SIdEM
Società Italiana di Emaferesi e Manipolazione Cellulare

17° Corso Nazionale di Aggiornamento SIdEM

Manipolazioni/lavorazioni dei prodotti da aferesi:

Inattivazione, congelamento e lavaggio

Dott. Angelo Ostuni

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Contents lists available at SciVerse ScienceDirect

Biologicals

Biologicals 40 (2012) 187–190

journal homepage: www.elsevier.com/locate/biologicals

Preparation of blood products for transfusion: Is there a best method?

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"An overarching driver for blood systems is the production of **high quality blood products** that present a **minimal risk to recipients**, and that are **collected without causing undue risk to the donor**. In seeking to establish and then optimize a system for component production there are many factors that come into play in the decision making".

"Blood components can either be produced from a manually collected unit of whole blood, or they can be collected as specific products **using automated collection devices**. In the latter case, little if **any manipulation of the product is required after collection** …"

"Apheresis offers some distinct **advantages**…. Despite its widespread use, apheresis technology is not without **drawbacks**..."

Prodotti da aferesi

ADVANTAGES	DRAWBACKS
•Collection of specific products; •Better match inventory supply and demand and reduction in wastage due to outdating. •Collection of plasma: donation interval can be much shorter than whole blood. •Increased frequency donation for donors who collect platelet by apheresis. •Produce specialty blood: platelet-specific antigen-matched and HLA-matched platelet products •Collection of a transfusion dose from single donor •Apheresis products were safer than whole blood derived (for viral transmission and bacterial contamination). •Use of apheresis equipment for collection of two units red cells in a single procedure has seen increasing adoption.	•Equipment and disposables cost •The operation of the devices requires a significant skill level so staff have a fairly high level of training such as a nursing or medical degree. •The most devices are not portable so use is restricted to permanent facilities. •The procedure itself presents a greater risk to the donor than whole blood donation due to the infusion of citrate into the donor and albeit rare but known fatality arising from misuse of the •equipment •The donation of a double unit of red blood cells: represents a challenge for iron management in donors (this strategy is only cost effective if done on a high proportion of donors)

Queen for a day … ?!

Apheresis collections must be part of platelet production options in order to meet the demand for specific products (HLA-matched, platelet antigen-matched) **as well as to supplement inventory**.

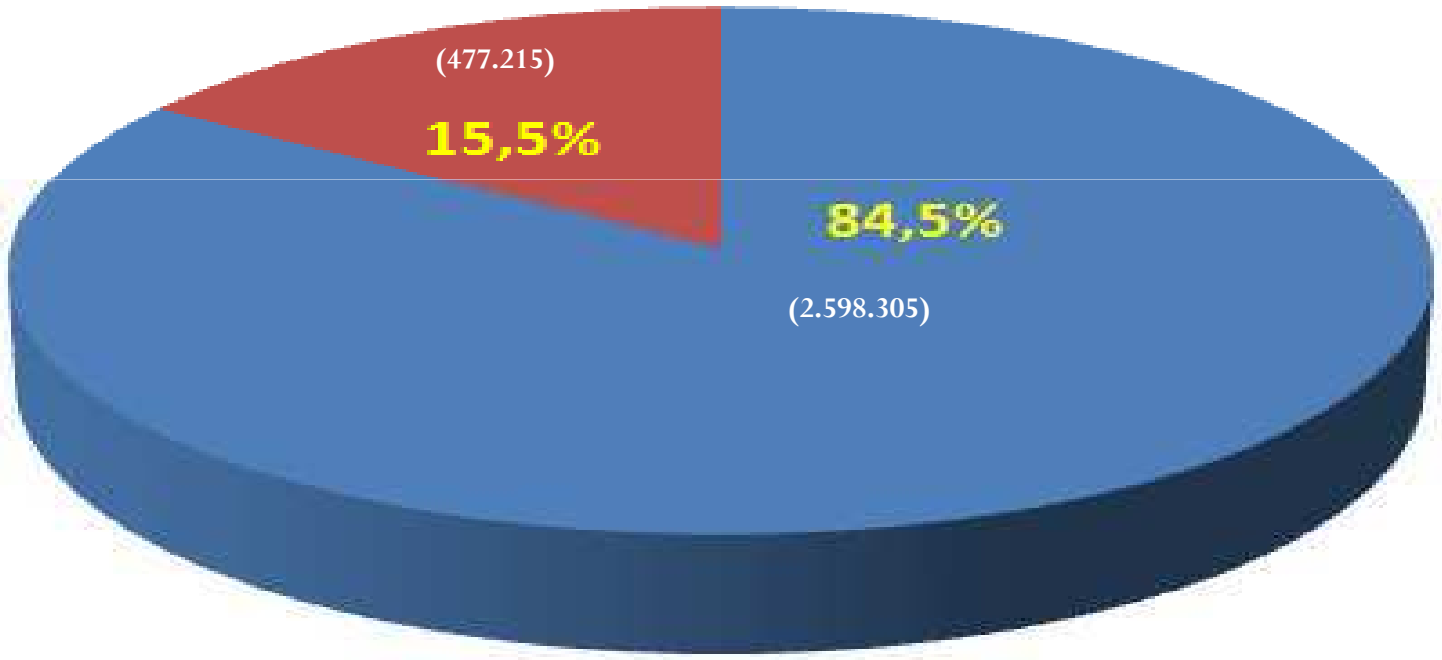
The percentage of **multi-dose collections of platelets** should be **maximized to optimize both the cost of this blood product and the donor's gift**.

…the authors advocate for more research effort applied to **identification of the donor factors that influence product quality** so that we can someday ensure better consistency of our starting material for the production of blood components.

