versus passive surveillance ($P < .001$). Including 2 index cases, bacterial contaminants included gram-negative bacilli in 4 units (3 of which were associated with fatal reactions), staphylococci in 44 units, streptococci in 4 units, and *Bacillus cereus* in 2 units. Endotoxin content of the 4 units that were contaminated with gram-negative bacilli ranged from 11,373 to 173,130 endotoxin units. Reaction severity was greater for units with bacterial counts of $\geq 10^5$ colony-forming units/mL and higher bacterial virulence. A detection method with a 10^3 colony-forming units/mL threshold would detect >90% of contaminants.

Conclusions. Active surveillance detected 32-fold more bacterially contaminated platelet units and 10.6-fold more septic reactions than did passive surveillance, and virulent species and bacterial counts of $\geq 10^5$ colony-forming units/mL were associated with more-severe transfusion reactions. Improved detection methods or use of pathogen inactivation technology are needed to eliminate this problem.

Administration of platelets is an important supportive measure in the treatment of patients in the hematology-oncology unit and is required to maintain hemostasis during surgical procedures associated with extensive blood loss. Although the risk of viral infection has greatly decreased in recent years, bacterial contamination of platelet products is currently the major remaining infectious hazard, with 60 deaths reported to the US Food and Drug Administration from 1995 through 2004 [1]. Although low numbers of bacteria may initially contaminate platelet products, storage at 22°C for 5–7 days allows growth, with high bacterial loads present at the time of transfusion [2]. Although platelets are best used as soon as possible after collection, recruitment of donors and necessary delays associated with testing for the presence of infectious agents, as well as delays in distribution and use, result in many platelet products being used when they are 4 or 5 days old. Few data are available on the bacterial loads present in transfused, contaminated units and the severity and outcome of any resulting septic reactions. However, understanding these relationships is vital to the development of detection methods.

To address these issues, we have performed extensive

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monitoring for bacterial contamination of platelet products at our institution for >15 years, using both active and passive surveillance, with quantitation of bacterial loads in contaminated units at time of use and association with reactions in patients [3]. This has allowed us to correlate reactions with bacterial species and bacterial loads present in contaminated units. These data are unique and provide a basis for recommendations for improving surveillance and preventing transfusion of contaminated units.

PATIENTS, METHODS, AND MATERIALS

Patient population. Patients receiving whole blood–derived, random-donor platelet (RDP) units, usually in pools of 5 units, and single-donor apheresis platelet (SDP) units from July 1991 through December 2006 at University Hospitals Case Medical Center (Cleveland, Ohio), a 900-bed academic tertiary-care center, comprised the study population. Most platelet units were obtained from local transfusion services, with some collected in-house, and were given primarily to adult patients in the hematology-oncology unit, especially patients undergoing bone marrow transplantation and patients with acute leukemia who were undergoing chemotherapy.

Surveillance for bacterial contamination of platelet units Passive surveillance was performed by investigation of reported clinical reactions in patients who received platelet transfusions, including patient evaluation, and Gram stain and aerobic and anaerobic culture of the remains of implicated platelet units from March 2000 through February 2004.

After 2 cases of bacterial contamination of platelets were detected by passive surveillance in June and July 1991 (index cases) [4], active surveillance was instituted, as described elsewhere [3]. Surveillance included testing all units for 20 months (July 1991 through February 1993), only 4-day-old or 5-day-old units for 84 months (March 1993–February 2000), and all units from March 2004 through December 2006. Active surveillance was performed by collecting 1–2-mL aliquots of SDP units and RDP pools for culture at the time of issue [3]. Gram stain was also performed from August 1991 through January 1999. Culture was performed by plating 0.1 mL onto blood agar plates, which were incubated for 48 h in 5% CO_2 at 35°C; isolates recovered were preserved at −70°C. All cultures with positive results were verified by isolation of the same organism by a second culture from the same source and, for RDP pools, from 1 of the source units. Quantitative cultures were performed if the initial cultures had positive results by plating 0.1 mL of serial 10-fold dilutions of platelet specimens, which had been kept at 4°C to prevent further bacterial multiplication [3]. Culture results were recorded as colony-forming units (cfu) per mL of platelet specimens. Patients were evaluated for evidence of transfusion reactions as soon as positive culture results were obtained.

Endotoxin assay. Gram-negative platelet contaminants found during the study were recovered from frozen storage and grown on agar plates overnight. Organisms were suspended in endotoxin-free water at 10^3–10^4 cfu/mL, and endotoxin content was determined by the limulus lysate assay method using the Endosafe-PTS system (Charles River Laboratories). Endotoxin levels, expressed in endotoxin units (EU), were determined, and the endotoxin content of the original contaminated platelet units was calculated on the basis of organism loads and the volume of platelets transfused.

Data sources. After approval from the University Hospitals Case Medical Center Institutional Review Board, data were extracted from available sources, including surveillance records, clinical charts, blood bank investigation logs, and laboratory records. Patients exposed to bacterially contaminated platelets were classified by type and severity of transfusion reaction. A classification and grading method for describing the occurrence and severity of septic transfusion reactions was developed by combining the definitions used in the Common Terminology Criteria for Adverse Events from the National Institutes of Health [5] and in the updated classification scheme for acute transfusion reactions of Sanders et al. [6], particularly as relating to septic reactions, with modifications appropriate to the patient population. Reactions were graded on a 5-point scale, as shown in table 1.

Data analysis. Reaction grades were analyzed against bacterial counts in transfused products, bacterial species, the patient's underlying condition (particularly neutropenia), and whether the patient was receiving antibiotics active against the contaminating species at the time of exposure. Data were initially analyzed using scatter plots of bacterial counts against reaction type to determine the presence of any trends. Bacterial contamination at various detection sensitivities was plotted as an accuracy plot. Data were examined for all exposed patients, using reaction type as a dichotomous variable (no reaction vs. any reaction and severe vs. nonsevere reactions) against potentially contributory clinical conditions, including the presence or absence of neutropenia, use of effective antibiotics at the time of the transfusion, virulence of the organism, and sensitivity thresholds for detection of bacterial contamination at various levels. *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Serratia marcescens,* and *Streptococcus bovis* were regarded as more virulent species, whereas other staphylococci and viridans group streptococci were regarded as less virulent species [7]. Differences were calculated as ORs with 95% CIs; *P* values were calculated by Pearson's χ^2 test, with Bonferroni correction for multiple comparisons of the same data set.

Table 1. Grading system for septic transfusion reactions.

Grade	Reaction type	Definition
0	None	Patients known to have received a bacterially contaminated platelet transfusion but who did not show either clinical or laboratory evidence of a septic reaction
1	Mild	A mild febrile reaction (1–2°C increase in temperature) or an asymptomatic clinical case with positive blood culture result or development of leukocytosis
2	Moderate	A transient change in vital signs (e.g., fever or hypotension) or clinical status that resolved within 24 h with minimal intervention (e.g., treatment with analgesics or antihistamine) or no intervention
3	Severe	A change in vital signs requiring intervention (e.g., intravenous fluid, antibiotic, or vasopressor administration) with resolution without persistent sequelae
4	Life threatening	A severe reaction with septic shock or impairment of vital organ functions
5	Fatal	A severe reaction with death partly or fully attributable to the contaminated platelet transfusion

RESULTS

Surveillance was instituted in July 1991 following the occurrence of 2 index cases in June 1991 [4]. During the 15.5-year study period (July 1991 through December 2006), 238,983 platelet units were used, of which 56,883 were SDP units and 182,100 were RDP units issued in 36,420 pools. Fifty-two bacterially contaminated platelet units were detected during the surveillance period; 50 were detected by active surveillance, and 2 were detected by passive surveillance. Contamination rates were similar for SDP and RDP units, with rates for RDP pools being 5-fold higher than rates for SDP units, because RDP units were usually administered in pools of 5 units. Data were, therefore, analyzed for combined SDP and RDP units. The rate of detection of bacterial contamination was 32-fold higher by active surveillance than by passive surveillance (485 vs. 15 contaminated units per million units; OR, 32.0; 95% CI, 8.0–135.7; $P < .001$) (table 2).

Transfusion of contaminated units. The 2 index case units and 44 of the 52 contaminated units identified during surveillance were transfused. The 8 contaminated units that were not transfused were all contaminated with *Staphylococcus epidermidis* and included 2 units for which transfusions were cancelled and 6 units that were interdicted because of positive Gram stain results during the 7.5-year period in which Gram stains were performed. Eighteen contaminated units were transfused during this 7.5-year period, including 17 units for which Gram stain results were negative (14 units contaminated with *S. epidermidis,* 1 unit contaminated with *S. aureus,* 1 unit contaminated with *S. marcescens,* and 1 unit contaminated with a viridans group streptococcus), and 1 unit for which Gram staining was not performed (this unit was contaminated with *P. aeruginosa*).

Detection of contaminated transfusions by active and passive surveillance during the surveillance period. Of the 44 contaminated units transfused during the surveillance period, only 2 were detected by passive surveillance (a rate of 15 contaminated units detected per million units transfused), compared with 42 units detected by active surveillance (408 contaminated units per million units transfused; OR, 27.7; 95% CI, 6.7–114.6; $P < .001$) (table 2). The rate of septic transfusion reactions from these 44 transfusions was 10.6-fold higher as determined by active surveillance, compared with passive surveillance (155 vs. 15 cases per million transfusions; 95% CI, 2.4–45.9 cases per million transfusions; $P < .001$), and the rate of septic reactions with bacteremia was 6.6-fold higher, but this difference did not reach statistical significance (49 vs. 7 cases per million transfusions; 95% CI, 0.77–56.5 cases per million transfusions; $P = .047$). However, the fatality rate did not differ between the 2 surveillance arms (10 vs. 7 deaths per million transfusions; 95% CI, 0.08–21.1 deaths per million transfusions; $P = .84$).

Bacterial species, loads, and transfusion reactions. Nine bacterial species were isolated from the 54 contaminated units (including the 52 units that were detected during surveillance and the units from the 2 index cases); 38 units were contaminated with *S. epidermidis,* 4 with *S. aureus* (including 1 unit that was contaminated with a methicillin-resistant strain), 2 with *B. cereus,* 2 with *P. aeruginosa,* 2 with *S. bovis,* 2 with *Staphylococcus lugdunensis,* 2 with *S. marcescens,* and 2 with viridans group streptococci (including 1 unit that was also contaminated with *Staphylococcus warneri*). Eight units or pools that were contaminated with *S. epidermidis* were not transfused, as noted above. Of the remaining 46 contaminated units or pools that were transfused, 20 were associated with transfusion reactions in recipients (figure 1).

Fatal reactions occurred in 3 of the 4 cases of contamination with gram-negative bacilli (*P. aeruginosa* in 2 cases and *S. marcescens* in 1 case); bacterial counts of platelets were $>10^6$ cfu/mL in these cases. Endotoxin levels, determined for organisms recovered from frozen storage, were 5.1 and 5.2 EU per million organisms for the 2 *P. aeruginosa* isolates and 2.5 EU per million organisms for the *S. marcescens* isolate, and the total endotoxin content in the units transfused, calculated from original bacterial levels in contaminated units and volumes transfused, were

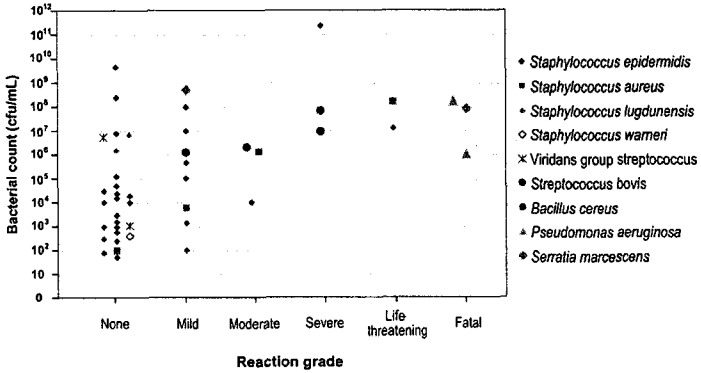

Figure 1. Relationship of bacterial species and bacterial load to occurrence and severity of transfusion reactions in 45 cases, 1991–2006. There are 46 data points shown, because 1 unit had 2 contaminants; quantitation was not performed for 1 case of *Staphylococcus epidermidis* contamination with no transfusion reaction.

182,700 EU, 11,373 EU, and 34,646 EU, respectively. In the fourth case of contamination with gram-negative bacilli, which was due to *S. marcescens* detected at a level of 5×10^8 cfu/mL, no clinically detectable reaction other than transient leukocytosis occurred in a 2-week-old, premature neonate with congenital neutropenia and thrombocytopenia; endotoxin content of the organism was 4.3 EU per million organisms, and the total endotoxin content transfused was 173,100 EU.

Reaction severity was associated with bacterial load and virulence, with all severe reactions (grade 3 and higher) associated with loads of $\geq 10^5$ cfu/mL and/or more-virulent bacterial species (*P. aeruginosa, S. marcescens, S. aureus, B. cereus,* and *S. bovis*) (figure 1). The mean bacterial load was higher in patients with transfusion reactions than it was in those without transfusion reactions (3×10^6 cfu/mL vs. 2.4×10^4 cfu/mL; $P < .002$) and was higher in patients with severe reactions than it was in those with mild or moderate reactions (9.2×10^7 cfu/mL vs. 3×10^5 cfu/mL; $P < .008$). Reaction rates for more virulent versus less virulent bacterial species were 3.5-fold higher (95% CI, 1.9–6.2-fold higher) in those with any reaction than in those with no reaction (91.7% vs. 26.5%; $P = .001$) and 8.5-fold higher (95% CI, 2.0–36.6-fold higher) for severe reactions

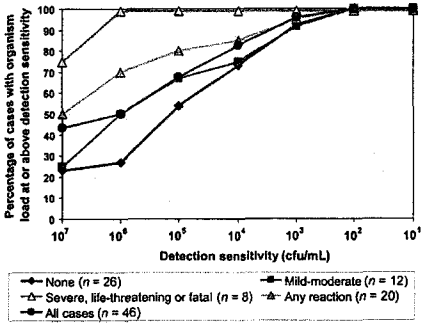

Figure 2. Plot of accuracy of detection of bacterial contamination of platelets based on detection limits of detection methods required to detect 46 bacterial contaminants in 45 cases for all cases and based on the presence and severity of transfusion reactions.

Table 2. Contamination of single-donor platelet (SDP) and random-donor platelet (RDP) units, by surveillance method, July 1991–December 2006.

Variable	No. of cases (no. of cases per million units)						Difference between rates for all units by active vs. passive surveillance	
	Active surveillance			Passive surveillance				
	SDP units (n = 24,309)	RDP units (n = 78,689)	All units (n = 102,998)	SDP units (n = 32,574)	RDP units (n = 103,411)	All units (n = 135,985)	OR (95% CI)	P
Bacterially contaminated units detected	12 (494)	38 (483)	50 (485)	1 (31)	1 (10)	2 (15)	32.0 (8.0–135.7)	<.001
Bacterially contaminated units transfused	10 (411)	32 (407)	42 (408)	1 (31)	1 (10)	2 (15)	27.7 (6.7–114.6)	<.001
Septic transfusion reaction	5 (206)	11 (140)	16 (155)	1 (31)	1 (10)	2 (15)	10.6 (2.4–45.9)	<.001
Septic transfusion reaction and bacteremia	1 (41)	4 (51)	5 (49)	1 (31)	0 (0)	1 (7)	6.6 (0.77–56.5)	.047
Death	1 (41)	0 (0)	1 (10)	0 (0)	1 (10)	1 (7)	1.3 (0.08–21.1)	.84

NOTE. A total of 56,883 SDP units and 182,100 RDP units in 36,418 pools were transfused during the surveillance period. $P \leq .01$, by Pearson's χ^2 test with Bonferroni correction, was considered to be statistically significant.

than for mild or moderate reactions (50% vs. 5.9%; $P = .002$) (table 3). These reaction rates were also significantly higher when bacterial load was $\geq 10^5$ cfu/mL than when it was $<10^5$ cfu/mL (4-fold and >34-fold higher, respectively) but did not differ significantly on the basis of the presence of neutropenia or the absence of antibiotics effective against contaminants (table 3). Age of platelet units also showed no difference, but this analysis was limited by the age of the platelet units being either 4 or 5 days in most cases.

Detection sensitivity. The sensitivity of methods required to detect bacterial contamination of platelet products on the basis of counts of the 46 bacterial species present in 45 transfused, contaminated units or pools is shown in figure 2 (quantitation was not performed for the remaining case). Whereas a sensitive detection method (with a cutoff value of 10^3 cfu/mL) would be needed to detect >95% of all contaminants and >90% of contaminants resulting in transfusion reactions, less sensitive methods (with a cutoff value of 10^6 cfu/mL) would have detected all severe, life-threatening, and fatal reactions.