Italia - dati 2009
SIdEM-SIMTI

Registro aferesi 2009

Carlo Malantrucco e Francesco Picardi

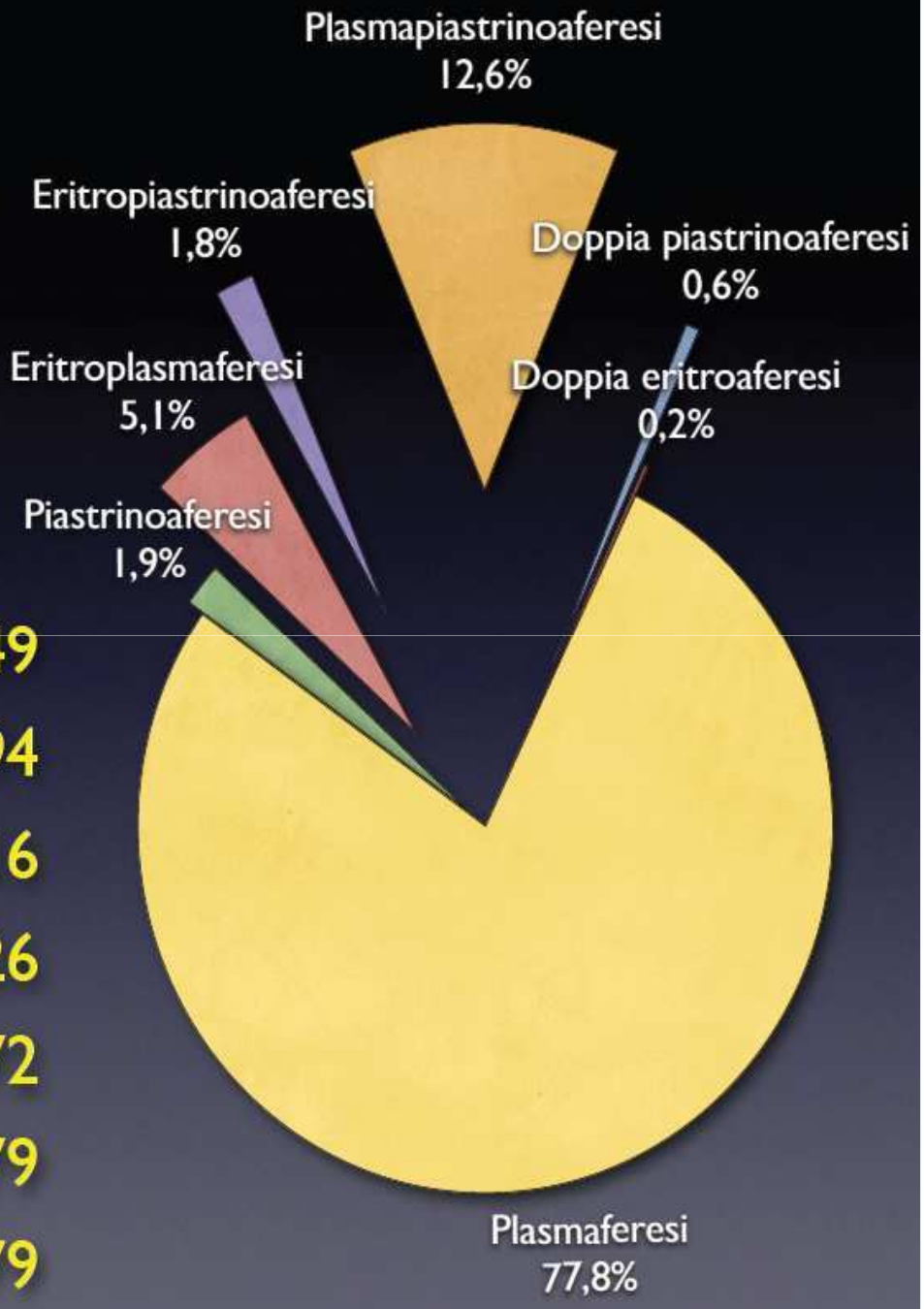


Registro aferesi 2009
SIdEM – SIMTI

Carlo Malantrucco , Francesco Picardi

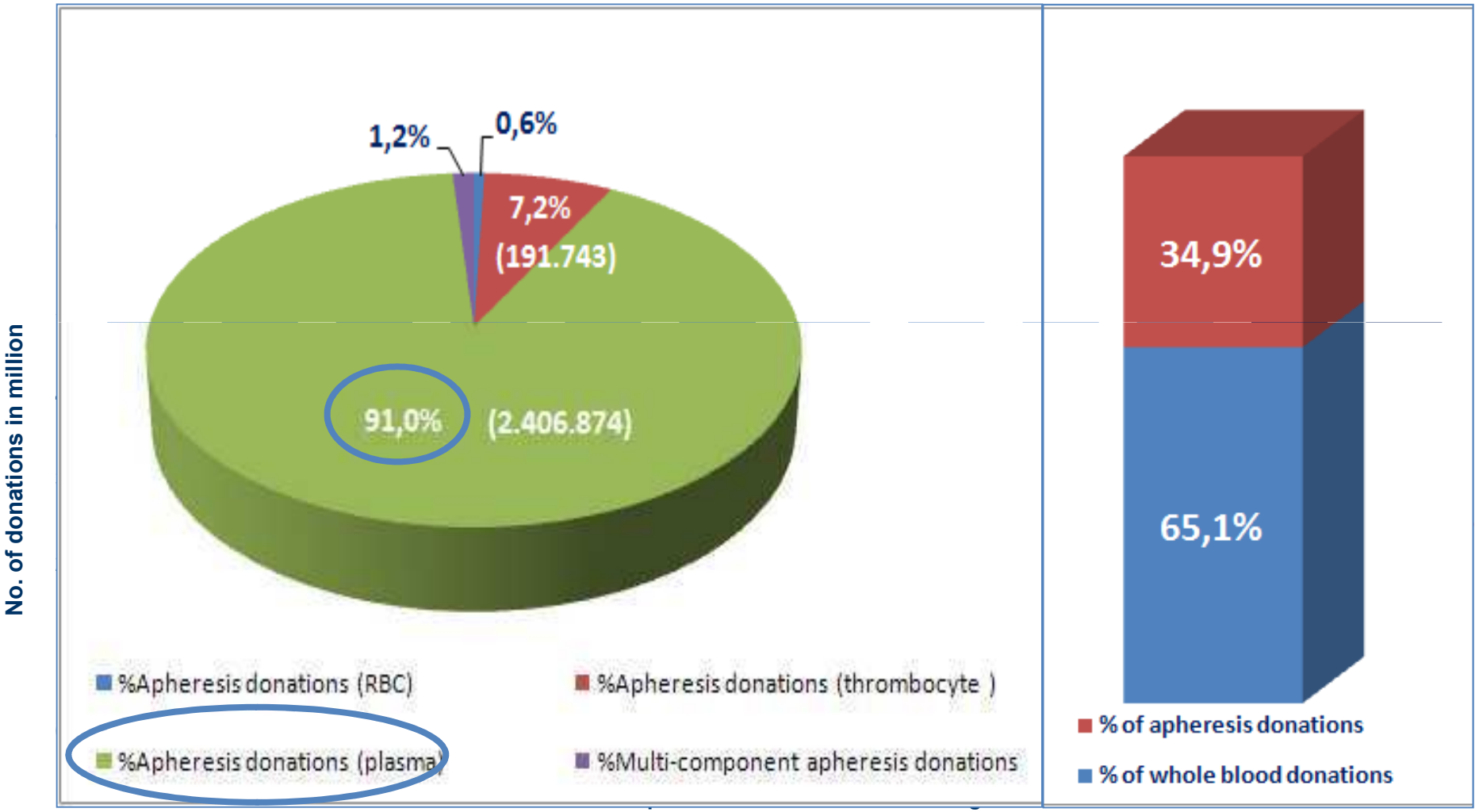
477.215
aferesi produttive

plasmaferesi	371.049
piastrinoaferesi	9.194
eritroplasmaferesi	24.216
eritropiastrinoaferesi	8.626
plasmapiastrinoaferesi	60.072
doppie piastrinoaferesi	3.079
doppie eritroaferesi	979



Paul-Ehrlich-Institut, Langen, Germany

http://www.pei.de/cln_330/nn_155724/EN/information/blood-supply/reports/report-blood-supply-2010-2011-21tfg-content.html



Blood Component Collection by Apheresis

Edwin A. Burgstaler*

Division of Transfusion Medicine, Mayo Clinic, Rochester, Minnesota

Journal of Clinical Apheresis 21: 142–151 (2006)

The use of apheresis equipment to collect blood components has rapidly increased in the last few years.