DISCUSSION

Our study documented that a 32-fold higher rate of bacterially contaminated platelet units, a 27.7-fold higher rate of bacterially contaminated platelet units transfused, and a 10.6-fold higher rate of septic transfusion reactions were detected by active surveillance, compared with passive surveillance (table 2). Only 2 (4.5%) of 44 bacterially contaminated units transfused and 2 (12.5%) of 16 septic reactions were detected by passive surveillance, and we conclude that the real prevalence of these occurrences is greatly underreported in studies relying on passive surveillance. The fatality rate, however, did not differ between the surveillance methods, suggesting that these were appropriately recognized (albeit, our study was limited by a relatively small sample size). In addition, our surveillance showed that septic reactions, as defined in our study, occurred in 18 (41%) of 44 patients with contaminated transfusions and that 2 (11.1%) of 18 septic reactions were fatal.

Based on 2004 data, nearly 3 million platelet units were transfused in the United States in the form of 1.4 million SDP units and 1.5 million RDP units, the latter administered in an estimated 0.26–0.38 million pools of 4–6 units [8]. Contamination rates are similar for SDP and RDP units, but the contamination rate per transfusion, as expected, is 4–6-fold higher for RDP pool transfusions [9]. The fatality rate associated with bacterial contamination of platelets is estimated, based on recent data obtained by passive surveillance, to be ~2 deaths per million units transfused (~6 deaths per year in the United States), and the rate of septic transfusion reactions is estimated to be 10–13 cases per million units transfused (30–40 cases per year in the United States) [1, 10, 11]. Our data generated by passive surveillance reflect similar findings, with detection rates of 15 cases of septic transfusion reaction and 7 fatalities from

Table 3. Differences in the prevalence and severity of transfusion reactions based on virulence of the bacterial species, leukocyte counts of transfusion recipients, treatment with antimicrobial agents active against the bacterial contaminant at the time of transfusion, platelet unit age, and bacterial load.

Virulence	Virulence				Neutorpenia				Receipt of antibiotics	
	More-virulent species	Less-virulent species	OR (95% CI)	P	Yes	No	OR (95% CI)	P	No	Yes
Any transfusion reaction	11/12 (91.7)	9/34 (26.5)	3.5 (1.9–6.2)	**<.001**	10/23 (43.5)	10/23 (43.5)	1.0 (0.5–1.9)	.23	6/8 (75.0)	14/38 (36.8)
Severe transfusion reaction	6/12 (50.0)	2/34 (5.9)	8.5 (2.0–36.6)	**.002**	6/23 (26.1)	3/23 (13.0)	2.0 (0.6–7.0)	.16	0/8 (0)	8/38 (21.1)

NOTE. Data are proportion (%) of transfusion recipients, unless otherwise indicated. $P \leq .01$, by Pearson's χ^2 test with Bonferroni correction, was considered to be statistically significant. Statistical significane is indicated by boldface type. cfu, colony-forming units; NA, not applicable.

bacterially contaminated platelet transfusions per million transfused units. However, our data generated by active surveillance suggest that as many as 900–1200 contaminated units could be transfused annually in the United States, resulting in 300–400 septic reactions and 6–20 deaths per year. These projections based on our data could also be underestimates, because the active surveillance culture method used had some limitations, including an analytical sensitivity of 10 cfu/mL, which would not have detected lower levels of contamination; using only aerobic incubation, which would have missed obligate anaerobes; and not culturing platelets that were <4 days old for 84 months.

Furthermore, our study demonstrated the relationships between bacterial species and levels, unit age, neutropenia, antibiotic administration, and the occurrence and severity of transfusion reactions (figure 1 and table 3). More-virulent bacterial species, particularly gram-negative bacilli, and higher bacterial loads were significantly associated with both the occurrence and severity of reactions. The presence or absence of neutropenia showed no significant association with reaction occurrence or severity. Receipt of appropriate antibiotic therapy at the time of transfusion showed some association with a lower occurrence of any transfusion reaction, with the 95% CI of the OR just reaching statistical significance. However, no association could be shown with severe reactions, because there were no patients with severe reactions who had not been receiving antibiotics. Unit age at time of use showed no association with occurrence or severity of reactions, but this analysis was limited by most units being either 4- or 5-days old at the time of use.

In the previous 3 decades, small doses of endotoxin (2–4 ng/kg, equivalent to 14–28 EU in a 70-kg adult), administered intravenously to thousands of volunteers to study acute inflammatory responses, have generally been found to be safe, although 4 cases of severe bradycardia or protracted asystole have been reported [12]. A study of patients with septic shock showed median plasma endotoxin loads of 13,000 EU [13], and a self-administered intravenous endotoxin dose of 1 mg (100,000 EU) resulted in shock and multiple-organ dysfunction in a patient [14]. The amounts of endotoxin present in the 3 units that were contaminated with gram-negative bacilli and were associated with septic shock and multiorgan failure were 11,373 EU, 34,646 EU, and 182,700 EU, with such levels expected to produce severe reactions. No reaction occurred in the case of contamination with *S. marcescens* in a neonate with congenital neutropenia who received the largest amount of endotoxin (173,130 EU), and deficiency in or desensitization of phagocytic effector cells or defects in the IFN-γ–IL-12 axis may explain the lack of response and survival of this patient [15].

Our data also provide unique information on the relationships between bacterial levels and transfusion reactions. The bacterial load that differentiated between the occurrence and severity of reactions was 10^5 cfu/mL, indicating that the detection threshold of a clinically useful detection method at time of use should be at least 10^5 cfu/mL (table 3 and figure 2). In addition, a method with a detection threshold of 10^3 cfu/mL would have detected >95% of all cases and >90% of all reactions, whereas all cases would have been detected at 10^2 cfu/mL. These parameters can be used to guide the development of methods for at-issue testing.

Two recent changes in transfusion medicine practices—culture of SDP units 24 h after collection, with release for use if culture results were negative after an additional 12–24 h [16] and use of a diversion pouch on the inlet line of the plateletpheresis collection kit to trap and eliminate skin contaminants [17]—have been beneficial, although it is difficult to assess the relative roles of these changes at this time. In a recent study of 1 million apheresis platelet donations, these measures appreciably decreased the rate of septic reactions from 18 to 5.4 reactions per million transfused units, but they did not decrease the fatality rate (2.1 vs. 1.8 deaths per million transfused units), because fatalities were associated with bacteria that were not typical of skin flora [11]. Because two-thirds of the 60 deaths reported to the US Food and Drug Administration from 1995 through 2004 were associated with Enterobacteriaceae [1], the risk of septic and fatal reactions, particularly with these more virulent, non–skin-associated contaminants, is therefore likely to remain. There is also a need for a national hemovigilance system for adverse events from blood product use in the United States [18], an approach that has been successfully used in other countries to coordinate the recognition and documentation of transfusion-associated adverse events and, thereby, to improve the overall safety of transfusions [19].

Although these trends are encouraging, the well-recognized modalities currently in use to limit bacterial contamination of platelets, such as the use of SDP units rather than RDP pools and the use of younger rather than older units [20], should be continued, with universal use of diversion pouches in collection systems. Several solutions have been proposed to further reduce these risks. The first is to increase the sensitivity of bacterial detection performed at 24 h by increasing the volume cultured or performing the culture at a later time [21]. However, increasing the volume cultured results in only a modest increase in detection rates of ~25% [2]. The second proposed solution is to use pathogen inactivation technology, which also obviates the need for bacterial detection, and although regulatory approval has been received in Europe, this is not likely to occur in the United States in the next few years [22]. The third solution is an at-issue bacterial-detection method, an approach that holds considerable promise, with several manufacturers working on a variety of methods [23]. Detection of endotoxin by the limulus lysate method, although limited to detecting gram-negative contaminants, would have detected all fatal cases in our series. Based on our findings, successful implementation of these solutions in the United States could prevent the transfusion of up to 900–1200 contaminated units annually, thus avoiding 300–400 septic reactions and 6–20 deaths per year.

Table 3. ***(Continued.)***

		Unit age				Bacterial count			
OR (95% CI)	*P*	5 days	<5 days	OR (95% CI)	*P*	⩾10^5 cfu/mL	<10^5 cfu/mL	OR (95% CI)	*P*
2.0 (1.1–3.6)	.048	10/19 (52.6)	10/27 (37.0)	1.4 (0.74–2.7)	.23	16/23 (69.6)	4/23 (17.4)	4.0 (1.5–6.8)	**.001**
<0.05 (NA)	.19	3/19 (15.8)	5/27 (18.5)	0.85 (0.23–3.1)	.70	8/23 (34.8)	0/23 (0)	>34 (NA)	**.002**

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A laboratory comparison of pathogen reduction technology treatment and culture of platelet products for addressing bacterial contamination concerns

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BACKGROUND: Concerns over the risk of bacterial contamination of platelet products have led to implementation of bacteria culture and other screening methods. New approaches for dealing with this issue have also been proposed.

STUDY DESIGN AND METHODS: A direct comparison of treatment with riboflavin and ultraviolet (UV) light (Mirasol pathogen reduction technology [PRT] system) versus bacterial culture testing (two-bottle system, 48-hour quarantine) was undertaken to compare their effectiveness. Thirteen clinically relevant bacterial organisms (20 strains) were used in this evaluation. Results were compared with spiking levels at 20 to 100 colony-forming units (CFUs) per product and at less than 20 CFUs per product.

RESULTS: At spiking levels of 20 to 100 CFUs per product, the riboflavin and UV light process demonstrated 91% effectiveness against a broad spectrum of bacteria. In comparison, the culture method demonstrated an ability to detect up to 91% of the same contaminants, when used in the two-bottle, 48-hour-to-release configuration. At lower initial titers of contaminating agents (<20 CFUs per product), the effectiveness of PRT increased to 98% whereas the culture method effectiveness decreased to 66%. Effectiveness of the culture method further decreased to 60% when a one-bottle system was used.

CONCLUSION: The results from this work suggest that the riboflavin and UV light process may provide up to 98% protection against transfusion of bacterially contaminated units at the most clinically relevant contamination levels (<20 CFUs per product). This compares favorably to the 60% to 66% effectiveness of bacterial culture testing using a 48-hour quarantine period before product release.

Bacterial contamination of platelet (PLT) products has been identified as one of the most significant risks associated with the transfusion of blood components, reportedly occurring at levels as high as 1:2000 to 1:3000 donated products.[1-3] The storage of PLT products at room temperature for extended periods of time provides a medium and a condition of storage that can sustain bacterial growth. Product contamination at the time of collection is typically from the donor, although contamination from external sources has been reported. Even though the contamination levels of bacteria are believed to be extremely low, bacteria can proliferate to high titers before transfusion.[4,5] The inability to detect these low levels of bacteria at collection can result in severe consequences to PLT transfusion recipients and include morbidity and fatal reactions. Given the frequency of bacterial contamination, the AABB promulgated a new Standard effective March 1, 2004, mandating the implementation of methods to detect and reduce bacteria in PLT units.[6]

The implementation of PLT product screening has been successful in identifying contaminated products and reducing their transfusion into patients.[7-13] The level of success that has been observed varies in accordance with the technique that is utilized and has several logistic consequences. Because the level of bacteria present at the time of PLT donation is low, detection is limited by several factors, including the initial titer of bacteria, the size and

ABBREVIATION: PRT = pathogen reduction technology.

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timing of the test sample, and the growth kinetics of the specific bacterial species. Low levels of bacteria at the time of donation, particularly for slow-growing organisms, pose a challenge for most culture methods.[10,12]

To compensate for these factors, a postdonation product incubation before sampling and increased sample volumes for testing were evaluated.[14] While these methods do increase the sensitivity of the detection of contaminated units, they require an additional prerelease storage interval in which products are held before testing and then subsequently held for an additional period after sample is withdrawn to allow detection of contaminated units. This may result in as much as a 48-hour reduction in the initial storage period, a period in which product quality may be at a maximum, although the intent was to increase the storage interval for an additional 2 days to compensate for the testing period before release.[14,15] Because most PLT products in routine use historically are used within a 48- to 72-hour period of collection, this change in practice could represent a major shift in possible clinical experience necessitated by the additional culture time requirements.[13,16,17]

An alternative approach that has been proposed for assuring a decrease in the transfusion of PLT units contaminated with bacteria has been the use of pathogen reduction technology (PRT). Several of these methods are currently in development or in actual clinical use in Europe.[18-21] The technologies are based on the use of photochemical agents, which can be activated by ultraviolet (UV) light in specific spectral regions and then carry out chemical modifications to DNA and RNA that prevent their subsequent replication.[22] These modifications in essence render bacterial agents present in these products incapable of growth during storage and thus also incapable of causing complications after transfusion.

The challenge for PRT methods, unlike that of bacterial detection, are not low titer levels that are present at donation, but rather higher titer challenges that may develop during storage. For PRT to be effective at preventing transfusion of units that may cause complications in this regard, it must be able to effectively prevent growth of low levels of contaminating bacteria, which may be present soon after donation. The inability of these techniques to perform adequately in this fashion might lead to subsequent growth of bacteria during storage. Because growth of bacteria may lead to the formation of pyrogenic agents and endotoxin, inactivation of products at time intervals considerably after collection would likely be ineffective in preventing pyrogen- or endotoxin-mediated clinical reactions. As a result, these methods are usually carried out at short intervals after collection (e.g., <22 hr in the case of the riboflavin and UV light system specifications).

This study was undertaken to assess in a laboratory setting the ability of the riboflavin and UV light process to inactivate bacteria in products sufficiently soon after collection to prevent growth of the bacteria during subsequent storage. Results from this work were compared directly to a culture method using a sufficient postcollection, presampling incubation period (24 hr) and postsampling detection window (24 hr) to assure the detection of low levels of contamination in spiked products. Comparisons were then made between the ability of the PRT method to inactivate bacteria spiked into these samples with the ability to detect low levels of these agents after the appropriate presample and postsample culture times.

MATERIALS AND METHODS

A panel of organisms identified in prior hemovigilance programs was selected for evaluation in this study.[23] These included the following species: *Staphylococcus epidermidis, Staphylococcus aureus, Propionibacterium acnes, Streptococcus mitis, Streptococcus agalactiae, Streptococcus pyogenes, Serratia marcescens, Acinetobacter baumannii, Yersinia enterocolitica, Bacillus cereus* (spore-forming agent), *Escherichia coli, Enterobacter cloacae,* and *Klebsiella pneumoniae.* Multiple strains of both *S. epidermidis* and *S. aureus* were tested: five strains of *S. aureus* and four strains of *S. epidermidis.* Several groups identified these organisms to be of interest.[24-26] A minimum of three replicates for each bacterial strain were tested in independent spiking studies. Although identified as an organism of interest, *Providencia rettgeri* was not tested in this study.

The range of contaminating bacteria used for this study was between 1 and 100 colony-forming units (CFUs) per product. Bacteria were grown in a nutrient broth for up to 36 hours, after which time they were centrifuged, concentrated, and then resuspended in a minimal nutrient medium. Bacteria stock culture concentrations were determined through the use of an endpoint plating scheme for all organisms except *P. acnes* and *B. cereus.* Bacteria cultures were stored at 4°C until they were ready to be used. An endpoint plating scheme was not used to determine the stock culture titer of *P. acnes* and *B. cereus* due to the relative instability of these organisms when stored at 4°C; instead, historical data were used to estimate the titer of the culture and the organism was used on the day it was harvested. Inoculating doses were determined mathematically using the initial culture titer value. Results from actual clinical experience with detection times and growth curves for these organisms in culture suggest that this level of contamination is consistent with actual clinical experience.[13,27-30] Notable exceptions with regard to the spike titer limits were *B. cereus* at 103 CFUs per product and *P. acnes* at 596 CFUs per product. The *B. cereus* titer was an unintended result; however, it was determined the 103 CFUs per product value was within the range of experimental error used to measure the titer of the stock culture. It also represented a worst-case scenario for riboflavin and UV light–treated units given the 1 to 100 CFUs per product range this study was investigating. The initial titer for *P. acnes* was increased to ensure that an adequate amount of organism would be present at Day 7 so that it could be detected in the positive control.

General study design

Figure 1 provides a diagram of the overall study design utilized in this work. A direct comparison of the riboflavin and UV light process to bacterial screening was performed using double PLT units. Each incoming double PLT product was split into 2 units and each unit was inoculated with a clinically relevant bacterial dose. This was done to allow ample volumes to be available for each test condition. One unit was treated with the riboflavin and UV light process according to methods described previously while the other unit underwent bacterial screening using a culture method.[31] The volume of the unit used for culture was maintained at 280 mL on average to simulate mean product volumes experienced in the clinical setting. For the riboflavin and UV light treatment, the units had on average a final volume of 225 mL. Additionally, a small volume of the original double PLT product (before inoculation with bacteria) was set aside and allowed to incubate at 22°C for 7 days. The sample served as the negative control. This was the study design that was followed when Gram-positive bacteria were tested. However, due to the susceptibility of Gram-negative bacteria to complement activity found in plasma-derived products, heat treatment was used to deplete the native complement activity. For Gram-negative bacteria, each incoming double PLT product was centrifuged and the non–complement-depleted plasma was expressed off. Pooled, recovered human AB+ plasma was heat treated at 56°C for 45 minutes in a water bath and clarified via centrifugation to remove any precipitate. The PLTs were then resuspended in a comparable amount of complement-depleted recovered human AB+ plasma and allowed to rest overnight. Because many organisms can be inactivated by complement, this process assured that we were evaluating a worst-case situation, which promoted optimal growth conditions. All work, not including collection of the apheresis PLTs, took place at CaridianBCT Biotechnologies (Lakewood, CO).

Bacteria culture testing

The bacterial screening procedure utilized here involved incubating all collected PLTs for 24 hours at 22°C on a

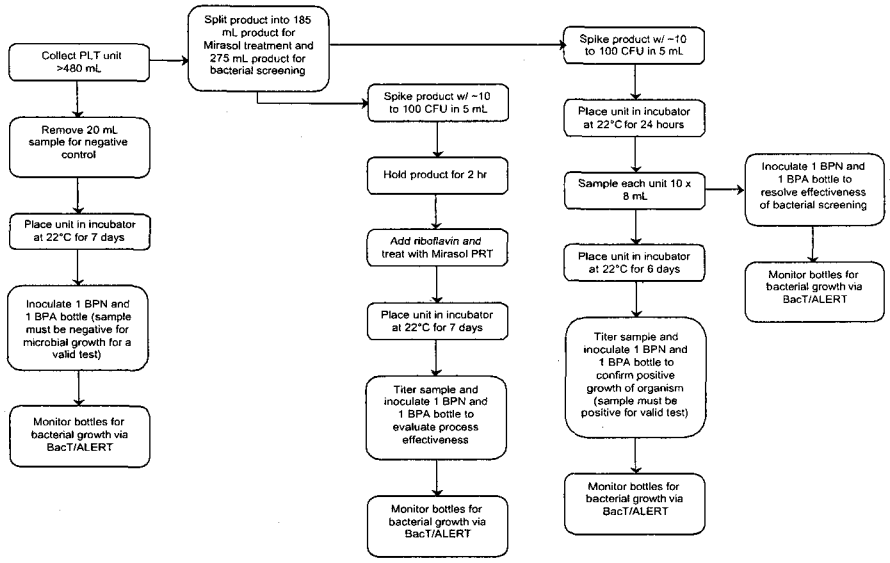

Fig. 1. Study diagram overview. The figure depicts the general sampling and treatment schemes used for both the bacteria culture testing and riboflavin and UV light treatment arms of the study.

PLT shaker before sampling to allow growth of contaminants to high enough titers to increase the probability of detection in small-volume samples. The specific sampling protocol followed was consistent with the recommendations of the PASSPORT protocol, which was under evaluation in the United States as a method for reducing transfusion of bacterially contaminated products stored for up to 7 days.[14] This protocol was carried out according to the following procedure: After 24 hours of incubation, 10 individual 8 mL samples were withdrawn from each unit and two 4 mL aliquots from each sample were inoculated into two bottles (aerobic and anaerobic media, BacT/ALERT, bioMérieux, Durham, NC) respectively. These individual samples were used to represent multiple sampling events. In the PASSPORT protocol, if the product remained negative after 24 hours of monitoring (after 24 hr of storage presampling; total of 48 hr), it was released for transfusion; however, monitoring for bacterial contamination continued for 7 days to allow an increased sensitivity to detect organisms later in storage that may have been at too low a titer level to detect earlier. This same approach was utilized in this study plan. The 10 sample pairs withdrawn from each unit were considered as 10 individual sampling events. Paired bottles that did not test positive during monitoring were considered as a failure to detect. The data were also reanalyzed to simulate the effectiveness of the procedure when only a single 4-mL aerobic bottle was used.

After sampling, the remaining PLT units were placed back into the PLT incubator at 22°C for an additional 6 days. An aliquot from each product was then sampled at Day 7 as a positive growth control for the paired PLT products. Inoculated bottles were then monitored for a period of 7 days after sampling. Any negatives that would have been observed during this period would have eliminated paired bacterial screening and riboflavin and UV light–treated units from the final data analysis due to a failure of the organism to proliferate in the positive control. There were no positive control units in any of the studies where the inoculated organism failed to survive. The positive control was also tested for the 7-day bacterial load using a conventional endpoint dilution-plating method with agar plates.

PRT-treated units

Each unit of the split double collection was spiked with 1 to 100 organisms, incubated for a minimum of 2 hours after spiking, and then treated according to the riboflavin and UV light method for PLTs (Mirasol PRT process, CaridianBCT) as described previously.[31] This entire unit was then placed into incubation at 22°C under standard PLT storage conditions for 7 days. At the end of the 7-day period, an 8-mL sample was removed and placed into culture using the method described above. Inoculated bottles were then monitored for a period of 7 days after sampling. Any positives observed during this period were counted as a positive event.

Test inclusion requirements

The following requirements had to be met or paired PLT units were removed from the study and a replacement pair used:

The collected double PLT product must have had a PLT concentration range of 1180×10^3 to 2100×10^3 cells per μL and a volume greater than 480 mL. Apheresis double PLT units were collected using the CaridianBCT Trima Accel platform. PLTs were allowed to rest 2 hours before manipulation.

- The negative control must have remained negative for bacterial growth.
- The positive control must have been positive for bacterial growth.
- The bacterial load dosed to each unit must have been between 1 and 100 CFUs per product as determined by an independent culture and titer determination, which involved titering of the actual dilution used to spike the product. Exceptions with regard to the spike titer limits were *B. cereus* at 103 CFUs per product and *P. acnes* at 596 CFUs per product.

Determination of overall efficacy

For the determination of overall performance, the contamination frequency reported in hemovigilance studies was used to calculate the expected clinical performance of the product.[23] For this calculation, the overall effectiveness of the method for detecting (culture method) or inactivating (PRT method) the species present was multiplied by the occurrence frequency reported in the literature. For multiple strains of the same organism, overall mean values for all strains were used to estimate effectiveness for that particular species. This multiple thus provides a rough estimate of the potential ability of each method to interdict a proportion of the expected contamination events in the clinical setting: Occurrences = Reports from hemovigilance studies of the number of cases observed, Frequency = Occurrences of this individual species normalized for total number of reported events, and % Effectiveness = Ability to detect or to inactivate the particular agent. Each sample was evaluated in a minimum of three separate replicate spiking experiments for each strain of a given species of bacteria: Overall effectiveness = The multiple of the % Effectiveness with the Frequency of Occurrence for this agent reported in the cited hemovigilance studies.

RESULTS

An overall summary of bacteria testing results is provided in Table 1. A total of 29 separate studies were conducted with at least three replicates for each strain tested. Emphasis was placed on organisms according to the prevalence reported in published hemovigilance studies to simulate actual clinical experience as much as possible.[23] Table 2 shows a comparison of overall efficacy of the culture and PRT methods evaluated in this study at spiking levels of 20 to 100 CFUs per product.

Both the culture method and the riboflavin and UV light treatment demonstrated 91% effectiveness at detection or inactivation respectively, when culture testing was performed using the two-bottle, 48-hour-to-release method. These results appear to be within the ranges of clinical observations for culture detection methods employing similar sampling and incubation techniques.[14,29,30] At lower levels of contamination (<20 CFUs per product), the effectiveness of the riboflavin and UV light treatment increased to 98% (Table 3), whereas the culture method decreased to 66% (Table 4), which is more consistent with clinical observations, possibly suggesting that actual clinical contamination levels are more frequently in this range. Using a one-bottle method, the effectiveness of the culture method under these conditions decreased further to 60% (Table 5). As one might expect, the culture method showed a reduced ability to capture a contaminant when the sample titer is lower, making detection less likely, while the ability of a PRT method to achieve complete inactivation increased at lower bacteria levels (see Fig. 2).

Several agents were not uniformly detected by the culture method employed in this study. Not unexpectedly, these are primarily organisms exhibiting a slow growth rate at 22°C. Such behavior makes detection by a culture method difficult due to the inability to obtain an adequate bacterial inoculum for culture detection. In this study, any detection that occurred in the bacteria culture arm of the study was counted as a positive detection event, even if this occurred outside of the 24-hour release window, with the exception of *P. acnes*, which took

TABLE 1. A summary table of organisms that were evaluated in this study*

Organism type	Gram (+/–)	ATCC number	Culture time until detection (mean hr)	Inoculum titer (CFUs/product)	7-day positive growth control titer (CFUs/mL)
A. baumannii	–	17961	7.6 ± 1.1	61	3.6×10^8
A. baumannii	–	17961†	NT	12	8.8×10^7
B. cereus	+	NA	4.4 ± 0.1	103	6.5×10^6
E. cloacae	–	29005	5.9 ± 0.5	41	1.5×10^9
E. cloacae	–	29005†	NT	12	1.4×10^9
E. coli	–	25922	6.6 ± 2.3	65	2.7×10^8
K. pneumoniae	–	8045	12.6 ± 0.7	55	6.2×10^8
P. acnes	+	51277	106.7 ± 45.8	596	<1.0
S. marcescens	–	43862	4.3 ± 0.2	79	$>3.0 \times 10^9$
S. aureus	+	29213	9.7 ± 1.1	75	2.2×10^8
S. aureus	+	29213†	12.1 ± 2.1	14	4.0×10^8
S. aureus	+	10832	14.4 ± 0.9	63	3.8×10^8
S. aureus	+	25923	9.3 ± 0.3	68	4.3×10^8
S. aureus	+	25923†	NT	12	3.8×10^7
S. aureus	+	700787	19.5 ± 2.6	53	1.6×10^8
S. aureus	+	700787†	NT	10	2.4×10^7
S. aureus	+	27217	9.0 ± 0.6	70	5.3×10^8
S. aureus	+	27217†	NT	12	3.1×10^8
S. epidermidis	+	12228	17.3 ± 1.7	54	3.6×10^8
S. epidermidis	+	12228†	19.1 ± 0.8	11	1.8×10^8
S. epidermidis	+	14990	19.0 ± 1.9	57	1.2×10^8
S. epidermidis	+	700578	23.2 ± 1.7	23	2.9×10^8
S. epidermidis	+	700578†	NT	11	2.0×10^7
S. epidermidis	+	35984	19.4 ± 1.5	39	2.7×10^7
S. agalactiae	+	700046	9.1 ± 0.8	54	6.1×10^6
S. agalactiae	+	700046†	NT	8	3.0×10^7
S. mitis	+	6249	16.2 ± 2.9	28	2.9×10^7
S. pyogenes	+	BAA-1064	10.2 ± 0.6	42	4.4×10^5
Y. enterocolitica	–	23715	10.1 ± 2.3	76	8.5×10^8

* These agents were selected from the reports of several hemovigilance studies (BACON, BacTHEM, SHOT), which provided estimates of the types and frequency of contamination of PLT products with bacteria. Several strains of predominant organisms were used to represent possible variation in the nature of the contaminants and test the effectiveness for both the culture and the inactivation method as a function of the strain within a given bacterial species. Incubation times after inoculation of culture bottles to detection using the BacT/ALERT method are listed as well as the final titers measured at Day 7 of incubation of the positive control products.
† Samples tested at less than 20 CFUs per product.
+ = positive; – = negative; NA = not applicable; NT = not tested.

TABLE 2. A summary table for comparison and calculation of overall efficacy of the two methods used in this study (PRT and culture)*

Occurrences	% Frequency	Organism	ATCC number	% Effectiveness		Overall effectiveness	
				Culture	Mirasol	Culture	Mirasol
20	33	*S. epidermidis*	12228	80	100	29	31
			14990	83	100		
			700578	97	67		
			35984	87	100		
8	13	*E. coli*	25922	100	100	13	13
7	12	*B. cereus*	NI-0001	100	100	12	12
6	10	*S. aureus*	29213	90	100	10	8
			10832	90	100		
			25923	100	62		
			700787	97	88		
			27217	100	67		
5	8	*S. agalactiae*	700046	100	67	8	7
		S. mitis	6249	100	100		
		S. pyogenes	BAA-1064	100	100		
4	7	*E. cloacae*	29005	100	67	7	4
3	5	*P. acnes*	51277	0	100	0	5
3	5	*S. marcescens*	43862	100	100	5	5
2	3	*K. pneumoniae*	8045	100	100	3	3
1	2	*A. baumannii*	17961	100	33	2	1
1	2	*Y. enterocolitica*	23715	100	100	2	2
				Effectiveness:		91	91

* All samples were spiked at 20 to 100 CFUs per product initially with each of the species indicated. Occurrences = Reports from hemovigilance studies of the number of cases observed; Frequency = Occurrences of this individual species normalized for total number of reported events; % Effectiveness = Ability to detect or to inactivate the particular agent. Each sample was evaluated in a minimum of three separate replicate spiking experiments for each strain of a given species of bacteria. Overall effectiveness = the multiple of the % Effectiveness with the Frequency of Occurrence for this agent reported in the cited hemovigilance studies.

TABLE 3. Summary table for riboflavin and UV light effectiveness

Occurrences	% Frequency	Organism	ATCC number	Mirasol	
				% Effectiveness	Overall effectiveness
20	33	*S. epidermidis*	12228	100	33
			14990	100	
			700578*	100	
			35984	100	
8	13	*E. coli*	25922	100	13
7	12	*B. cereus*	NI-0001	100	12
6	10	*S. aureus*	29213	100	9
			10832	100	
			25923*	50	
			700787*	100	
			27217*	100	
5	8	*S. agalactiae*	700046*	100	8
		S. mitis	6249	100	
		S. pyogenes	BAA-1064	100	
4	7	*E. cloacae*	29005*	100	7
3	5	*P. acnes*	51277	100	5
3	5	*S. marcescens*	43862	100	5
2	3	*K. pneumoniae*	8045	100	3
1	2	*A. baumannii*	17961*	66	1
1	2	*Y. enterocolitica*	23715	100	2
				Effectiveness:	98

* Samples tested at less than 20 CFUs per product.

significantly longer to detect (Table 1). As the data in Table 1 suggest, this window may be inadequate for several of the slow growing organisms given that mean detection times for several strains of *S. epidermidis* were on the order of 19 to 23 hours and well beyond this value for *P. acnes* (107 hr). The late positives detected in the case of *P. acnes* were not included in the effectiveness sums that are reported here.

Agents not uniformly inactivated by the PRT method employed in this evaluation included *S. aureus* and *A. baumannii*. Interestingly, separate high spike titer studies with *A. baumannii* and *S. aureus* (data not shown) have

TABLE 4. Summary table for culture method effectiveness using two-bottle culture method

Occurrences	% Frequency	Organism	ATCC number	Culture method	
				% Effectiveness	Overall effectiveness
20	33	*S. epidermidis*	12228*	27	9
8	13	*E. coli*	25922	100	13
7	12	*B. cereus*	NI-0001	100	12
6	10	*S. aureus*	29213*	53	5
5	8	*S. agalactiae*	700046	100	8
		S. mitis	6249	100	
		S. pyogenes	BAA-1064	100	
4	7	*E. cloacae*	29005	100	7
3	5	*P. acnes*	51277	0	0
3	5	*S. marcescens*	43862	100	5
2	3	*K. pneumoniae*	8045	100	3
1	2	*A. baumannii*	17961	100	2
1	2	*Y. enterocolitica*	23715	100	2
				Effectiveness:	66

Results demonstrate the reduced ability to detect these organisms, likely due to the lower initial titers present at the 24-hour sampling time point. Occurrences = Reports from hemovigilance studies of the number of cases observed; Frequency = Occurrences of this individual species normalized for total number of reported events; % Effectiveness = Ability to detect or to inactivate the particular agent. Each sample was evaluated in a minimum of three separate replicate spiking experiments for each strain of a given species of bacteria. Overall effectiveness = the multiple of the % Effectiveness with the Frequency of Occurrence for this agent reported in the cited hemovigilance studies.

* Samples tested at less than 20 CFUs per product.