The introduction of new equipment and the need to optimize the contribution of valuable blood donors has prompted the increase.

Apheresis equipment allows **EFFICIENT COLLECTION** of specific components with **GOOD PURITY**, **CONSISTENT VOLUMES** and **PREDICTABLE DOSES**.

The blood components currently collected by apheresis include **GRANULOCYTES**, **PLATELETS**, **RED BLOOD CELLS**, and **PLASMA**.Multiple doses of platelets, red blood cells and plasma can be collected from single donor,…

Blood components are separated by apheresis equipment **using centrifugation to separate by weight (specific gravity), filtration to separate by size, or a combination of both**…

Blood Component Collection by Apheresis

Edwin A. Burgstaler*

Division of Transfusion Medicine, Mayo Clinic, Rochester, Minnesota

Journal of Clinical Apheresis 21: 142–151 (2006)

United States apheresis collection:

GRANULOCYTES → Fenwal CS3000, Fenwal CS3000 Plus, COBE (Gambro) Spectra, Haemonetics LN9000, and Fresenius AS 104.

PLATELETAPHERESIS → Fenwal Amicus, COBE (Gambro) Spectra, Gambro Trima Version 4, Gambro Trima Accel (Version 5), and Haemonetics LN9000.

RED BLOOD CELL (**RBC**) → Haemonetics MCS+LN8150, Gambro Trima Version 4, Gambro Trima Accel (Version 5), Amicus, and Baxter Alyx.

PLASMA → Fenwal Autopheresis C and Haemonetics PCS2.

Best practice for peripheral blood progenitor cell mobilization and collection in adults and children: results of a Società Italiana Di Emaferesi e Manipolazione Cellulare (SIDEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO) consensus process.

Luca Pierelli, Paolo Perseghin, Monia Marchetti, Patrizia Accorsi, Renato Fanin, Chiara Messina, Attilio Olivieri, Marco Risso, Laura Salvaneschi, and Alberto Bosi - SIDEM e GITMO

Volume 52, Issue 4, pages 893–905, April 2012

PBPC MOBILIZATION

A. Allogeneic setting

1.Which is the best schedule of myeloid growth factors?
2.Which is the target PBPC dose?
3.How should peculiar donor subgroups, for example, elderly, children, and donors with comorbidities, be managed?

B. Autologous setting

4.Which is the best schedule of myeloid growth factors in PBPC mobilization with chemotherapy?
5.Which is the best schedule of myeloid growth factors in PBPC mobilization without chemotherapy?
6.Are G-CSF biosimilars recommended for PBPC mobilization?
7.Which is the target PBPC dose?
8.How should technical issues, for example, venous access, be managed?

PBPC COUNT

9.Which is the optimal method for CD34+ cell count?
10.Which is the optimal timing for CD34+ cell count?

PBPC COLLECTION

Which is the timing to start PBPC collection?

RACCOMENDATIONS. *The first PBPC collection should be performed in patients showing at least 20 × 106/L CD34+ cells in peripheral blood, until reaching the prescribed target cell dose… Donors may undergo the first collection on the fourth to fifth days of G-CSF administration,provided that at least 20 × 106/L CD34+ cells are counted in peripheral blood.*

How should an ideal apheresis procedure, for example, flows, volumes, and tailoring, be performed?

RACCOMENDATIONS. *A large-volume apheresis (at least 3 vol) is preferred, excepted for children. A tailored procedure according to published or local algorithms, is advisable in patients showing a high circulating CD34+ cell count (higher than 80 × 106/L) and an increasing trend… The recommended cell separator should be a high-efficiency, flow (<1-2 mL/kg/min in children and <70 mL/min in adults) device with low extracorporeal blood volumes. The ideal separator, moreover, should possibly adopt PLT- and RBC-sparing programs. Automatized PBPC collection strategies are recommended in centers with low-volume PBPC collection activity.*

Prodotti da aferesi "*convenzionali*"

DECRETO 3 marzo 2005
Caratteristiche e modalità per la donazione del sangue e di emocomponenti.

CONCENTRATO DI EMAZIE DA AFERESI.

E' ottenuto da un **singolo donatore** … è costituito da **una o due unità** … contenuto minimo di **Hgb > 40 g**; **Hct ≥ 65-75%** o 50-70% se risospeso in soluzione additiva …

CONCENTRATO PIASTRINICO DA AFERESI.

… è ottenuto da un singolo donatore sottoposto a piastrinoaferesi utilizzando un separatore cellulare. Ad un controllo di qualità a campione, il contenuto di piastrine del concentrato deve essere ≥ **3x10e11** piastrine in almeno il 75% dei campioni.
Il concentrato ottenuto da **plasmapiastrinoaferesi** o da **prelievo multicomponente** deve contenere almeno **2x10e11** piastrine. L' emocomponente, se preparato in sistema chiuso, può essere conservato a 22°C ±2 °C in agitazione continua per un periodo di tempo variabile in funzione del contenitore impiegato, e comunque non superiore a cinque giorni dal prelievo. La conservazione deve essere effettuata con modalità che garantiscano la vitalità e l'attività emostatica delle piastrine contenute. Il volume di plasma o di liquido conservante deve essere in quantità tale da garantire, durante tutto il periodo di conservazione, un pH compreso fra 6,4 e 7,4.

PLASMA FRESCO CONGELATO.

È ottenuto attraverso il congelamento di plasma, dopo separazione del sangue intero o mediante aferesi (plasmaferesi), che deve avvenire entro limiti di tempo e a temperature tali da preservare adeguatamente l'attività dei fattori labili della coagulazione. Tale preparazione contiene normali livelli di fattori stabili e labili della coagulazione albumina e immunoglobuline, per una quantità totale di proteine superiore a 50 g/L.
Il preparato dovrebbe contenere meno di 6x10(elevato a 9/l) di emazie, meno di 0,1x109/l di leucociti e meno di 50x109/l di piastrine.
[…] Qualora il plasma sia ottenuto da aferesi, deve essere congelato utilizzando una apparecchiatura che lo completi entro un'ora a temperatura inferiore a -30°C.
Ad un controllo di qualità a campione, il preparato deve contenere almeno il 70% del contenuto originale di fattore VIIIc.

Procedure di manipolazione / lavorazione

LAVAGGIO	INATTIVAZIONE PATOGENI	CONGELAMENTO
• RBC • PIASTRINE • *(CSE)*	• PLASMA • PIASTRINE • *(RBC)*	• RBC • PIASTRINE • PLASMA • *(CSE)*

Manipolazione

MANIPOLAZIONE CELLULARE→ insieme di procedure che permettono alle cellule ematiche di essere utilizzate ai fini trasfusionali ottimizzandone l'impiego in relazione alle diverse necessità clinico/pratiche…

MC"MINIMA" → manipolazioni che non portano ad una attivazione delle cellule e/o ad una stimolazione della proliferazione cellulare…non alterano le caratteristiche principali delle cellule… cellule/tessuti non manipolati o manipolati in modo minimo […], andrebbero assimilati per gli aspetti di controllo di processo e di qualità ai trapianti d'organo o alle trasfusione di sangue.