TABLE 5. Summary table for culture method effectiveness using one-bottle (aerobic) culture method

Occurrences	% Frequency	Organism	ATCC number	Culture method	
				% Effectiveness	Overall effectiveness
20	33	*S. epidermidis*	12228*	13	4
8	13	*E. coli*	25922	100	13
7	12	*B. cereus*	NI-0001	100	12
6	10	*S. aureus*	29213*	43	4
5	8	*S. agalactiae*	700046	100	8
		S. mitis	6249	93	
		S. pyogenes	BAA-1064	100	
4	7	*E. cloacae*	29005	100	7
3	5	*P. acnes*	51277	0	0
3	5	*S. marcescens*	43862	100	5
2	3	*K. pneumoniae*	8045	100	3
1	2	*A. baumannii*	17961	100	2
1	2	*Y. enterocolitica*	23715	100	2
				Effectiveness:	60

* Samples tested at less than 20 CFUs per product.

demonstrated the ability to inactivate up to 2.6 and 3.6 logs/mL of these agents. For the culture method, *A. baumannii* was more readily detected due to its rapid growth characteristics. This observation raises the point that the effectiveness of eliminating bacteria contamination also needs to consider the type of organism involved, as some Gram-negative species tend to be of greater concern in terms of potential adverse outcomes in patients. For this particular Gram-negative species (*A. baumannii*), however, no fatalities were observed in the hemovigilance programs referenced above.[23] This analysis is not capable of taking clinical outcomes into full account.

Surprisingly, a spore-forming agent tested in this study, *B. cereus*, did show complete inactivation in the PRT-treated samples, suggesting that the ability to inactivate this class of agents in general may be dependent on the levels of spores that are present in the product at the time of treatment and not on whether the agent can be classified as spore-forming. The culture method consistently failed to detect *P. acnes* even at the higher spiking levels studied. In all other cases where failures occurred, partial efficacy was demonstrated with either the bacterial detection or PRT method.

DISCUSSION

The results from this study allow comparison of the riboflavin and UV light PRT method for pathogen reduction of PLT products and the bacterial culture method for

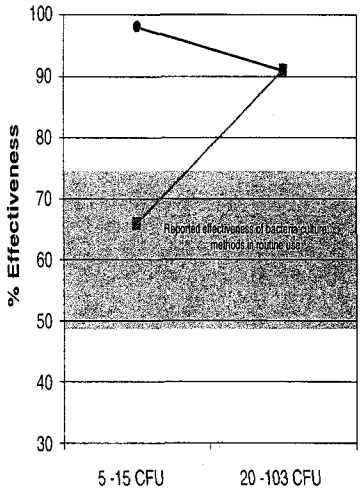

Fig. 2. Illustration of the effectiveness of PRT (●) versus bacteria culture testing (■) at two bacterial contamination levels. Shaded area reflects reported effectiveness of bacteria culture methods in routine clinical use.[14,29,30]

detection of bacteria contamination in PLT products. Not surprisingly, the bacterial culture method requires extended incubation times of the product before and after sampling to increase the likelihood of detection events. Such a requirement may quarantine PLTs when they are at the height of their clinical performance. Although studies have shown that PLTs stored for an extended period to compensate for this time loss during quarantine exhibit similar in vivo recovery and survival properties, there is a paucity of information concerning the clinical performance of these products when they are used in the routine treatment of patients with thrombocytopenia.[32] Little information is available on the performance of these older products relative to count increments, transfusion frequency, transfusion requirements, and efficacy in preventing bleeding.[32] The data that are available in this regard demonstrate reduced levels of corrected count increment values for products stored for extended time, dependent on the storage medium utilized.[33-37]

The PRT methods that have been proposed also have the potential to affect PLT quality and performance. Their propensity to alter cell metabolic and activation properties as measured in vitro has been well documented in the literature.[31,38] Recovery and survival studies of these products also demonstrate differences relative to untreated controls at the same time point in storage, although these values fall within the limits established for historical products currently in routine clinical use.[39] These products have also been evaluated in randomized prospective clinical studies for several of the parameters listed above.[17,19,20] For riboflavin and UV light–treated products, clinical data obtained in a randomized, prospective, blinded clinical study demonstrated that there was no change in PLT or red cell transfusion requirements and no increase in bleeding or adverse events in thrombocytopenic patients receiving PRT-treated PLT products as compared to patients receiving untreated products.[40]

Proof of the efficacy of a PRT method in the clinical setting would require tens of thousands of samples in order to establish the ability of the procedure to prevent transfusion of bacterially contaminated units. Some authors have suggested that the lack of such data justifies not implementing PRT methods in clinical settings.[41] This seems, however, to be a circular argument in that such data will not be available until these methods are more routinely applied in the clinical setting and appropriate analyses can be carried out. Since such a condition does not presently exist, we undertook this study to evaluate the effectiveness of the process for inactivating the most commonly identified bacterial species from several hemovigilance reports on contamination of PLT products with bacteria. A direct comparison with bacterial culture methods was employed to compare effectiveness levels and provide a reference with current practices designed to decrease transfusion of bacterially contaminated PLT products.

The effectiveness of both methods was compared for two different levels of bacterial contamination: less than 20 CFUs per product and 20 to 100 CFUs per product. At the higher contamination levels, equivalent performance (91% effectiveness) was observed for the two methods studied here when bacteria detection was performed using a two-bottle method with 24-hour incubation and a 24-hour minimum culture period. However, at lower initial bacteria titers (<20 CFUs per product), the riboflavin and UV light procedure (performed on the day of collection) demonstrated significantly greater effectiveness as compared to bacteria culture using the methods described here, that is, 98% versus 66%. Interestingly, outcomes reported from larger-scale clinical evaluations of bacteria culture performance in detecting and abrogating transfusion of bacterially contaminated units suggest that these methods are only about 50% to 70% effective (see Fig. 2). This has been reported by Dumont and colleagues,[14] Benjamin and colleagues,[29] and Foley and colleagues[30] based on extensive routine use experience with bacteria culture methods. The approach used by Foley and colleagues involved holding apheresis products only 12 hours before culture and thus may explain the lower detection rates reported in that study. A similar performance is also predicted based on modeling of available

data from several sources.[42] When combined with these reported findings and as suggested by Eder and coworkers[13] and with results from the PASSPORT study,[14] the results from our study indirectly confirm that contaminating events are likely to occur at levels of less than 20 CFUs per product and hence that the outcomes for this range of bacteria titers may be the most clinically relevant.

It is important to point out that the bacterial detection method used in our study involved a two-bottle culture method with a 24-hour postcollection sampling period and a 24-hour postsampling culture period (total of 48-hr quarantine). Not all culture methods currently in use in the routine clinical practice employ this approach.[13,43,44] Reduction in the amount of sample tested (one bottle vs. two bottles) or reduction in the presampling hold time or the postsampling culture time to release are likely to significantly decrease the ability of culture methods to detect contaminated units. The outcome for bacteria detection efficacy in our study (66% effectiveness) is comparable to actual observations made in the clinical setting (approx. 50%-70% effectiveness).[14,29,30] Our results indeed demonstrated that the bacteria detection effectiveness dropped from 66% to 60% when using only a one-bottle test.

The data contained in this report also provide evidence that neither method may be expected to provide 100% protection against all septic transfusion events. Given that all contaminated units do not lead to sepsis,[45] the frequency of significant clinical events that are observed should decrease dramatically with a PRT method in place.

The reason for failures in the case of the bacterial detection system seems clearly based on the growth kinetics of the organisms employed and the titer of the bacteria that are present in these preparations. Lower titers of slow-growing organisms at donation are not only harder to detect initially, but also less likely to reach detection limits during quarantine.

In the case of PRT-treated products, the reasons for failures are less clear. It would be reasonable to assume that low titers of a given species present during treatment (20-100 CFUs per product) should easily be inactivated by these techniques given results from high-titer studies, which report efficacy levels exceeding 4 to 6 log/mL (equivalent of 300 million CFUs per product for a 300-mL product). The results in this work suggest that this is not the case. Some suggestion for a possible mechanism of action here comes from the work of Clawson and White,[46] who described as long as 30 years ago the propensity for certain species of bacteria to interact with PLT surfaces, primarily through protein A receptors. We speculate that the possible adherence or engulfment of certain bacterial strains by PLTs via these types of mechanisms may act as a shield to sensitizer uptake and light exposure, affording protection of these agents against the PRT method being applied. If such is the case, a correlation should exist between the ability of these organisms to interact with PLTs via these mechanisms and their ability to be inactivated to completion even at low spike titers, where a larger proportion of the population may actually be bound. Internal studies conducted separately using these species spiked into plasma products (no PLTs present) demonstrate complete inactivation, consistent with this possible mechanism (data not shown).

It is also possible that interactions with other bacteria at higher concentrations create shielding effects against treatment. One may also not rule out the potential of bacteria defense mechanisms against PRT treatment. Regardless of the precise mechanism involved, the results do suggest that inactivation of the larger proportion of free organisms may provide an inadequate or incomplete picture of the ability to maintain culture negative products during storage.

Similar concerns are also present when addressing spore-forming bacteria in their spore form. These agents would be expected to be naturally highly impermeable to photosensitizers and thus resistant to these treatments. If such is the case, this work has profound implications regarding appropriate methods for evaluating the effectiveness of both PRT treatment methods and bacteria detection methods. Because these properties may vary from strain to strain within a given species of bacteria, it would be important for such work to employ several strains of test organisms and not extrapolate results from a single strain to cover all strains within a given species. Such was the case for several organisms studied in this work where results varied as a function of strain within a given species of the same organism. As demonstrated by the results of this work, selection of a particular strain with low interaction potential could provide a false estimate of inactivation efficacy extrapolated to all other strains. Detection methods may also be biased in a similar fashion depending on the presence or absence of cellular agents in the testing matrix during culture or detection. Clearly the particular strains seen clinically would appear to be those most relevant for this type of analysis. Likewise, the use of high-titer spike studies alone for demonstrating efficacy against bacteria in general can be only a partial and potentially misleading estimation of clinical efficacy, given that this is far removed from the actual clinical experience with regard to contamination levels in donated products at the point at which PRT methods may be practically and appropriately applied. Inactivation of high titers would also seem clinically irrelevant if accompanied by high endotoxin levels that are not reduced by these processes, which, upon infusion, can generate severe reactions even in the absence of viable bacteria.

The work of Nussbaumer and coworkers[47] with an alternative, approved PRT system describes 100% efficacy of the process for all of the clinical strains that were tested.

The use of clinical strains isolated from contaminated blood samples may afford samples that are more relevant to the actual clinical setting, but the ability to demonstrate reproducible results with such strains, due to their limited characterization and availability, is often constrained. As suggested from our work as well, the use of single strains of a given organism may provide a false sense of overall effectiveness for a given species of bacteria. The use of multiple strains, as demonstrated here, may yield variable results. Prowse[48] described several concerns with the approach utilized in prior analyses of the effectiveness of inactivation with other PRT methods. This included the lack of a positive control. In this study, samples which failed to grow in the positive control caused the elimination of both samples from the test and control groups from the study population as it would be impossible to determine if inactivation had occurred due to the treatment process or if the bacteria were simply inactivated by complement or other blood product components alone, not directly associated with the treatment method. In addition, in this study, for Gram-negative bacteria, which are known to be sensitive to complement, we eliminated the possibility of bacteria elimination by complement action alone through heat inactivation of test sample plasma before spiking with bacteria.

In summary, the purpose of this work was to address the ability of the riboflavin and UV light method to inactivate bacteria in contaminated PLT products and to compare outcomes with those obtained using a culture method that is being employed routinely today. The results from this work suggest that the riboflavin and UV light process may provide up to 98% protection against transfusion of bacterially contaminated units at clinically relevant contamination levels (<20 CFUs per product) as compared to the 60% to 66% effectiveness of bacterial culture testing observed in this study. Thus, use of a PRT method may afford greater levels of protection than bacterial detection by any particle-based detection method, even with larger sampling volumes and extended incubation times. A true determination of the validity of this analysis and this approach for approximating clinical outcome can only be provided once more routine application of PRT methods occurs.

This analysis does not take into account other properties that the PRT methods may provide relative to prevention of viral, parasitic, or white cell–mediated complications of transfusion of blood products.[31,49-53] These may be additional targets for hemovigilance analysis of products treated with these processes once they are used in a more routine fashion. The PRT approach may thus afford a means to address multiple transfusion-related concerns related to blood safety in a single platform. Clearly, bacterial detection methods are not intended to provide prevention of viral, parasitic, or white cell–mediated complications or other utility. This too may be a consideration in decision-making processes by transfusion medicine professionals.

CONFLICT OF INTEREST

All authors are employees of CaridianBCT Biotechnologies, LLC.

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Infectivity in chimpanzees (*Pan troglodytes*) of plasma collected before HCV RNA detectability by FDA-licensed assays: implications for transfusion safety and HCV infection outcomes

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Infectivity in chimpanzees (*Pan troglodytes*) of plasma collected before HCV RNA detectability by FDA-licensed assays: implications for transfusion safety and HCV infection outcomes

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Serial plasma aliquots (50 mL) obtained from 10 commercial donors who converted from hepatitis C virus (HCV) RNA negative to positive were transfused into 2 chimpanzees to assess infectivity during early HCV infection. Plasma, obtained 4 days before HCV RNA detectability by licensed assays, transmitted HCV infection to chimpanzee X355. The infectious PCR-negative plasma was subsequently shown to be positive in 2 of 23 replicates using a sensitive transcription-mediated amplification (TMA) assay, and estimated to contain 1.2 HCV RNA copies/mL (60 copies/50 mL transfused). Plasma units obtained up to 8 weeks earlier were not infectious in a second susceptible chimp, even when from donors with low-level, intermittent HCV RNA detection. Chimp x355 developed acute viremia with subsequent seroconversion, but cleared both virus and Ab in 17 weeks. When rechallenged 38 months later with 6000 RNA copies/mL from the same donor, X355 was transiently reinfected and again rapidly lost all HCV markers. We conclude that: (1) transfusions can transmit HCV infection before RNA detection, but the interval of test-negative infectivity is very brief; (2) early "blips" of HCV RNA appear noninfectious and can be ignored when calculating residual transfusion risk; and (3) markers of HCV infection can be lost rapidly after exposure to low-dose inocula. (*Blood*. 2012;119(26):6326-6334)

Introduction

The phase between the onset of hepatitis C virus (HCV) infection and sustained systemic viremia is known as the eclipse or previremic window phase of infection.[1-3] At its conclusion, plasma HCV RNA concentrations rise exponentially in what has been termed the ramp-up phase of infection.[1,2] The eclipse phase, by definition, is characterized by lack of detectable plasma viremia by commercially available HCV RNA assays, which are primarily based on PCR technologies. However, by performing quadruplicate analyses of plasma donations from acutely infected source plasma donors using more sensitive qualitative transcription-mediated amplification (TMA) assays for HCV RNA, we found previously that 108 of 225 eclipse-phase donations from 50 donors unexpectedly demonstrated the presence of intermittent, low-level HCV RNA.[1] A second study of source plasma donors found a similar phenomenon,[4] as did studies in injection drug users (IDU)[5,6] and transfusion recipients.[7] Thus, HCV dynamics in the eclipse phase may follow one of 2 patterns: no viremia until a sustained rapid increase in serum RNA levels occurs (ramp-up) or intermittent low-level HCV RNA detection (previously referred to in HIV and SIV infections as "blip" viremia)[8,9] that precedes ramp-up by varying intervals up to 2 months.

Because HCV RNA levels are very low in this very early phase of infection, it has not been possible to determine whether the RNA is contained in infectious virions or to characterize the sequence integrity of HCV RNA genomes during these "blips" relative to the sequences observed during the ramp-up period from the same subjects. With respect to blood safety, if the "blips" are found to be infectious, then current residual risk modeling might be underestimating HCV transfusion-transmission risk because such modeling assumes that donations given before the extrapolated beginning of the ramp-up period are noninfectious.[10-12]

The chimpanzee model of HCV infection has played a key role in our understanding of determinants of viral transmission and replication, the development of antiviral immune responses, assessment of potential viral and host factors that lead to resolved versus chronic infection, and evaluation of therapeutics and vaccines. Of particular note, the chimpanzee model is considered an extremely sensitive model for assessment of parenteral infectivity relevant to transfusion safety.[13-24] For this reason, we conducted multistep inoculation experiments in 2 chimpanzees, designed to study the infectivity of serially collected plasma donations from source plasma donors recently infected with HCV. First, we evaluated infectivity from donations initially assessed as occurring immediately before the onset of ramp-up–phase viremia. Next, we evaluated infectivity of nonviremic and low-level HCV RNA-positive (blip) donations from donors who demonstrated intermittent HCV RNA detection in the eclipse phase of HCV infection. We also studied the early immunology of HCV infection after a low-dose exposure and the influence of this exposure on subsequent reinfection with the same HCV isolate. In addition to contributing to our understanding of transfusion-transmission, these experiments provide data relevant to broader issues of HCV transmissibility, the ability to document previous HCV infection using RNA and Ab diagnostics, and the early immunologic events in HCV infection.

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Methods

Preparation of HCV-RNA–positive plasma donor panels

Plasma for chimp inoculation studies was selected from a collection of 50 source plasma panels used in a previous study of early HCV viral dynamics (see supplemental Methods, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).[1] Each panel consisted of aliquots of a series of donations (serial donations) from a given donor. These donations occurred at approximately twice-weekly intervals in both the eclipse and ramp-up phases of acute HCV infection. We based the classification of ramp-up phase infection on the specific rate of increase in HCV viral load for each individual donor.[1] We then back-extrapolated from the slope of ramp-up viremia for each donor to establish a time when the estimated viral load would have been < 0.05 copies/mL; all RNA-positive donations collected before this time point were classified as occurring in the eclipse phase (ie, before the extrapolated beginning of the ramp-up phase). Donations given before quantifiable ramp-up viremia were then further evaluated for HCV RNA by performing TMA testing (Procleix discriminatory HCV [dHCV] assay; Gen-Probe) on 4 replicates of 0.5 mL of plasma for each donation.[1] Multiple additional replicates (n = 20 or 23) of selected eclipse-phase samples were also further tested for HCV RNA either by the dHCV assay or by the Procleix duplex (HIV-HCV) assay (Gen-Probe) as part of a previously published study.[25] Based on this testing, we characterized 37 of the 50 panels as having intermittent HCV RNA-positive donations (at least 1 dHCV TMA-positive replicate result) during the eclipse phase interspersed with nonviremic (all replicates negative by TMA) donations.

Selection of plasma donor panels for infusion into chimps

We selected 10 ABO-compatible serial donation panels for chimpanzee infectivity studies. We used 5 of the 13 panels that did not have evidence of intermittent viremia as well as 5 of the 37 that showed intermittent HCV RNA detection (blips). The 5 cases without intermittent viremia were selected to have the most frequent serial donations over the 3 weeks preceding RNA detection by quantitative HCV PCR and qualitative HCV TMA assays. The 5 cases with intermittent HCV RNA during the eclipse phase were selected to have at least 3 reactive results among the 4 replicate HCV TMA results on 2 or more specimens collected > 2 weeks before ramp-up viremia; they were also selected to include intervening donations that tested negative on a total of up to 27 replicate TMA assays.

General format of chimp inoculation experiments

Two healthy adult female chimpanzees (X331 and X355) were housed in a containment facility maintained by the Texas Biomedical Research Institute and Southwest National Primate Research Center, an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility. Housing conditions and animal manipulations were approved by the institutional animal care and use committee and all animal procedures were performed with prior administration of appropriate sedatives and anesthesia. Fifty milliliters of donor plasma units were intravenously administered; these could be either individual unit infusions, multiple units infused one after another, or a premixed pool of 5 U (250-mL total volume). Further details of the timing of the infusions in the various experiments are provided in Figure 1 and supplemental Figure 1, and in "Results."

The animals were monitored by testing blood samples for HCV RNA by PCR, anti-HCV by enzyme-linked immunoassay (EIA), liver enzyme levels, and T-cell assays. If infected, weekly or biweekly monitoring continued; however, if there was no detectable infection after 6 weeks, the animal was rested for an interval of up to 3 weeks and then became eligible for infusion with another donor plasma sample. This sequence was repeated with several different donor plasma samples until evidence of infection could be ascertained. After confirmation of infection, animals were monitored for a period of 1 year to determine the outcome of infection. No additional infusions with donor plasma were performed until completion of this 1-year period.

Laboratory assays

HCV RNA quantification in plasma donor panels. Two different assays were performed as previously described.[1] The COBAS Amplicor HCV Monitor (Version 2.0 assay; Roche Molecular Systems) HCV PCR assay was able to quantify HCV RNA down to a lower limit of 600 IU/mL. Samples that were negative on this assay were tested using the dHCV TMA assay using multiple 0.5-mL replicates of plasma. The limit of detection (LOD) of each replicate assay is 12.1 copies/mL (50% LOD; 95% confidence interval [CI] 11.1-13.2).[26] HCV RNA concentration was determined based on the percentage of replicates that gave positive results using probit analyses.

HCV RNA detection and quantification in the recipient chimps. HCV RNA detection was performed using the COBAS Amplicor Hepatitis C Virus Test (Version 2.0; Roche Molecular Systems) and quantification of HCV RNA was performed using the COBAS Amplicor HCV Monitor (Version 2.0 assay).

HCV Ab detection. Initial screening of plasma donor panels was done using a third-generation HCV Ab EIA (Ortho Diagnostics). Repeat reactive samples were further evaluated by the Recombinant Immunoblot Assay (RIBA; Version 3; Novartis Diagnostics). Initial screening of chimp sera was done using an anti-HCV 2.0 EIA (Abbott Laboratories) with confirmation by RIBA.

HCV RNA extraction, amplification, cloning, sequencing, and phylogenetic analysis. HCV RNA was extracted from 140 μL of plasma from one viremic time point from each of the 5 plasma donors implicated in HCV transmission to chimp X331 and from 2 viremic time points from the infected chimp using the QIAamp Viral RNA Mini Kit following the manufacturer's instructions (QIAGEN). Extraction and amplification of donor and chimp samples were performed on different days to eliminate the possibility of cross-contamination. Details of the amplification, cloning, sequencing, and phylogenetic analysis procedures are in supplemental Methods.

ALT and AST measurements. Serum samples collected from the study animals were analyzed for alanine aminotransferase (AST) and asparate aminotransferase (ALT) levels with a Unicel DXC600 serum chemistry analyzer (Beckman Coulter). Enzyme levels were considered normal if they were within the normal range (AST 11-25 U/L; ALT 21-55 U/L) established at the primate center.

CD4 proliferative responses to HCV Ags. PBMCs were isolated from ACD-anticoagulated chimpanzee blood via gradient centrifugation as described.[27] Triplicate cultures of 200 000 PBMCs were stimulated with 1 μg/mL HCVcore, NS3, helicase, NS4, NS5A, NS5B proteins (Mikrogen) or buffer control as previously described.[28] Cultures were labeled with 1 μCi [^{3}H]thymidine (Amersham) on day 5 and harvested 16 hours later. Separate cultures stimulated with or without PHA (1 μg/mL; Murex Biotech Limited) were labeled with [^{3}H]thymidine on day 2. The stimulation index (SI) was calculated as a ratio of the average number of counts per minute of 4 replicate cultures in the presence of Ag compared with control buffer or medium.

Results

Three chimp inoculation experiments were performed in multiple phases as described below and in Figure 1.

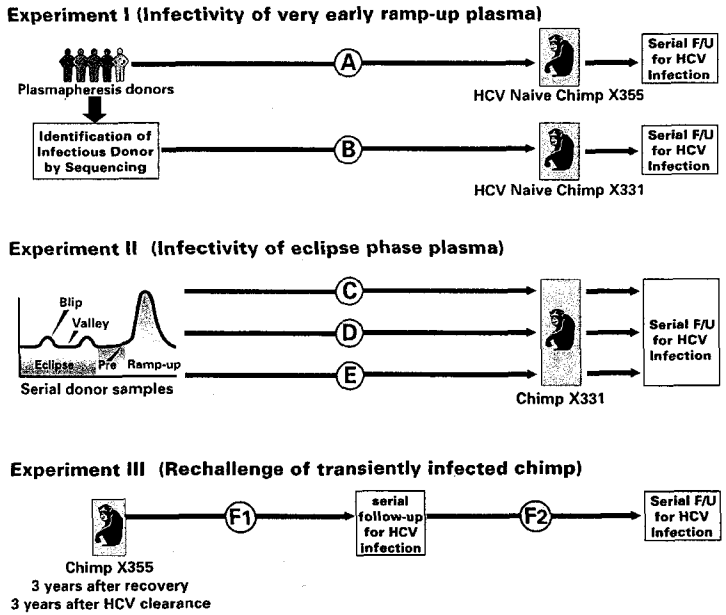


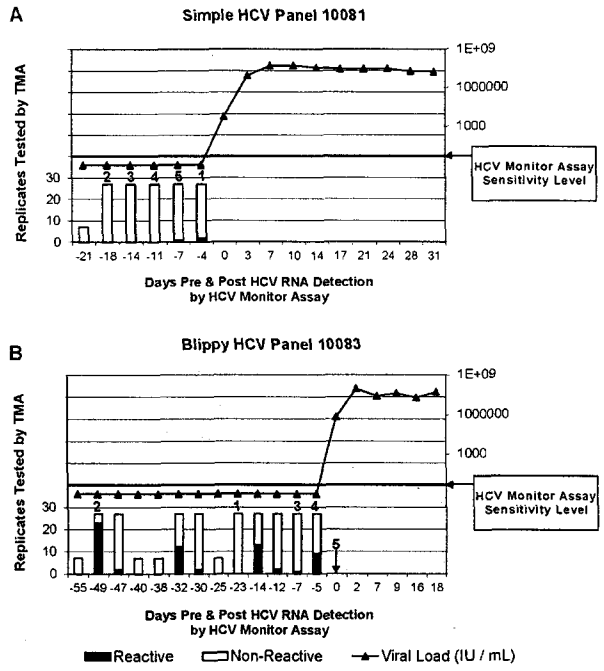
Figure 1. Experimental design and sequence of plasma infusions and follow-up of chimpanzees. Experiment I assessed the infectivity of plasma that tested HCV RNA negative by licensed diagnostic assays and was obtained in the days just before ramp-up viremia. (A) Fifty milliliters of pre–ramp-up phase plasma from each of 5 commercial apheresis donors was infused sequentially during a single experimental procedure into chimp X355. (B) When transmission was linked to 1 donor by phylogenetic sequencing, 50-mL plasma samples from each of 4 earlier donations from that implicated donor were transfused to a second animal (X331) at 9-week intervals. Both animals were followed for virologic, serologic, and cell-mediated immune responses to assess evidence of HCV infection. Experiment II examined the infectivity of samples from 5 donors who had intermittent low-level HCV RNA ("blips") detected during the eclipse phase of HCV infection by infusions into chimp X331. (C) Phase 1: Infusion of a pool of 250 mL (50 mL of plasma/donor from donations collected subsequent to blips) of HCV RNA-negative plasma from the eclipse phase. (D) Phase 2: 50-mL plasma samples from blip viremic units from the eclipse phase from the same 5 donors were sequentially infused at 6-week intervals. Phase 1 and phase 2 infusions did not transmit HCV infection to the recipient animal (chimp X331). (E) Phase 3: To confirm this chimp's susceptibility to HCV infection, 50-mL plasma samples from each of 3 progressively higher titer HCV RNA-positive donations collected during the early ramp-up phase of infection from one of these 5 donors were infused at 8-week intervals. In experiment III, chimp X355 who had spontaneously recovered from HCV infection and lost anti-HCV as well as virus, was rechallenged 3 years later to determine whether prior infection conferred protection against reinfection. (F1) Infusion of 50 mL of plasma containing an estimated 80 HCV RNA copies (1.6 copies/mL) from the previous infecting donation. (F2) Rechallenge with 50 mL of plasma containing an estimated 300 000 HCV RNA copies (6000 copies/mL) from a subsequent early ramp-up–phase donation from the same donor.

Experiment I: testing the infectivity of plasma immediately before the ramp-up phase

Experiment I, phase 1 involved infusions of 50 mL of plasma (during a single infusion episode) from each of 5 different source plasma donors into an HCV naive chimp (X355). The infused donations were from donors in whom there was no HCV RNA detected during the eclipse phase and were selected so as to be the donations that immediately preceded the first ramp-up viremia donation (Figure 2A, and supplemental Figure 1A-E). These donations, which tested negative for HCV RNA by quadruplicate dHCV TMA and viral load assays, were estimated by back-extrapolation from measured viral loads during the subsequent ramp-up phase of each donor to have been collected from 4 to 16 days before the onset of the ramp-up phase (defined as one HCV RNA copy present in 20 mL of plasma).

After infusion, chimp X355 was evaluated weekly for the first 11 weeks, then biweekly until week 29 after infusion, with a final assessment at 1 year after infusion. Tests performed were HCV RNA (qualitative and, when indicated, quantitative), HCV Ab, ALT, and AST. Assays for PBMC proliferative responses to HCV proteins were performed before infusion and biweekly until week 10 after infusion. As shown in Figure 3, chimp X355 developed mild hepatitis (peak ALT 68 IU/L) and transient HCV infection with viremia developing at week 2 and persisting until week 9. Peak viral load was 5.2×10^4 copies/mL at week 4. HCV Ab was first detected at week 8 and persisted until week 17, after which it became undetectable throughout follow-up (seroreversion). Samples collected from weeks 9 through 13 were RIBA positive but never showed Ab reactivity to the core (c22) Ag. Samples at weeks 15 and 17 showed very low Ab reactivity by RIBA (c33 at 1+, 511 and NS5 at +/−, and c22 nondetectable). ALT was elevated from weeks 5 through 8 with a peak value of 68 U/L. Proliferation of PBMC to HCV Ags was not detectable preinfusion or at week 2, but a clear peak was detectable at week 4. Responses were exclusively targeted against nonstructural HCV Ags as evidenced by SIs of 33 and 27 against NS5B and NS5A, respectively, 29 against NS4 and 31 against NS3. Responses were maintained at week 6 and 8 after infusion, and declined to slightly above baseline at week 10. By 17 weeks after infusion, HCV RNA and HCV Ab were undetectable and ALT levels were normal, thus leaving no residual evidence of prior

Figure 2. Representative viremia results from 2 of the 10 plasma donors used in chimpanzee infection experiments (data from all 10 panels is presented in supplemental Figure 1). (A) TMA and viral load results over time from plasma donor panel 10081, who did not demonstrate intermittent blips of HCV RNA before detection of ramp-up–phase viremia. Numbers above bars represent the order in which aliquots were infused into chimps. Infusion 1 was part of a transmitting pool that was infused into chimp X355. Infusions 2-5 were each infused into chimp X331 at intervals of 9 weeks between infusions. ▲ indicates viral load in the ramp-up and post–ramp-up phase of HCV infection in donor 10081. (B) TMA and viral load results over time from plasma donor panel 10083 who manifested intermittent blips of HCV RNA reactivity during the eclipse phase of acute HCV infection. Both HCV RNA-negative "valley" and HCV RNA-positive "blip" samples were infused into chimp X331 (see Figure 1, experiment II). Numbers above bars identify aliquots and the order infused into chimps. Note that the units were infused in a different order than they were collected and that infusion 1 was part of a nontransmitting pool. ▲ indicates viral load in donor 10083.



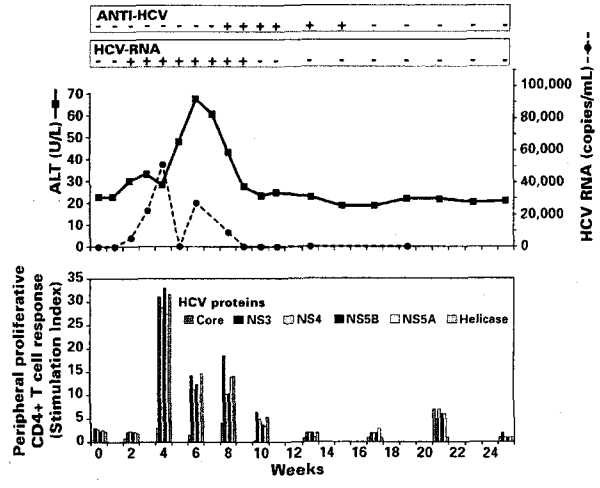


Figure 3. Results of transmission experiment I in chimp X355. Fifty milliliters of plasma from each of 5 donors in the pre–ramp-up phase of HCV infection were infused in a single experimental procedure into chimp X355 (see Figure 1, experiment I) and infectivity monitored by weekly or biweekly measurement of ALT level (solid line), HCV RNA (dashed line and box), anti-HCV (box), and cell-mediated immune response (bottom panel). Transient HCV infection is demonstrated. Note that HCV RNA was no longer detectable by week 10 and that Ab to HCV disappeared by week 18 after infusion. Cell-mediated immune responses, measured in a proliferation assay, peaked at week 4 and considerably diminished by week 10.