Per tutti gli altri casi, in cui le manipolazioni effettuate possono, o hanno lo scopo di modificare le caratteristiche biologiche o la funzione di cellule/tessuti, sarà necessario il rilascio di un'autorizzazione preventiva all'utilizzo clinico da parte di una autorità preposta, sulla base di una descrizione dettagliata del processo, dei materiali, dei test e dei criteri di validazione impiegati →→ → **MANIPOLAZIONE ESTENSIVA** *(non minima)*

Procedure di manipolazione / lavorazione: **lavaggio**

LAVAGGIO	INATTIVAZIONE PATOGENI	CONGELAMENTO
• RBC • PIASTRINE • *(CSE)*	• PLASMA • PIASTRINE • *(RBC)*	• RBC • PIASTRINE • PLASMA • *(CSE)*

Lavaggio emazie

+

soluzione
salina 4°C

Centrifugazione con salina e scarto del sovranatante.

Si ottiene un concentrato eritrocitario privo di plasma.

Conservazione: a 2-6°C

L'UTILIZZO DOPO IL LAVAGGIO DEVE AVVENIRE NEL PIÙ BREVE TEMPO POSSIBILE

non oltre 24 ore se la procedura ha comportato l'apertura del sistema

LAVAGGIO

- **RBC**
- PIASTRINE
- *(CSE)*

Raccomandazioni SIMTI 2008

Emazie lavate:

Hct compreso tra 65-75%

Hgb > 40g

Recommendations for the transfusion of red blood cells

Giancarlo Liumbruno[1], Francesco Bennardello[2], Angela Lattanzio[3], Pierluigi Piccoli[4], Gina Rossetti[5]

Blood Transfus 2009; 7: 49-64 DOI 10.2450/2008.0020-08

Washed RCCs

- Patients with IgA deficiency *(Grade of recommendation: 2C)...*
- Prevention of allergic reactions not sensitive to antihistamine drugs *(Grade of recommendation: 2C)…*
- Post-transfusion febrile reactions present even when leucodepleted RBCs are used *(Grade of recommend. 2C)*

Up to 21-day banked red blood cells collected by apheresis and stored for 14 days after automated wash at different times of storage

VOX SANGUINIS (2006) 90, 40–44

C. Grabmer,[1] J. Holmberg,[2] M. Popovsky,[2] E. Amann,[1] D. Schönitzer,[1] S. Falaize,[2] H. Hanske,[2] E. Pages[2] & W. Nussbaumer[1]

Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population

Volume 51, May 2011 TRANSFUSION

Mandy Flannery O'Leary, Penny Szklarski, Thomas M. Klein, and Pampee Paul Young

The *in vitro* quality of washed, prestorage leucocyte-depleted red blood cell concentrates

VOX SANGUINIS (2004) 87, 19–26

V. Weisbach, W. Riego, E. Strasser, J. Zingsem, J. Ringwald, R. Zimmermann & R. Eckstein

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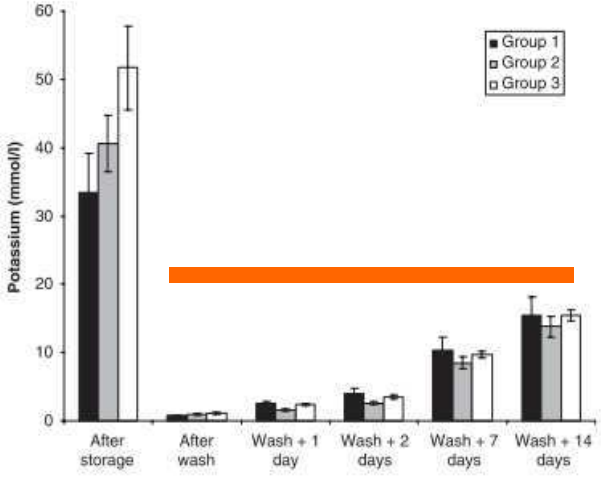
device automated washing system ACP-215 Haemonetics	Post-wash gr.1 (+7g)	Post-wash gr.2 (+14g)	Post-wash gr.3 (+21g)
Hgb % recovery	**90.9±1.7 (p<0.05)**	88.8±1.7	**87.3±1.6**

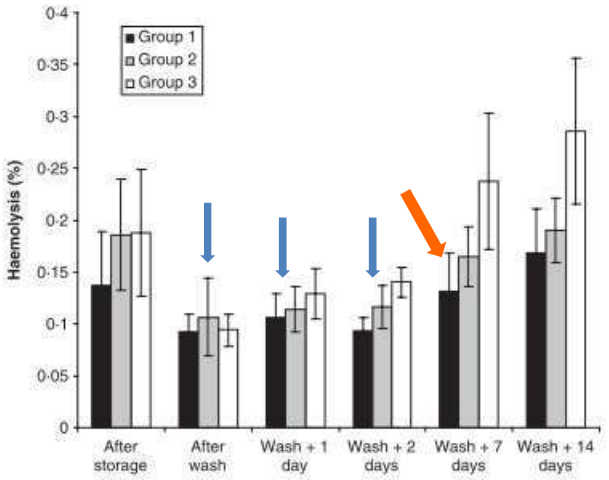
- Mean serum **IgA** of washed units was below the definition of IgA deficiency (IgA < 0·05 mg/dl).
- **Potassium** was effectively depleted … reincrease did not reach prewash values (after 14 days of storage)
- Depletion of **lactate** was dependent of prewash level and reduction was about threefold from prewash values.
- Removal of **proteins** and of cumulating potassium by ACP 215 was comparable to that of manually washed RBC
- Product **volume** and **haematocrit** of the final washed RBC product showed no significant differences between the three groups and confirmed **increased standardization** as a result of automated washing.
- Differences in **haemoglobin recovery** may result from differences in storage period prior to washing.
- Comparing **pH values** of washed and unwashed (prewash) units stored for the same period of time…pH can be significantly decreased by washing.

- **Product-related haemolysis in all groups was < 0.8% at the end of the postwash storage period and met the recommendations of the Council of Europe.**
- **However, our finding of approximately 15% loss of Hb confirms previously published data and reflects an important disadvantage of washing**
- **This loss increases both costs and donor exposure.**
- **Therefore, indications for washed products should be examined and suggested on a per patient basis.**

Potassium was significantly lowered in all three groups (P < 0·001) and remained significantly below the prewash value even after day 14 of postwash storage (P < 0·001)



% Haemolysis - free Hgb was significantly (P < 0·05) washed out in all three groups and remained not significantly different from prewash values for groups 1 and 2 after 14 days of postwash storage (P > 0·05)…



Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population

Volume 51, May 2011 TRANSFUSION

Mandy Flannery O'Leary, Penny Szklarski, Thomas M. Klein, and Pampee Paul Young

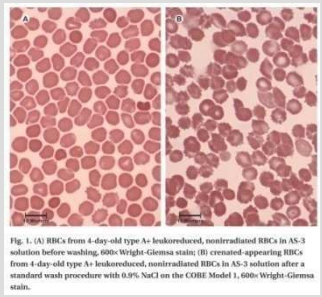


Fig. 1. (A) RBCs from 4-day-old type A+ leukoreduced, nonirradiated RBCs in AS-3 solution before washing, 600× Wright-Giemsa stain; (B) crenated-appearing RBCs from 4-day-old type A+ leukoreduced, nonirradiated RBCs in AS-3 solution after a standard wash procedure with 0.9% NaCl on the COBE Model 1, 600× Wright-Giemsa stain.

…compare the quality of the washed RBCs generated with Fresenius Continuous AutoTransfusion System (CATS) device, COBE-2991 (Model 1) and COBE-2991 (Model 2).

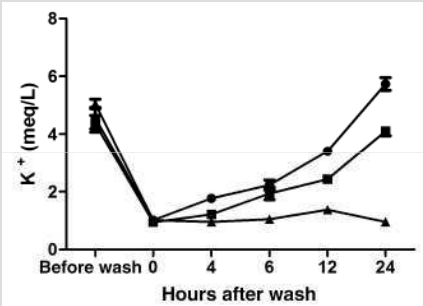


Fig. 2. Mean extracellular K and SDs over 24 hours after wash. (●) COBE Model 1; (■) COBE Model 2; (▲) Fresenius CATS.

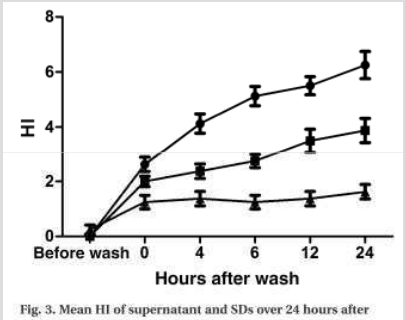


Fig. 3. Mean HI of supernatant and SDs over 24 hours after wash. (●) COBE Model 1; (■) COBE Model 2; (▲) Fresenius CATS.

At 12 hours, there was a significant difference ($p < 0.001$) between the extracellular K concentration for all devices

the CATS showed the lowest amount of hemolysis among the three devices with a clinically significant difference ($p < 0.001$)

•increase in extracellular K concentration in fresh RBC units over time after cell washing with the COBE Model 2991 blood cell processor and the Fresenius CATS.

•increase in hemolysis with all devices, particularly with the COBE Model 1.

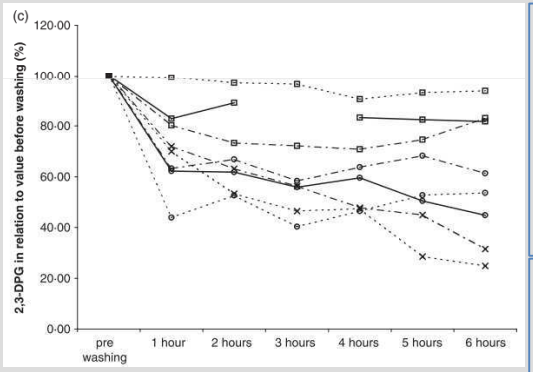
•<u>Our data support a decrease in the expiration time of washed units to 6 hours or less</u>.

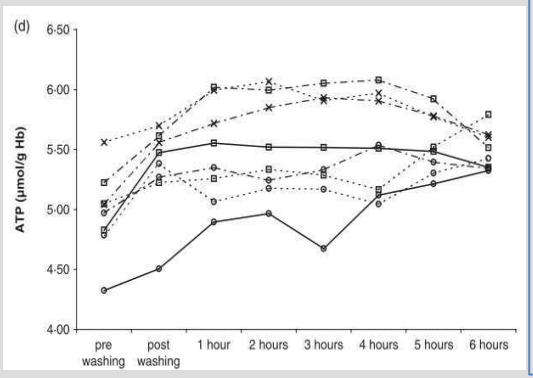
The *in vitro* quality of washed, prestorage leucocyte-depleted red blood cell concentrates

VOX SANGUINIS (2004) 87, 19–26

V. Weisbach, W. Riego, E. Strasser, J. Zingsem, J. Ringwald, R. Zimmermann & R. Eckstein

According to current guidelines**, there are few reasons to wash red blood cell concentrates** (RCCs)… One widely accepted indication for washing RCCs is **prior to the massive transfusion of neonates**… The application of **large amounts of potassium during the transfusion** of older RCCs is a major concern in this setting. Furthermore, the **removal of additive storage solutions** has been strongly recommended before massive transfusion in neonates… Therefore, **the best possible quality of RCCs, with a low potassium content and optimum erythrocyte quality, should be made available for these patients to avoid any risk.**



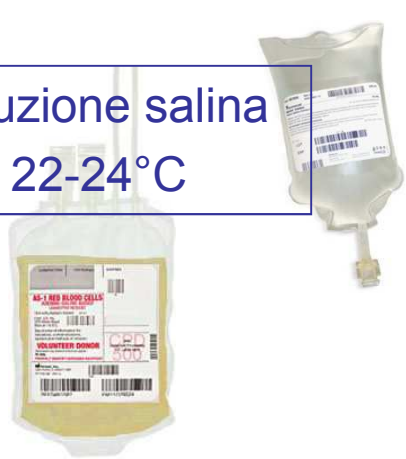


Reduction rate of potassium: 90.6 ± 5·7%. Potassium concentrations did not increase substantially in any group during the 6-h postwash storage period.

•slight **increase of lactate**
•increase free Hb
•decrease in pH
•loss of 2,3-DPG during washing →resulting in a **diminished oxygen availability for tissues**, might cause **additional respiratory problems** in small infants, especially under conditions of extracorporal circulation

Lavaggio piastrine

soluzione salina
22-24°C



CONCENTRATO PIASTRINICO LAVATO

Centrifugazione del concentrato piastrinico con soluzione salina (fisiologica) a 20-24°C,
Scarto del sovranatante e risospensione del preparato in soluzioni additive.

La tecnica comporta una riduzione del contenuto piastrinico.
Conservazione: in agitazione a 22-24°C.

L'UTILIZZO DOPO IL LAVAGGIO DEVE AVVENIRE NEL PIÙ BREVE TEMPO POSSIBILE
non oltre 24 ore se la procedura ha comportato l'apertura del sistema

LAVAGGIO

- RBC
- **PIASTRINE**
- (*CSE*)

http://www.hse.ie/eng/about/Who/clinical/natclinprog/blood.html

Blood Transfusion Programme

BLOOD TRANSFUSION

4.6 Use of washed platelets

Recurrent urticarial or other anaphylactic reactions to platelets that are not prevented by pre-medication with anti-histamines **may be prevented by washing the platelet component** prior to issue from the Blood Centre. **This is only occasionally necessary** – use of a preparation suspended in >50% crystalloid additive solution during manufacture is often as efficacious and should be specified in the prescription **if the patient has had a febrile or urticarial reaction in the past.** Washing platelets is labour intensive, but more importantly, will often result in **loss of some of the platelets in the final product**. Sometimes this loss is considerable, and can delay effective treatment. **Washed platelets have a reduced shelf life**. In **patients with a history of severe anaphylactic reactions and congenital IgA deficiency**, washing is unlikely to be effective, and platelets from IgA deficient donors will be required…

Recommendations for the transfusion of plasma and platelets

Giancarlo Liumbruno[1], Francesco Bennardello[2], Angela Lattanzio[3], Pierluigi Piccoli[4], Gina Rossetti[5] as Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Working Party

Washed PCs can be prepared for patients who have repeated reactions after transfusion of platelets or for patients with anti-IgA antibodies. Washing also reduces the content of the platelets, which must be resuspended in an additive solution (Grade of recommendation: 2C).