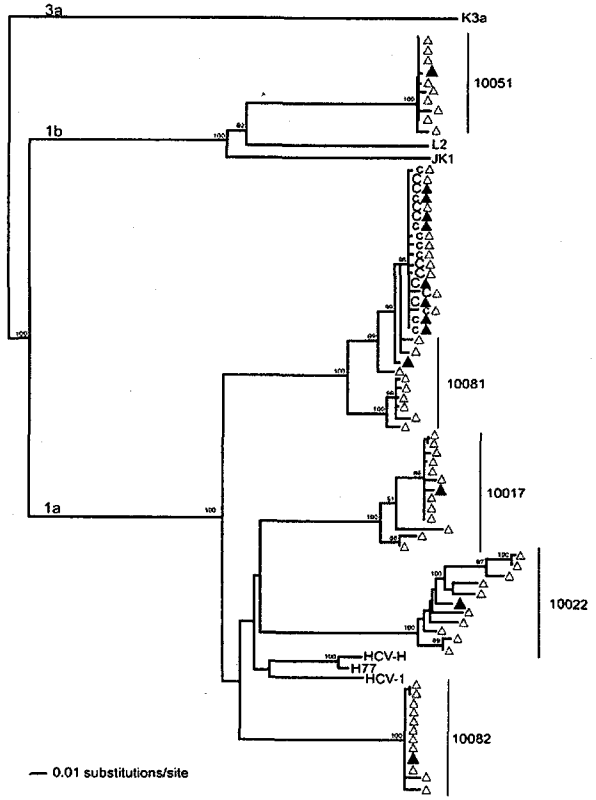


Figure 4. Phylogenetic reconstruction of HCV E1/E2 region sequences from 5 plasma donors (10081, 10051, 10082, 10017, and 10022) and a chimpanzee (X355) infused with a pool of 50 mL of plasma from each of the donors. ▲ indicates population sequences corresponding to the first hypervariable region (HVR-1) of E1/E2 (404nt) for donors and the chimp; and △, cloned sequences. Samples from 2 time points 19 days apart were obtained from the chimpanzee. The first time point sequences are indicated with c and the second time point sequences by C. Viral sequences in the chimp are shown to be closely related to donor 10081. Reference sequences from genotypes 1a (HCV-H, H77, and HCV-1), 1b (L2, JK1), and 3a (K3a) were included. The maximum likelihood tree was rooted with the 3a reference sequence. Bootstrap values > 70% are indicated.

HCV infection except for the cell-mediated immune response which remained detectable as late as week 21 after infusion.

Sequencing of HCV RNA and phylogenetic analysis revealed that the chimp HCV isolate was very closely related to an HCV isolate obtained from a subsequent highly viremic donation from one of the 5 source plasma donors used in the infecting inocula (Figure 4). The transmitting donation had been collected 4 days before the first quantifiable HCV RNA-positive donation (viral load of 6×10^3 HCV RNA copies/mL) from this donor (donor 10081). To increase the sensitivity for detection of HCV RNA in the transmitting and nontransmitting source plasma donations, 23 additional 0.5-mL plasma replicates from the infused donations were tested under code for HCV RNA by the TMA assay. In total, 2 of the 27 replicate TMA assays were positive with plasma from the donation implicated as infectious by phylogenetic analysis, whereas similar multiple replicate TMA testing performed on identically processed frozen-thawed plasma from the other 4 donations in the transmitting pool gave negative results. A probit analysis indicated that the dHCV TMA-positive donation had an estimated HCV RNA concentration of 1.2 copies/mL (95% CI 0.5-1.9 copies/mL). In retrospect, this initially HCV RNA-negative donation was probably in the very early ramp-up phase of infection such that multiple replicate testing was required to detect the very low level of HCV RNA that was present. Because 50 mL of plasma from this donation was infused, we estimate that chimp X355 acquired HCV infection after being transfused with a total of ~ 60 HCV RNA copies (95% CI: 25-95 copies).

To confirm that the donation that initially infected chimp X355 was the earliest infectious donation in the panel of serial donation samples from the implicated donor, we conducted phase 2 of this experiment. A second HCV naive chimp (chimp X331) was infused on separate occasions with 50 mL of plasma from HCV RNA-negative units donated by the transmitting donor that had been collected 14, 10, 7, and 3 days before the transmitting donation (Figure 2A). Chimp X331 was monitored weekly for 6 weeks after each 50-mL infusion (total of 24 weeks) to document evidence of HCV infection by HCV RNA, HCV Ab, and ALT/AST testing. There was no evidence for HCV infection in this second recipient chimp.

Table 1. Estimated HCV RNA concentrations of eclipse-phase donations with blip viremia infused into chimp X331 (experiment II, phase 2)

Donor/donation no.	Days preceding ramp-up*	No. of TMA-positive replicates (%)	Imputed HCV RNA copies/mL (95% fiducial limits)†	Infused HCV RNA copies
10050-04	41	6/27 (22)	2.22 (1.23-3.17)	111
10083-02	49	23/27 (85)	11.22 (9.35-13.67)	561
10011-02	49	2/27 (7)	1.18 (0.53-1.91)	60
10029-01	48	10/27 (38)	3.37 (2.15-4.47)	185
10085-02	49	10/27 (37)	3.29 (2.08-4.38)	164

TMA indicates transcription-mediated amplification.

*Plasma from HCV RNA-negative donations from each of these five donors were previously infused into chimp X331 and resulted in no evidence of HCV infection. These donations were collected prior to ramp-up viremia at days −31, −23, −19, −21, and −18, respectively.

†RNA copy levels were estimated from probit analysis of replicate testing of the HCV WHO International Standard (NIBSC Codes: 97/690); copies per milliliter were based on a conversion factor of 3.4 RNA copies/IU.

Experiment II: testing the infectivity of plasma during the eclipse phase

In experiment II, eclipse-phase RNA-positive and RNA-negative donations were selected from 5 of the 37 source plasma donors who had intermittent HCV RNA detected in the eclipse phase of infection (Figure 1, supplemental Figure 1F-J). Chimp X331, who had not been infected in phase 2 of experiment I, was used for these studies, which were begun 22.5 months after the completion of experiment I. In phase 1 of experiment II, a 250-mL plasma pool from nonviremic donations obtained during the eclipse phase of infection from 5 different source plasma donors was infused into chimp X331 (see supplemental Figure 1F-J for time points used in these infusions); these HCV RNA-negative donations were collected during the RNA-negative "valleys" between intermittent HCV RNA-positive eclipse-phase donations (blips). This chimp was monitored weekly for 6 weeks for HCV RNA, HCV Ab, ALT, and AST. There was no evidence for viremia, Ab seroconversion, or transaminase elevation, indicating that these HCV RNA-negative eclipse-phase donations were not infectious.

One month later, phase 2 of this second experiment involved infusions of 50 mL of low-level HCV RNA-positive eclipse-phase plasma from each of the same 5 source plasma donors into the same chimp (Table 1, supplemental Figure 1F-J). The infusions of the 5 units were separated by 6-week intervals during which the recipient chimp was monitored for evidence of HCV infection. There was no virologic evidence for transmission of HCV infection, and no evidence for humoral or cellular immune responses.

Finally, after an additional 6-month interval, phase 3 of this experiment was conducted. In light of the multiple exposures to HCV RNA from eclipse-phase infusions, the aim of this phase was to establish that chimp X331 was susceptible to HCV infection when infused with a sufficient HCV RNA dose. Three 50-mL plasma infusions from HCV RNA-positive units obtained either immediately before ramp-up or during the ramp-up phase of infection from one of the 5 donors (plasma donor 10083) used in phases 1 and 2 were infused at 8-week intervals (Figure 2B, Table 2). These 3 units had increasing HCV viral loads (1.5 copies/mL, 3.0 copies/mL, and 6.8×10^5 copies/mL). The chimp was monitored weekly for 8 weeks for HCV RNA, HCV Ab, and ALT/AST after the first 2 infusions and then weekly for 12 weeks and biweekly until week 24 after the third infusion. Liver biopsies were performed immediately before the infusion containing 6.8×10^5 copies/mL and at weeks 2, 3, 5, and 7 after that infusion. No HCV infection was detected over an 8-week follow-up period after each of the first 2 infusions (infusion of 74 HCV RNA copies [1.5 copies/mL × 50 mL] and 149 HCV RNA copies [3.0 copies/mL × 50 mL]). In contrast, after infusion of the donation with 6.8×10^5 HCV copies/mL (total infusion of 3.4×10^7 copies [6.8×10^5 copies/mL × 50 mL]), the recipient chimp developed viremia at 1-week postinfusion (peak titer 1.3×10^5 copies/mL) which persisted until week 16 (Figure 5). All liver biopsies were normal. Unexpectedly, HCV Ab never developed over the 24 weeks of follow-up.

Experiment III: testing whether transient HCV infection protected against rechallenge

Experiment III (Figure 1) was conducted to determine whether the acquisition of HCV infection from a low-dose inoculum, followed by viral clearance, would protect against rechallenge by the same HCV strain. In this experiment, performed 34 months after recovery from the initial experimental transmission of HCV to chimp X355, this same chimp was challenged with a 50-mL plasma infusion from the same low-viremic (1.6 copies/mL), early window-phase donation that had transmitted the initial HCV infection. The chimp was monitored weekly for 12 weeks during which time there was no evidence of a second HCV infection, implying some level of immunity even though anti-HCV and HCV-specific cell-mediated immunity were not detectable before or after the HCV challenge.

***Table 2. Estimated HCV RNA concentrations* of donations infused into chimp X331 to prove susceptibility to HCV infection (experiment II, phase 3)**

Donor/donation no.	Phase of infection	Days preceding ramp-up	No. of TMA-positive replicates (%)	HCV RNA copies/mL (imputed from replicate TMA testing* or measured by VL assay)	Infused HCV RNA copies
10083-12	Prior to ramp-up	7	3/27 (11)	1.48 (0.71-2.28)	74
10083-13	Prior to ramp-up	5	9/27 (33)	2.99 (1.83-4.05)	149
10083-14	Ramp-up	0	NA†	6.8×10^6	3.4×10^7

TMA indicates transcription-mediated amplification; VL, viral load; and NA, not applicable.

*RNA copy levels were estimated from probit analysis of replicate testing of the HCV WHO International Standard (NIBSC Codes: 97/690); copies per milliliter were based on a conversion factor of 3.4 RNA copies/IU.

†This donation was positive by quantitative HCV RNA testing at a concentration of 6.8×10^5 copies/mL and was not subjected to replicate TMA testing.

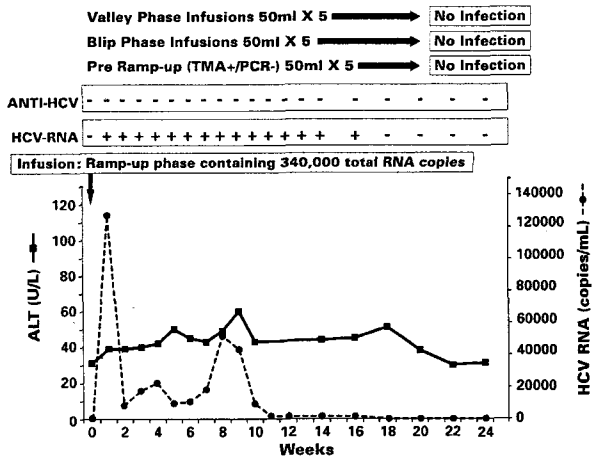


Figure 5. Results of transmission experiments in chimp 331 (see Figure 1, experiment II). After demonstrating that valley phase, blip phase, and pre–ramp-up phase infusions (50 mL) from each of 5 plasma donors were not infectious in chimp X331, this animal was challenged with a ramp-up phase inoculum containing 340 000 total RNA copies in a volume of 50 mL. Chimp X331 was infected as indicated by the detection of HCV RNA from weeks 1 to 16, but then HCV RNA cleared; HCV Abs and cell-mediated immune responses to HCV were not detected and the ALT level was not elevated. By week 18, there was no residual evidence that this infection had occurred.

To further assess protective immunity, 4 weeks later, the chimp was rechallenged with a 50-mL plasma infusion from the subsequent ramp-up phase donation (donated 4 days later) given by this same donor, which contained 6000 HCV RNA copies/mL (for a total infusion of 300 000 HCV RNA copies). The chimp was monitored weekly for 10 weeks, then biweekly until week 24, and then monthly until week 52. Chimp X355 was reinfected with HCV as evidenced by development of low-level HCV viremia that was only present at weeks 1, 2, and 3 and only quantifiable at week 1 (1620 copies/mL). HCV Ab developed at week 5 and persisted through week 20, was intermittently detected at weeks 28 and 32 and then reverted to negative on 3 subsequent measurements. ALT remained normal throughout the 1-year follow-up period and this chimp did not mount any PBMC proliferative responses to the challenge inoculum. At week 52, the chimp expired from an unrelated pyelonephritis and *Escherichia coli* sepsis precluding any further follow-up.

Discussion

We studied the infectivity of human plasma collected in various stages of acute HCV infection to define the relationship between HCV RNA as detected by the most sensitive RNA assays and *infectivity in* the well-established chimp infection model. We demonstrated that infusions of 50 mL of plasma from each of 5 human source plasma donors thought to be in the late eclipse phase of infection (just before or at the beginning of the ramp-up phase) at the time of donation into an HCV-naive chimpanzee resulted in a mild and transient HCV infection characterized by low-level viremia, HCV Ab production, and HCV-specific T-cell immune responses. This was followed by viral clearance, rapid loss of HCV Ab, and marked diminution of the cellular immune response. Sequencing of isolates established that the infection was from a single plasma donor in the group of 5 used in the experimental infusion. Based on performing HCV TMA on 27 replicate samples from the transmitting donation, we established that this plasma had an estimated HCV RNA concentration of 1.2 copies/mL and hence the donor was probably in the early phase of HCV ramp-up viremia (rather than the eclipse phase) at the time of this donation. The definition of the transition between the eclipse and ramp-up phases is not precise and the mechanisms leading to this transition are not known, as discussed in Glynn et al.[1] Based on the 50-mL plasma infusion from this donation, we estimated that 60 HCV RNA copies were infused into the recipient chimp. Samples collected from 3 to 14 days previously from the same donor were infused in 50-mL aliquots into another HCV naive chimp and did not cause infection, indicating that the transmitting donation was obtained at or near the time when the donor first became infectious. This also indicated that the duration of infectious viremia before detectable RNA by donor screening nucleic acid assay technology (NAT) assays was very brief.

This experimental transmission has some parallels with the first reported human case of transfusion-transmitted HCV infection from a unit retrospectively shown to be HCV RNA negative by a sensitive individual donation NAT (ID NAT) assay.[29] In this human transmission, HCV RNA could be demonstrated inconsistently in the transmitting donation when evaluated in multiple replicates by dHCV TMA testing, leading to the conclusion that HCV RNA was present, but at a very low level (estimated at < 10 copies/mL). The donor of this unit may have been in the very early ramp-up phase with HCV RNA levels below the level of consistent detection by even the most sensitive ID NAT screening method, as occurred in our *chimp transmission* experiment. Data from this study in chimpanzees, other chimp experimental studies using serially diluted ramp-up phase plasma infusions,[14,16] and the clinical case report of a breakthrough transmission[29] demonstrate that ID NAT cannot completely eliminate the risk of transfusion transmission of HCV, given the high level infectivity of ramp-up phase virus. Of relevance to transfusion safety, it appears that blood transfusions from donors in the early stage of acute HCV infection can be infectious before the time of RNA detection by routinely performed NAT screening, including the most sensitive ID NAT test currently available which has a 50% limit of detection of 12.1 copies/mL (95% CI 11.1-13.2).[26] Hence, conversion to individual donation NAT from currently used mini-pool NAT (MP NAT) screening will not close the infectious window period completely and may not be warranted given its projected very low incremental yield and poor cost-effectiveness.[10,25,30]

A further set of experiments was performed to evaluate the infectivity of 2 types of donations from the surprisingly prolonged eclipse phase of infection observed in 74% of the plasma donor panels we studied. These included donations that were HCV RNA positive and estimated to contain from 1 to 20 HCV RNA copies/mL (60 to 561 HCV RNA copies per infusion) and other intervening donations from the same *donors that were* HCV RNA negative by sensitive multireplicate testing. Infusions of 50 mL of plasma from either of these 2 donation types from each of 5 donors with such intermittent HCV RNA "blips" did not cause infection when transfused into a single chimp, who was later proven to be susceptible to HCV infection when infused with a ramp-up phase viremic sample from one of these same donors.

Thus, although HCV RNA was intermittently detected during the eclipse phase of infection for up to 8 weeks before ramp-up viremia, our results show that transfusion of such units was not infectious in chimpanzees. In contrast, we demonstrated infectivity with an early ramp-up phase sample at a total infusion dose of 60 HCV RNA copies. Japanese investigators have shown that even lower doses of HCV RNA (total inocula of 20 HCV RNA copies) from an acutely infected chimp transmitted HCV infection to recipient chimps.[14] Hence, during the ramp-up phase of early HCV infection, infusions with very low copy numbers are highly infectious whereas infusions having equal or higher copy numbers of HCV RNA obtained during the eclipse phase are not infectious. This suggests critical differences in the presentation or packaging of HCV RNA during the eclipse and ramp-up phases of HCV infection as discussed below.