The impact of apheresis platelet manipulation on corrected count increment

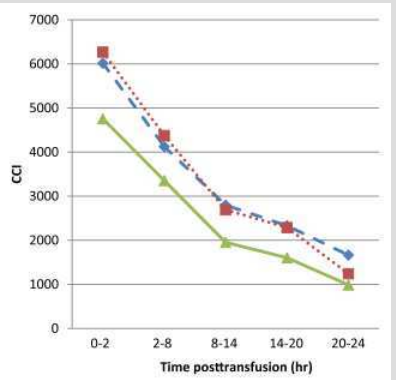
Matthew Karafin, Alice K. Fuller, William J. Savage, Karen E. King, Paul M. Ness, and Aaron A.R. Tobian

Volume 52, June 2012 **TRANSFUSION**

TABLE 2. The impact of product manipulation on PLT count*

	Volume (mL)			PLT count			
Manipulation	Before manipulation	After manipulation	p value	Before manipulation	After manipulation	p value	Percent recovery
Concentrated PLTs (n = 27)	303.8 (67.1)	107.6 (15.9)	<0.001	354,421.9 (70,305.4)	276,866.5 (54,135.5)	<0.001	79.0 (10.5)
Washed PLTs (n = 83)	281.8 (72.5)	173.1 (11.5)	<0.001	349,098.9 (87,937.7)	278,825.9 (71,554.9)	<0.001	80.1 (7.8)

* Data are reported as mean (SD).



Washing APs appears to **shorten the survival time of PLTs in vivo**.

concentrating or washing APs be undertaken only for clearly defined indications that cannot otherwise be prevented and only after the careful assessment of a transfusion medicine specialist and monitoring chronic PLT recipients receiving washed PLTs with serial PLT counts. Additional prospective, comparative studies are needed to evaluate the effects of AP function and survival in vivo, as are new approaches for preparing and monitoring plasma-reduced PLT products.

Procedure di manipolazione / lavorazione: **congelamento**

LAVAGGIO	INATTIVAZIONE PATOGENI	CONGELAMENTO
• RBC	• PLASMA	• RBC
• PIASTRINE	• PIASTRINE	• PIASTRINE
• *(CSE)*	• *(RBC)*	• PLASMA
		• *(CSE)*

Congelamento emazie: razionale

1. **È una valida alternativa alla conservazione *standard* 1-6°C,** dal momento che le emazie congelate possono essere conservate per diversi anni senza che sia compromessa "apparentemente" vitalità e sicurezza.

- È una strategia di notevole interesse clinico-pratico perché può "**diminuire" le problematiche connesse con le carenze stagionali, le richieste di gruppi rari e le situazioni di calamità (diffusione in ambito militare)**

Congelamento emazie: metodiche

Glicerolo 40%

↓

-60 / -80°C

Congelatore meccanico

Entro 7 gg dalla raccolta

Glicerolo 15-20%

↓

Azoto liquido (-150 °C)

In azoto liquido

Fino a 10 anni

Scongelamento

Deglicerolizzazione

Risospensione in soluzione nutriente SAG-M

Utilizzare nel più breve tempo possibile

Sistema aperto

Entro 24 ore (+2 - +6°C)

Sistema chiuso/automatico

Max. 14 giorni

(+2 - +6°C)

CONGELAMENTO

- **RBC**
- PLT
- (CSE)

Congelamento emazie

Caratteristiche emazie congelate:

- L'unità ricostituita di emazie congelate è praticamente priva di proteine, granulociti e piastrine.
- L'unità ha un volume maggiore di 185 mL.
- Hb superiore a 36 g/unità.
- Ht 65 - 75%.
- Leucociti residui inferiori a 1 x 109 cellule/unità.
- Osmolarilità inferiore a 340 mOsm/l.

Indicazioni per l'uso:

Le emazie congelate dovrebbero essere utilizzate per particolari situazioni, quali:

- trasfusioni in pazienti con gruppi rari o con molteplici alloanticorpi;
- utilizzazione per immunizzazioni volontarie; i globuli rossi congelati, conservati per almeno sei mesi, consentono di ricontrollare i test dei donatori;
- autotrasfusioni (in casi speciali).

Congelamento emazie: aspetti biochimici e fisiologici

Cryostored RBCs apparently do not show any classic "storage lesion", in contrast to that observed in RBCs stored at 1–6 °C: Hemoglobin structure, methemoglobin levels, membrane and cellular energetics are unaffected by extended storage in the frozen state since very low temperatures suppress molecularmotion and arrest metabolic and biochemical reactions.

"Red cell changes during Storage"
Transfusion Apheresis Science 43 (2010) 51–59.

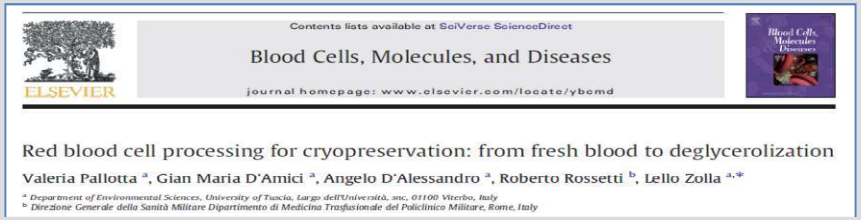
…after glycerolization step: increase of MCV and RDW-SD
…after washing step: these values back to normal range but higher than in fresh blood controls
…decrease of Hct and MCHC is consistent→not all of RBCs present at the start of processing survive to the end of cryostorage → this loss measured approximately 5%…

"The effects of cryopreservation on red blood cell microvesiculation, phosphatidylserine externalization, And CD47 Expression"
Transfusion 48 (2008) 1658-1668.

"Altered processing of thawed red cells to improve thein Vitro quality during postthaw storage at 4 degrees C"
Transfusion 47 -(2007) - 2242–2249.

Thawed RBCs are more fragile than conventionally liquid-stored and fresh RBC, as they display higher Osmotic fragility.

"The effects of cryopreservation on red blood cell rheologic properties" Transfusion. 2010 Nov;50(11)


Contents lists available at SciVerse ScienceDirect

Blood Cells, Molecules, and Diseases

journal homepage: www.elsevier.com/locate/ybcmd

Red blood cell processing for cryopreservation: from fresh blood to deglycerolization

Valeria Pallotta [a], Gian Maria D'Amici [a], Angelo D'Alessandro [a], Roberto Rossetti [b], Lello Zolla [a,*]

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[b] Direzione Generale della Sanità Militare Dipartimento di Medicina Trasfusionale del Policlinico Militare, Rome, Italy

Cryostorage itself in presence of glycerol does not significantly affect RBCs.
Most of the alterations observed were **related to cell processing** and, in particular, **to the increase of cytosolic glycerol as a consequence of the glycerolyzation step.**

Further studies might profitably investigate replacing glycerol with non-penetrating cryoprotectants.

Congelamento emazie

CONGELAMENTO

- **RBC**
- PLT
- (CSE)

PRO

- Conservazione prolungata (10 anni!?)
- Riduce i problemi connessi con le carenze stagionali, con le richieste di gruppi rari e le situazioni di calamità
- Possibilità di automatizzare la procedura

CONTRO

- Complessità procedura
- Costi legati alla procedura ed alla conservazione
- Glicerolizzazione: Possibili alterazioni della permeabilità di membrana con aumento della fragilità degli RBC criopreservati.

VALUTAZIONI

Sono auspicabili ulteriori studi per valutare le alterazioni sulla membrana eritrocitaria con valutazioni relative all'impiego di nuovi prodotti in sostituzione del **glicerolo** (**agenti crioprotettivi in grado di non penetrare nella cellula**, per es. il "trealoso", molecola naturale vegetale, utilizzata dalle piante in situazione di stress da freddo)

Congelamento piastrine: razionale

CONGELAMENTO

- RBC
- **PLT**
- (CSE)

La vita piastrinica è limitata a 5 giorni negli Stati Uniti ed a 7 gg in altri paesi…a causa del possibile insorgere di contaminazioni batteriche e del normale decremento funzionale delle piastrine stesse durante la conservazione.

La possibilità di **criopreservare le piastrine per periodi più lunghi potrebbe risolvere in parte gli aspetti legati alla gestione delle scorte oltre e contribuire alla soluzione di problematiche particolari**

Piastrine criopreservate: metodiche

Entro 24 ore dalla raccolta

DMSO 6% (peso/vol.) / Glicerolo 5% (peso/vol.)

-80°C

Congelatore meccanico

Fino a 1anno

Azoto liquido (-150 °C)

In vapori di azoto

Fino a 10 anni

Scongelamento

Lavaggio / Risospensione
in plasma autologo o soluzioni isotoniche

Conservare in agitazione fino all'impiego (+22°C +/- 2°C)

Utilizzare nel più breve tempo possibile

Congelamento piastrine

CONGELAMENTO

- RBC
- **PLT**
- (CSE)

Piastrine congelate: caratteristiche

Una unità ricostituita di piastrine crioconservate è praticamente priva di eritrociti e di granulociti.

Volume da 50 a 200 mL.

Conta piastrinica> 40% del contenuto piastrinico prima del congelamento

Leucociti residui < 0,2x10e6 per 60x10e9 piastrine

La dimostrazione del fenomeno dello swirling rappresenta un controllo affidabile della qualità del prodotto e va attuata al momento del rilascio del prodotto e della trasfusione.

Indicazioni

Le piastrine criopreservate dovrebbero essere riservate esclusivamente per la disponibilità di piastrine HLA e/o HPA compatibili (donatore compatibile non sia immediatamente disponibile).
Le forme cliniche che possono avvalersi di questo emocomponente sono:

- piastrinopenia alloimmune pre-natale e neonatale;
- alloimmunizzazione HLA;
- trapianto di midollo osseo.

Piastrine criopreservate:
aspetti tecnico – qualitativi -1

Cryopreservation of human platelets with dimethyl sulfoxide: changes in biochemistry and cell function

M. Böck, M. Schleuning, M.U. Heim, and W. Mempel

Background: The shelf life of liquid-stored platelet concentrates is limited to 5 days. Therefore, much work has been carried out in an attempt to establish the optimum method for cryopreservation. Among the various cryoprotectants, dimethyl sulfoxide (DMSO) has been shown to be the most effective. However, DMSO-frozen platelets are characterized by a number of cell lesions. This report describes metabolic and functional changes that should give rise to some concern about the functional integrity of these cells.
Study Design and Methods: Single-donor platelet concentrates were frozen in liquid nitrogen by use of DMSO (5%). After thawing, the cells were washed and resuspended in autologous plasma. Before, during, and after the freezing process, samples for analysis of metabolic measures (e.g., pH; calcium, potassium, and lactate dehydrogenase concentrations; plasma complement factors) and functional measures (e.g., aggregometry, in vitro bleeding time, α-granule membrane protein-140 expression) were taken.
Results: Mean platelet volume increases during the deep-freezing process. Potassium, calcium, and lactate dehydrogenase are released from the intracellular space to the extracellular space. A strong activation of complement, which is mainly due to the addition of DMSO, is observed. Platelets become activated as indicated by the expression of α-granule membrane protein-140. Accordingly, decreased platelet function can be observed.
Conclusion: DMSO-frozen platelets are characterized by several metabolic and functional changes. Although these cells have been shown to exert hemostatic effects in vivo, it is conceivable that those effects could be improved by further development of platelet-freezing techniques.

TRANSFUSION 1995;35:921–924.

A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide–frozen autologous platelets in healthy volunteers

Larry J. Dumont, Jose A. Cancelas,* Deborah F. Dumont, Alan H. Siegel, Zbigniew M. Szczepiorkowski, Neeta Rugg, P. Gayle Pratt, D. Nicole Worsham, Elizabeth L. Hartman, Susan K. Dunn, Margaret O'Leary, Janet H. Ransom, Rodney A. Michael, and Victor W. Macdonald*

Transfusion May 2012

TABLE 3. Recovery and survival by elution correction method and laboratory

Sample	Elution correction (×1000 *g*)	Overall (n = 24)		Overall (n = 21)†		Laboratory 1 (n = 12)†		Laboratory 2 (n = 12)		Laboratory 2 (n = 9)†	
		Recovery (%)	Survival (days)	Recovery (%)	Survival (days)	Recovery (%)	Survival (days)	Recovery (%)	Survival (days)	Recovery (%)	Survival (days)
Fresh	16*	74.2 ± 18.5	8.2 ± 1.3	68.4 ± 8.2	8.4 ± 1.2	68.6 ± 9.0	9.0 ± 0.4	79.8 ± 23.7	7.4 ± 1.4	68.1 ± 7.6	7.7 ± 1.5
Fresh	2	70.1 ± 14.1	8.4 ± 1.3	65.5 ± 6.4	8.5 ± 1.3	63.7 ± 5.3	9.1 ± 0.4	76.5 ± 17.2	7.7 ± 1.6	67.9 ± 7.3	7.7 ± 1.7
Fresh	0	62.6 ± 14.1	8.3 ± 1.3	58.0 ± 6.2	8.5 ± 1.3	57.5 ± 4	9 ± 0.4	67.7 ± 18.6	7.7 ± 1.6	58.7 ± 8.5	7.7 ± 1.7
CPPs	16*	41.6 ± 9.1	6.8 ± 2.1	41.6 ± 9.7	7 ± 2.1	44.3 ± 10.3	7.9 ± 1.7	39.0 ± 7.1	5.8 ± 1.9	38.0 ± 7.8	5.9 ± 2.1
CPPs	2	35.1 ± 7.8	7.4 ± 1.6	34.7 ± 8.2	7.4 ± 1.5	34.3 ± 8.0	8.0 ± 1.5	35.9 ± 7.8	6.7 ± 1.5	35.2 ± 8.9	6.7 ± 1.1
CPPs	0	22.7 ± 7.1	7.3 ± 1.7	22.6 ± 7.6	7.4 ± 1.6	22.0 ± 7.0	7.9 ± 1.7	23.4 ± 7.4	6.7 ± 1.5	23.4 ± 8.6	6.7 ± 1.1

* 16,000 × *g* elution corrections were used for primary hypothesis testing as described in the text.
† n = 21 was used for primary hypothesis testing as described in the text.