There are multiple possible explanations for the finding of intermittent HCV RNA detection in some donors during the eclipse phase. This may represent persistent low-level viremia that occurs at a concentration that is at or near the limit of assay detection and thus, based on assay limitations, is detectable in some samples and nondetectable in other serially collected specimens. Another explanation is that periods of HCV viremia alternate with nonviremic periods as a result of intermittent release of viral particles into the plasma from focal replication sites such as the liver and possibly even peripheral blood cells. Third, these episodes could represent repeated HCV exposures or reinfections which abort, until a particularly fit variant is finally transmitted and disseminates. Perhaps the most likely explanation is that early in HCV infection, HCV RNA can be released into plasma as nonvirion-associated nucleic acid or as defective, nonreplication competent virions. The data appear to be most consistent with the hypothesis that complete infectious particles likely do not circulate until immediately before the ramp-up phase of HCV infection, leaving a very narrow window of infectivity before detectability by sensitive blood donor screening and diagnostic assays.

Whatever the explanation, this series of experiments, and those of others,[14,16] establish that HCV RNA intermittently detected during the eclipse phase of HCV infection can be ignored when performing mathematical modeling of residual transfusion-transmitted HCV risk. This lack of infectivity (or perhaps very rare infectivity, which we were not able to demonstrate in our small study) is consistent with reports of only a very few transfusion-transmissions from HCV NAT-screened units. Thus, risk modeling can use the time period from the extrapolated beginning of ramp-up phase viremia to detectable virus by the screening method used (ID NAT, MP NAT, fourth generation Ag/Ab assays), as reviewed in detail elsewhere.[2]

We have shown that a chimp infected with HCV from infusion of a low-dose early ramp-up inoculation developed a transient HCV infection as evidenced by low-level and short-lived viremia, and both humoral and cellular immune responses; by week 17, virologic, serologic, and biochemical evidence of HCV infection had disappeared and very low-level T-cell responses were the only residua of past HCV infection. A second chimp also inoculated with plasma from the early ramp-up phase of HCV infection developed transient viremia but failed to *seroconvert*. Similar examples of transient infections without seroconversion or with seroconversion followed by seroreversion have been reported in experimental chimp infectivity studies,[15] and rarely in human HCV infections after transfusion and injection drug-use exposures.[6,7,31-36] Our experimental findings corroborate these previously reported human observations in establishing that more HCV infections occur in persons than are clinically, virologically, or serologically identified by cross-sectional serologic or NAT screening.

Lastly, these experiments in a single chimp indicate that HCV infection from a low-dose inoculum did not confer protective immunity to rechallenge, as we demonstrated only limited protection when the chimp who developed but then lost detectable adaptive humoral and cellular immunity was rechallenged with a higher dose of the identical HCV strain.

A noteworthy limitation of our study is that we had access to only 2 HCV naive chimps to evaluate the infectivity of a large number of donations from 10 donors with different patterns of eclipse and ramp-up phase viremia. Consequently, we had to judiciously design experiments with reuse of the same chimps for multiple infusion experiments. This could have resulted in either immunization or tolerance to HCV, perturbing our ability to ascertain infectivity of donor plasma. However, we do not believe this seriously compromised interpretation of our experiments because all the chimps were carefully evaluated using sensitive assays for detection of HCV RNA and both humoral and cell-mediated immunity and each animal was negative by all these parameters before viral challenge. Furthermore, in the chimp (X331) that was uninfected after challenge with blip and valley eclipse-phase infusions, we confirmed HCV susceptibility by transfusing a high titer inoculum from the ramp-up phase of infection.

In summary, these studies demonstrate that: (1) Large volume plasma transfusions can transmit HCV infection before RNA detection by current donor screening assays, but the interval of infectivity before RNA detection appears to be very brief (4 days in this experiment). (2) The noninfectivity of eclipse-phase "blips" indicates that these may represent incomplete virions and can be ignored when calculating HCV residual transfusion risk. (3) Markers of HCV infection can be rapidly lost after exposure to low-dose inocula, suggesting that more HCV infections occur than are currently documented. (4) HCV infection from a low-dose inoculum does not necessarily confer protective *immunity to rechallenge* from a higher dose of the homologous strain.

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Authorship

Contribution: M.P.B., S.H.K., B.R., and H.J.A. designed the study; K.K.M. and H.J.A. conducted and monitored the animal research; D.F.H., B.L.H., E.L.D., V.R., J.C.Y., and B.R. conducted research on samples, and compiled and analyzed data; M.P.B., K.K.M., S.H.K., E.L.D., B.R., and H.J.A. analyzed data, and contributed to the final design and the development of the manuscript; and M.P.B., S.H.K., and H.J.A. wrote the manuscript.

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（別紙２－参考文献④）

Symptomatic parvovirus B19 infection caused by blood component transfusion

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BACKGROUND: Although a risk of transfusion-transmitted human parvovirus B19V (TT-B19V) infection has been a concern, there have been very few reports of clinically relevant TT-B19V caused by the transfusion of a B19V-containing blood component. It has therefore been a matter of debate whether a universal B19V screening with an appropriate sensitivity is required.
STUDY DESIGN AND METHODS: Through the Japanese Red Cross hemovigilance system, clinical reports on possible TT-B19V were collected from 1999 to 2008, during which B19V donor screening (sensitivity, 10^{10} IU/mL) was conducted and repository blood samples from donors were available.
RESULTS: Eight patients with TT-B19V caused by component transfusion have been identified. Four patients developed sustained anemia and pure red blood cell (RBC) aplasia and one patient developed pancytopenia. The underlying diseases in these five patients were either hematologic malignancy or hemolytic diseases. The viral loads of the responsible components for these cases ranged from 10^3 to 10^8 IU/mL. Two patients who underwent surgical treatment without any hematologic disorder exhibited only moderate symptoms. The B19V DNA sequence identity between a patient and the linked blood donor was confirmed in five of the eight patients. All of the components responsible for the eight cases were positive for anti-B19V immunoglobulin (Ig)M.
CONCLUSION: Vulnerability to serious B19V-related hematologic disorders depended on the patient's underlying disease state of an enhanced erythropoiesis, not on the viral load of the component transfused. To prevent clinically relevant TT-B19V, a strategy is suggested in which patients at risk of acquiring RBC aplasia or pancytopenia are targeted.

Infection by human parvovirus B19 (B19V) is common in any geographic area and causes erythema infectiosum or fifth disease in childhood. The infection in adults is usually asymptomatic but may cause a transient red blood cell (RBC) aplasia in patients in the state of an enhanced erythropoiesis or may cause persistent anemia in immunocompromised patients.[1-3] The prevalence of anti-B19V immunoglobulin (Ig)G increases steadily even after childhood, suggesting that B19V infection occurs frequently during adulthood.[3-6] In acute infection, the viral load in peripheral blood reaches as high as 10^{12} IU/mL.[7] The frequent primary infection among adults and the high viral load without symptoms imply the presence of a considerable number of blood donors with a high viral load, which raises the possibility of a considerable risk of transfusion-transmitted B19V infection (TT-B19V).

Although TT-B19V cases have often been reported after the transfusion of plasma derivatives,[8-10] there have been very few reports of clinically relevant B19V infection that is considered as a result of the transfusion of a B19V-containing blood component (i.e., RBCs, fresh-frozen plasma {FFP}, or platelet {PLT} concentrates).[11-15] It has

ABBREVIATIONS: AML = acute myeloid leukemia; B19V = human parvovirus B19; JRC = Japanese Red Cross; nt = nucleotide(s); RHA = receptor-mediated hemagglutination; TT-B19V = transfusion-transmitted human parvovirus B19; TTI(s) = transfusion-transmitted infection(s).

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therefore been a matter of debate whether a universal B19V screening with an appropriate sensitivity is indeed required for securing blood component safety. In this regard, several studies have been conducted to establish the frequency of blood products harboring TT-B19V risk and the frequency of clinically relevant TT-B19V cases. In most such studies, a reason to implement a B19V screening method that covers all blood donations has not been verified because no case of clinically relevant TT-B19V was identified during the study period.[16-18]

Japanese Red Cross (JRC) blood centers established a hemovigilance system in 1993 and have been collecting voluntary reports on transfusion-transmitted infections (TTIs) and other adverse effects as a result of transfusion. Through the system, JRC has so far obtained five established and three probable cases of TT-B19V. In this article, we describe the details of TT-B19V cases, each of which represented a typical clinical course of B19V infection.

PATIENTS AND METHODS

Hemovigilance system

JRC blood centers are the sole facilities in Japan that deal with blood procurement and processing, testing, and delivery of blood components. The JRC hemovigilance system has been functioning since 1993 and covers the entire country with 1 million patients being transfused every year. Although the reporting of suspected TTI cases to JRC is not mandatory, it is obligatory for every physician to report TTI cases to the Japanese Ministry of Health, Labour and Welfare, which in turn refers this information to JRC. Eventually, JRC is able to obtain all information on suspected TTIs and other serious adverse reactions caused by blood transfusion. The system also includes a complete sample archive from all blood donations since 1999, which enables us to investigate the cause of a TTI using the repository blood samples obtained from the donation associated with the TTI. For TT-B19V cases, a lookback study for the possibility of previous donation with viremia has not been performed.

Receptor-mediated hemagglutination assay

In 1998, JRC implemented a receptor-mediated hemagglutination (RHA) assay as a screening test for B19V and used it for all donated blood until 2007. The theoretical basis of the RHA assay system was described elsewhere[19,20]; briefly, it is a B19V antigen detection method in which the indicator RBCs agglutinate via B19V particle binding to globosides on the RBC membrane at a critical pH (pH 5.6 ± 0.1). The sensitivity of RHA is approximately 10^{10} IU/mL, and by this method, 300 to 400 B19V-positive donors with very high titer viremia have been identified every year.[21] Although the sensitivity of RHA is not satisfactory, it has greatly contributed to the lowering of the viral load in a plasma pool that is manufactured into plasma derivatives.[22] In 2008, JRC implemented a chemiluminescence enzyme immunoassay–based screening assay that is also an antigen detection assay with a sensitivity of approximately 10^7 IU/mL.

Polymerase chain reaction analysis of B19V and antibody detection

On receiving a report of a suspected case of TT-B19V, polymerase chain reaction (PCR) analysis was carried out to detect the B19V DNA in patient sera obtained before and after the index blood transfusion as well as in repository tube(s) obtained from the donation(s) from which the blood component(s) suspected of causing TT-B19V had been processed. B19V DNA was extracted using a total nucleic acid isolation kit (MagNA Pure LC, Roche Diagnostics, Tokyo, Japan) and amplified and quantified using a B19V quantification kit (LightCycler, Roche Diagnostics, Tokyo, Japan). The forward and reverse primers were located at Nucleotides (nt) 2046 to 2064 and nt 2110 to 2092, respectively. The probe used was mapped at 24 bp of nt 2067 to 2090. The 95% detection limit of the PCR system is 289 IU/mL, as determined by probit analysis. Direct B19V DNA sequencing was performed targeting 1069 bp (nt 1884-2952) in the NS1/VP1 region for Cases 1 to 4.[23] As for Case 5, B19V DNA was sequenced for a total of 1913 bp covering the NS (489 bp [nt 1396-1884] and 225 bp [nt 1962-2187]), NS-VP1 (597 bp [nt 2370-2966]), and VP1 (602 bp [nt 2984-3585]) regions. Sequence identity was assessed for these regions between the patient sample and the blood donor sample. IgM and IgG specific for B19V were detected by enzyme-linked immunosorbent assay (Parvo-IgM and Parvo-IgG, Denka Seiken, Tokyo, Japan).

RESULTS

The annual number of blood donations in Japan is approximately 5 million and the annual number of components released to medical facilities for RBCs, FFP, and PLT concentrate are 3.3 million, 960,000, and 730,000, respectively. Data presented in this article were collected during the period from 1999 through 2008 when repository blood samples from donors were available. During that period, JRC received only 15 reports of suspected TT-B19V from physicians, among which we were able to identify eight cases of TT-B19V. This indicates that the case frequency of documented TT-B19V is eight per 50 million or approximately one in 6 million donations. Details of the five TT-B19V cases that were confirmed by B19V-DNA sequence analysis are described below (Table 1).

Case 1

A 41-year-old man with hairy cell leukemia underwent a treatment regimen including a course of cladribine (2-chlorodeoxyadenosine) for 7 days in May 2005. On Day 0, 9 days after the completion of cladribine treatment, he was transfused with 1 unit of RBCs because of anemia. The reticulocyte proportion in his peripheral blood decreased from 3.6% on Day 6 to 2.4 and 0.3% on Days 8 and 10, respectively. B19V PCR analysis was performed on his Day 11 serum, which revealed a viremia level of 1×10^{12} copies/mL. The JRC central laboratory also detected B19V DNA and anti-B19V of both IgM and IgG classes in sera obtained on Day 22. On the basis of these findings, the diagnosis of RBC aplasia due to TT-B19V was made. Reticulocyte counts ranged from 0.1% to 0.2% since then, and the patient remained RBC transfusion dependent requiring occasional granulocyte-colony-stimulating factor (G-CSF) administration. His reticulocyte count started to increase in late June and the complete resolution of anemia was confirmed in late August. The repository sample from the index donation for the RBCs was found to contain 1.8×10^5 IU/mL B19V DNA as well as anti-B19V of both IgM and IgG classes. The patient was administered G-CSF because of existing leukopenia caused by the preceding chemotherapy with cladribine, not by B19V infection (Y. Tsukada, manuscript in preparation).

TABLE 1. Cases established as having TT-B19V infection

Case	Patient profile	Before transfusion	After transfusion	Symptoms and laboratory findings	Transfused components
1	41, male Hairy cell leukemia After chemotherapy		DNA (+) IgM (+) IgG (+)	RBC aplasia (3 months) Reticulocytopenia (1 month) Viremia level of 1×10^{12} copies/mL	RBCs (irradiated) DNA (+) 1.8×10^5 IU/mL* 2×10^8 IU/bag† IgM (+) IgG (+)
2	57, male AML (M4) After chemotherapy	DNA (–) IgM (–) IgG (–)	DNA (+) IgM (+) IgG (+)	Pure RBC aplasia (approx. 2 months)	PC (irradiated) DNA (+) 9.7×10^8 IU/mL 2×10^{10} IU/bag IgM (+) IgG (–)
3	35, female Placenta previa	DNA (–) IgM (–) IgG (–)	DNA (+) IgM (+) IgG (+)	Fever Systemic eruption (3 weeks)	RBCs (irradiated) DNA(+) 3.0×10^5 IU/mL 3×10^6 IU/bag IgM (+) IgG (+)
4	59, male Rectal cancer	DNA (–) IgM (–) IgG (–)	DNA (+) IgM (+) IgG (+)	Sustained high fever (5 days)	RBCs (irradiated) DNA(+) 5.1×10^3 IU/mL 5×10^4 IU/bag IgM (+) IgG (+)
5	61, male AML After chemotherapy	DNA (–)	DNA (+)	High fever Disseminated erythema Pure RBC aplasia (7 weeks) Reticulocytopenia	PC (irradiated) DNA (+) IgM (+) IgG (+)

* Viral concentration in the donated blood.
† Estimated viral load in the component.
PC = PLT concentrate.

Case 2

A 57-year-old man received chemotherapy for acute myeloid leukemia (AML, M4). After the completion of chemotherapy in May 2005, he received multiple blood transfusions because of sustained marrow suppression. The index blood transfusion of the PLT concentrate responsible for TT-B19V was carried out on June 14 (Day 0). A delayed recovery of RBC generation was noted despite a complete recovery of white blood cell and PLT generation. Marrow examination was carried out on Day 21 revealing pure RBC aplasia as well as a complete remission of AML. His peripheral blood sample collected on Day 24 was positive for B19V DNA and anti-B19V of both IgM and IgG classes. He became negative for anti-B19V IgM on Day 35 and RBC generation recovery was recognized 1 month later. A pretransfusion sample obtained on Day –21 was negative for both B19V DNA and anti-B19V of both classes. The repository sample from the index donation for the PLT concentrate contained 9.7×10^8 IU/mL B19V DNA and the anti-B19V of the IgM class but not that of the IgG class. Seventeen blood components had been transfused to this patient before the marrow examination, but only the repository sample from the index PLT concentrate was positive for B19V DNA.

Case 3

A 35-year-old woman with placenta previa received a transfusion of 5 units of RBCs in June 2005 because of massive bleeding on delivery. On Day 7 after the index transfusion, she developed a fever of 38°C. Four days later she developed systemic small eruptions, which led her physician to suspect a viral infection. Laboratory testing revealed the presence of anti-B19V IgM in the blood sample collected on Day 12. All the symptoms disappeared 1 month after transfusion without specific medication. The pretransfusion sample collected on Day 0 was found to be negative both for B19V DNA and anti-B19V of both IgM and IgG classes. Posttransfusion samples obtained on both Day 47 and Day 60 contained B19V DNA and anti-B19V of both classes. A retrospective study revealed that a repository sample from 1 of the 5 units of the RBCs contained 3.0×10^5 IU/mL B19V DNA as well as anti-B19V of both classes.

Case 4

A 59-year-old man with rectal cancer received a transfusion of 2 units of RBCs during a surgical operation in April 2006. Although his postoperative course was uneventful for 5 days, he developed a high fever of 38 to 40°C on Day 6 postoperation and the fever remained for 5 days despite medication with antibiotics and antipyretics. The fever thereafter resolved spontaneously. It was reported that a surgical complication was unlikely from the viewpoints of the operative procedure and postoperative course. A posttransfusion sample collected on Day 22 was found to be positive for B19V DNA and anti-B19V of both IgM and IgG classes: these three markers were all negative in a pretransfusion sample obtained 1 day before operation. A repository sample from the donation for 1 of the 2 units of the RBCs transfused contained 5.1×10^3 IU/mL B19V DNA as well as anti-B19V of both IgM and IgG classes.

Case 5

A 61-year-old man undergoing chemotherapy for AML received 24 blood transfusions for 4 months in 2002. He developed a high fever and disseminated erythema 22 and 26 days after the index transfusion (Day 0), respectively. Reticulocytopenia developed and pure RBC aplasia was confirmed by marrow examination. B19V DNA was not detected in his pretransfusion sample collected on Day −49 but was detected in his posttransfusion sample collected on Day 25. He recovered from pure RBC aplasia 7 weeks after the transfusion. The responsible blood component was a PLT concentrate transfused in mid-May, which was found to be positive for anti-B19V of both IgM and IgG classes and B19V DNA. Data on the B19V DNA concentration in the repository sample from the index component are not available.

In the first four cases presented above, complete genome sequence identity was established for nt 1884 to 2952 of B19V between the patient posttransfusion samples and the associated donor repository samples. For Case 5, viral sequence identity was established for a total of 1913 bp (see Patients and Methods). Figure 1 presents a phylogenetic tree of the B19V DNA sequences for the five established cases.

In addition to these five cases with established B19V DNA sequence identity between donors and recipients, JRC blood centers have three reports of probable cases of TT-B19V (Table 2). In all three cases, B19V DNA was detected in posttransfusion samples and linked donor repository samples, but data on B19V DNA sequence analysis were not available. The first case was reported elsewhere in detail[15]; briefly, a 52-year-old woman with paroxysmal nocturnal hematuria was regularly receiving RBC transfusions with prednisolone administration. She developed pancytopenia with high fever and general malaise 13 days after the index transfusion (Day 0). She thereafter received a continuous course of transfusions with RBCs and PLT concentrates together with G-CSF. She recovered from pancytopenia approximately 1 month after the index transfusion, although the reticulocyte proportion remained low for a longer period. Anti-B19V IgM was detected in her posttransfusion samples with increasing titer in the following months. Before the onset of pancytopenia, the patient had received transfusion of 1 unit of RBCs on Days −56, −32, 0, and +1. Repository blood samples from the four components were subjected to PCR analysis and only one sample from the index blood component was verified to be positive for B19V DNA. Strikingly, the B19V DNA–positive blood component attributed to this infection was a washed RBC unit processed from a donation containing 6.8×10^3 IU/mL B19V and anti-B19V of both IgM and IgG classes. The blood obtained 4 months later from the same donor still had a viral load of 1.6×10^3 IU/mL but no specific IgM. The second patient was a 28-year-old woman with hemolytic anemia. She sustained a prolonged severe anemia after transfusion of 1 unit of RBCs. Marrow examination verified hypoplasia of a RBC lineage. The proportion of peripheral blood reticulocytes remained as low as 0.1% for 1 month. B19V DNA was not detected in her pretransfusion sample. The blood component responsible for this case was an RBC unit positive for anti-B19V of both IgM and IgG classes. The third patient was a 79-year-old man who received transfusion of 1 unit of RBC during pelvic tumor resection. He did not develop any clinical symptoms after the transfusion but routine postoperative blood analysis revealed a decreased reticulocyte count that lasted for 1 week. Further investigation verified the anti-B19V IgM conversion. The responsible RBC unit contained anti-B19V of both IgM and IgG classes.

In one of the three probable cases presented above, B19V DNA was negative in the pretransfusion sample, whereas in the other two cases, anti-B19V IgM was positive in the posttransfusion samples, which supports the notion that these three are TT-B19V cases as well.

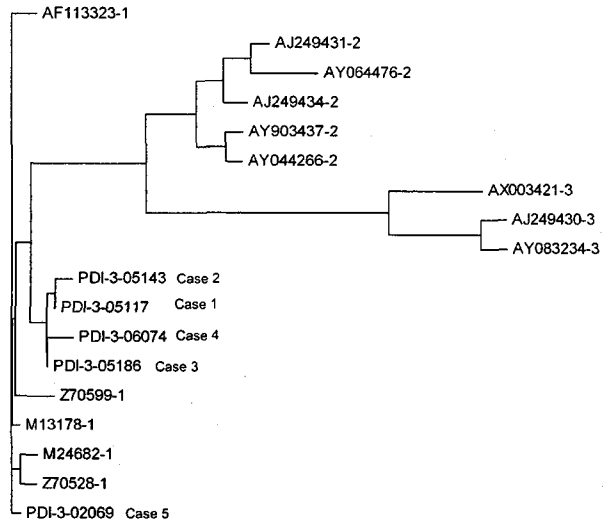

Fig. 1. Phylogenetic tree of B19V DNA for five established cases. PDI-3-05117, -05143, -05186, -06074, and -02069 correspond to Cases 1, 2, 3, 4, and 5, respectively. For these cases, the B19V DNA sequence was identical between those obtained from blood donors and those from transfused patients for the region indicated under Patients and Methods. As references, other sequences published in GenBank from Genotypes 1 to 3 are also shown.

TABLE 2. Probable cases of TT-B19V infection

Case	Patient profile	Before transfusion	After transfusion	Symptoms and laboratory findings	Transfused components
1	52, female Paroxysmal nocturnal hematuria		DNA (+) IgM (+)	High fever General malaise Pancytopenia (1 month)	Washed RBCs (irradiated) DNA (+) 6.8×10^3 IU/mL* IgM (+) IgG (+)
2	28, female Hemolytic anemia	DNA (−)	DNA (+)	Prolonged severe anemia RBC hypoplasia Reticulocytopenia (1 month)	RBCs (irradiated) DNA (+) IgM (+) IgG (+)
3	79, male Pelvic tumor		DNA (+) IgM (+)	*No symptoms* Reticulocytopenia (1 week)	RBCs (irradiated) DNA(+) IgM (+) IgG (+)

* The washed RBCs were processed from blood containing 6.8×10^3 IU/mL B19V DNA.

DISCUSSION

We described in this report five established and three probable TT-B19V cases, seven of which showed overt clinical illness. The association of the B19V infection with transfusion was proved by serologic data available and viral sequence homology analysis. From a phylogenetic tree of B19V DNA for the five established cases (Fig. 1), it is apparent that the five pairs of B19V DNA all belonging to Genotype 1 are very close but distinct genomes.

There have been only five previously reported cases of TT-B19V caused by the transfusion of blood

components,[11-15] one of which was cited in this article as a probable case.[15] Although the TRIP Dutch National Hemovigilance reported one possible case of TT-B19V in 2007,[24] there has been no report of TT-B19V in the literature published by hemovigilance systems in other countries including SHOT in the United Kingdom and hemovigilance in France. One asymptomatic case of TT-B19V has recently been reported during a donor-linked prospective study.[18] The possible reasons for the paucity of the reports are as follows: 1) a considerable proportion of transfusion recipients are immune to B19V. 2) B19V infection usually does not cause a serious illness even in nonimmune adults but results in a deleterious outcome only in a small proportion of patients who are in the specific conditions described above. 3) Passive immunization by the transfer of neutralizing anti-B19V often occurs with concurrent transfusion.[6] 4) The risk factors that increase susceptibility of recipients to severe B19V disease, and the signs and symptoms of TT-B19V infection, are not well recognized among physicians.

In view of almost the same B19V seroprevalence in Japan and western countries,[2,13,25] it is inconceivable that we encountered these cases because B19V infection is more common in Japan. It is also unlikely that the B19V genotype commonly found in Japan is more virulent and causes more serious illnesses in transfusion recipients because the B19V genome variance is small in a defined genotype and the B19V genotypes found in the established cases are all Genotype 1, the type commonly found in western countries. Moreover, B19V-related serious illnesses as a result of infusion of B19V-contaminated plasma derivatives have often been reported in western countries.[2] We speculate that we were able to collect *TT-B19V cases owing to the efficient hemovigilance* system of JRC. It should also be noted that physicians are very cooperative in reporting suspected TTIs to blood centers, which is possibly facilitated because JRC laboratories are capable of investigating the causality of TTIs using complete sample archives from all blood donations.

If the implementation of a universal B19V screening is under consideration or required by a national authority regulating blood program, it is necessary to establish a cutoff level for screening. In this regard, the infectious dose of B19V DNA in blood components that might cause TTIs has been a subject of debate.[17,26-28] In general, a viral load of approximately 10^5 IU/mL is becoming to be accepted as an infectious dose for TT-B19V. In our clinical observation, the viral concentrations of the components that caused RBC aplasia in susceptible patients were 1.8×10^5 IU/mL in Case 1 and 9.7×10^8 IU/mL in Case 2. The viral concentrations of the components that caused less severe symptoms such as febrile reaction or skin eruption were 3.0×10^5 IU/mL in Case 3 and 5.1×10^3 IU/mL in Case 4. Another patient with probable TT-B19V in our case series developed a sustained pancytopenia after the transfusion of a washed RBC unit processed from a donation containing 6.8×10^3 IU/mL B19V. Although these data may indicate that the components with very low viral loads are infectious, it is yet to be determined whether the lower limit of infectivity is 10^3 or 10^5 IU/mL, because most previous studies have not dealt with the blood components with viral loads in this range and few data are available to determine the lower limit.

While data necessary to determine the infectious dose of B19V in the blood components are still lacking, clinical symptoms due to B19V infection acquired by blood component transfusion appear to be related to the clinical state of the transfusion recipient. Each clinical course of the five established cases described in this report represents typical characteristics of acute B19V infection after a respiratory tract infection that have been described in the literature.[1,3] When a patient with an enhanced erythropoiesis receives a transfusion with a B19V-containing component, the cell production of a lineage susceptible to B19V, mainly the RBC lineage,[29,30] is totally impaired by B19V, which directly leads to RBC aplasia in the marrow and anemia in the peripheral blood because the RBC count or hemoglobin level in their peripheral blood was maintained by the enhanced erythropoiesis as presented in Cases 1, 2, and 5. If a patient has been in the state of immunosuppression, it will delay the elimination of B19V and the recovery of the affected cell lineage in the marrow. The underlying diseases for the two of the three probable cases (i.e., paroxysmal nocturnal hematuria and hemolytic anemia) also represent the typical conditions that could lead to serious hematologic disorders after B19V infection. On the other hand, in recipients who are immunologically competent and not in the state of an enhanced erythropoiesis, B19V transmission may not occur or if TT-B19V occurs it may result in outcomes limited to laboratory findings (e.g., seroconversion or DNA conversion) or moderate symptoms not more severe than a sustained fever, generalized skin lesions, or arthropathy, as shown in Cases 3 and 4. These symptoms subsequently resolved without medication possibly owing to a rapid viral neutralization by their intact immune response before anemia developed. It is possible that the patient in Case 4 presented only mild symptoms not because the viral load of the component was low but because he was not in the state of an enhanced erythropoiesis when transfused. It is, thus, more explainable to consider that the clinical state of the patient rather than *the viral load of the component determines* the clinical course of TT-B19V. In Cases 2 and 5, there is a slight possibility that the clinical course of acute B19V infection was modified by the passive transfer of neutralizing antibodies from other PLT concentrates transfused. None of the patients presented above received FFP transfusion.

It could, however, be argued that we have identified TT-B19V cases associated with a relatively low viral load and specific IgM because these cases were identified under the screening by RHA, which detects and excludes very high titer viremic donations. It is therefore possible that TT-B19V associated with low-viral-load component transfusion is only a portion of all TT-B19V cases that could be represented by cases transfused with high-viral-load components.

It is of note that all the components for the three probable TT-B19V cases as well as the five established cases were positive for anti-B19V IgM. In the four cases of TT-B19V reported in the literature except for one probable case described in this article, 2 of the 3 units of transfused components tested were also specific IgM positive.[11,12,14] These findings strongly suggest that the presence of specific IgM in the component with or without specific IgG is a risk factor for TT-B19V. The positivity for specific IgM implies that the donors were in the early recovery phase from an acute primary infection. It is therefore conceivable that the seven blood donors in this study who were also positive for specific IgG had anti-B19V IgG of insufficient titer or an *immature specificity that is incapable of* full neutralization of B19V, although it has been considered that infectivity is absent or at least modulated once specific IgG is present.

Deep insight is needed to determine whether universal preventive measures should be implemented to eradicate TT-B19V. First, the degree of seriousness of B19V-related illnesses has to be taken into consideration. Sustained fever is uncomfortable and skin lesions are painful to patients but they are essentially benign and self-limited with bearable duration. Although the condition of TT-B19V–related pure RBC aplasia sometimes requires RBC transfusion, patients eventually recover from anemia. Pancytopenia presents a real problem necessitating a course of intensive therapy.[31] It must be carefully deliberated which of these illnesses should be the target of a new preventive measure that might be implemented. Second, cost-effectiveness must be considered relative to the frequency of TT-B19V. In Japan, more than 5 million people donate blood and approximately 1 million patients receive blood transfusion annually. In such a circumstance, TT-B19V was found to occur at such a low frequency that only seven cases with symptoms were reported during the past 10 years, four of which had an impaired RBC production and one had pancytopenia. These cases were among only 15 reports of suspected TT-B19V, suggesting either that TT-B19V occurrence is very rare or that most clinicians are not aware of TT-B19V. Moreover, most symptoms eventually spontaneously resolve, which might lead to the overlooking or misdiagnosis of the infection. These clinical outcomes of TT-B19V may not support the idea of implementation of a universal donor screening strategy to cope with TT-B19V. However, if more evidence is accumulated showing TT-B19V–related serious illnesses, its implementation may be required in the future.

The universal screening of donated blood for B19V by nucleic acid testing (NAT)-based algorithm is currently carried out in Germany.[27] With the detection limit of 10^5 IU/mL, it is surely contributing to the decrease in not only the viral load in pooled source plasma but also the frequency of seroconversion or symptomatic infection after component transfusion. Our finding of the infectivity of blood components with 10^3 IU/mL viral load suggests that this measure may not completely eliminate TT-B19V cases with serious hematologic disorders. The implementation of NAT screening with a much higher sensitivity for B19V is, however, unlikely because it would impair the current blood program because it would result in the discarding of a considerable number of components.

Another strategy may be feasible in which an indication for the transfusion of B19V-safer blood components is defined and components with negligible B19V infectivity are identified, the strategy currently being recommended in the Netherlands.[32] Our experience with TT-B19V enabled us to define patients at risk of B19V-related serious illnesses; that is, patients with the indication of TT-B19V–safer components, namely, B19V-seronegative patients with an enhanced erythropoiesis or with hereditary RBC disorders having an increased RBC turnover.[3] Seronegative pregnant women are another population at risk because of the high risk of hydrops fetalis.[1] Blood components with no or negligible viral loads will be identified by screening a proportion of the current component inventory using NAT with high sensitivity. To avoid a donation during the NAT window period and early recovery phase after acute infection, components should be selected among donations that show positivity for specific IgG as well as negativity for specific IgM continuously over a long period (e.g., >6 months or 1 year).[32]

Pathogen reduction and/or inactivation is a novel strategy for the prevention of TTIs.[33] It is, however, considered to be difficult in general to mitigate the B19V infectivity of blood components because of the rigid viral capsid of B19V that hinders the entry of the photosensitizer and the extremely high viral load found in blood donors in acute-phase B19V infection.

In conclusion, eight cases of TT-B19V caused by transfusion with B19V-contaminated blood components have been identified through the hemovigilance system. Whether a patient developed a serious B19V-related hematologic disorder as a result of component transfusion depended on the patient's underlying disease state such as an enhanced erythropoiesis, not on the viral concentration of the component transfused.

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CONFLICT OF INTEREST

The authors have no conflict of interest for this article.

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