RESULTS: After frozen storage, the crio preserved pletelets (CPPs) 24-hour recovery (41.6 ± 9.7%) was lower than for fresh PLTs (68.4 ± 8.2%; $p < 0.0001$) CPPs had diminished survival compared to fresh PLTs (7.0 ± 2.1 days vs. 8.4 ± 1.2 days, respectively; $p = 0.018$), but did meet and exceed the FDA criterion for survival.

Congelamento piastrine

CONGELAMENTO

- RBC
- **PLT**
- (CSE)

PRO

Gestione dei problemi associati alla breve durata, soprattutto in situazioni particolari

CONTRO

- **Complessità procedura**
- **Costi legati alla conservazione**
- **Cambiamenti metabolici e funzionali che potrebbero ripercuotersi sulla attività emostatica**
- **Ridotto recupero piastrinico**

VALUTAZIONI

In considerazione di quanto sopra, **la criopreservazione di piastrine andrebbe riservata a casi limitati…anche per quanto riguarda il congelamento di piastrine autologhe**. Va comunque sempre fatta una valutazione costi/benefici in relazione alla situazione ed al contesto nel quale ci si trova ad operare.

Plasma fresco congelato

Separato entro 6 ore (non oltre 18) dal prelievo
Congelamento a -30° completato entro 1 ora

CONGELAMENTO

- RBC
- PLT
- **PLASMA**
- (CSE)

Congelato entro 6 ore dal prelievo
- Tipo A (aferesi)
- Tipo B (scomposizione)

Congelato tra 6 e 72 ore dal prelievo
- Tipo C (scomposizione)

Albumina
Immunoglobuline
ATIII
FVIII
FIX
Complesso protrombinico

Plasma fresco congelato

CONGELAMENTO

- •RBC
- •PLT
- •**PLASMA**
- •(CSE)

Utilizzo per uso clinico entro

- •24 mesi se conservato ad almeno -25°C
- •3 mesi se conservato tra -18°C e -25°C

Scongelato a 30 - 37°C con idonea strumentazione

Utilizzato nel più breve tempo possibile

Entro 24 ore se conservato a 0-4°C

Plasma fresco congelato

Scongelamento a 1-6°C

CONGELAMENTO

- RBC
- PLT
- **PLASMA**
- (CSE)

Crioprecipitato

FVIII, FXIII, vWF, fibrinogeno, fibronectina

Plasma privo di crioprecipitato (cryosupernatant)

vWF metalloproteasi (ADAMTS13)

Ricongelamento entro 1 ora

24 mesi se conservato ad almeno -25°C

3 mesi se conservato tra -18°C e -25°C

Utilizzato nel più breve tempo possibile

Entro 24 ore se conservato a 0-4°C

Procedure di manipolazione / lavorazione: **inattivazione**

LAVAGGIO	INATTIVAZIONE PATOGENI	CONGELAMENTO
• RBC • PIASTRINE • *(CSE)*	• PLASMA • PIASTRINE • *(RBC)*	• RBC • PIASTRINE • PLASMA • *(CSE)*

Inattivazione dei patogeni: razionale

1 ***The blood components continue to carry risk of infectious diseases*** *(associated with transfusion-transmitted-infections (TTI).*

	Doubling time	Assay Ab/Ag	NAT (pool 16-24)	NAT (single sample)	Chronic Infection	Residual Risk Update
HCV	15 hours	66 days (range 54-192)	12 days	7 days	70%	0,2 (2,7) x 10^6
HIV	20 hours	22 days (range 6-39)	11 days	7 days	100%	0,4 (2,2) x 10^6
HBV	2,56 days	59 days (range 37-87)	35 days	20 days	5%	

(Soldan K C *et al*, 2003; Tosti ME *et al*, 2002; Jackson BR *et al*, 2003; Pereira A, 2003; Laurer GM, Walker BD, 2001; Velati C *et al*, 2008)

2 ***The present reactive approach to avoid them is limited to specific known pathogens, is not effective against bacterial contamination, does not test for all pathogens, fails to prevent transmission of CMV*** *despite testing and tests for new pathogens, such as West Nile virus or Chikungunya virus, can only be implemented after the new agent is identified… the list of infectious agents causing life threatening infections is continuously increasing.*

3 *Moreover, with* ***increasing globalization*** *combined with* ***climate changes****, previously localized* ***transfusion-transmitted infections*** *are now becoming more widespread or* ***appearing in places where they did not exist before*** *.*

4 *For* ***Bacterial contamination*** *of* ***PLATELET CONCENTRATES*** *different strategies have been implemented, including* ***sensitive bacterial screening*** *of PCs. However, transfusion-related sepsis continues to occur and the US Food and Drug Administration determined that in spite of performing bacterial screening,* ***5 days should be the maximum shelf life of PCs*** *due to residual bacterial septic risk.*

5 ***…proactive strategies have been developed to treat the blood components in a way that will inactivate viruses, bacteria, protozoa and contaminating leucocytes but retain therapeutic efficacy of blood components…***

Figure 20.1
Number of bacterial TTI incidents, by year of report and type of unit transfused (Scotland included from 10/1998)

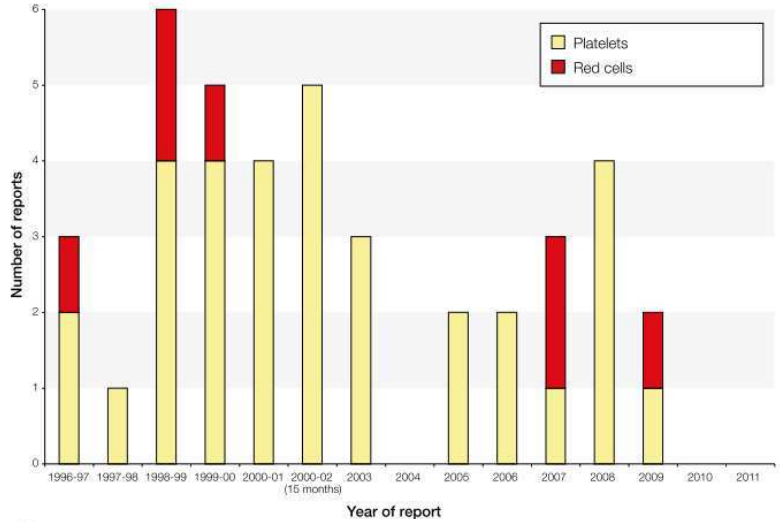


Figure 20.2
Number of viral and parasitic TTI incidents, by year of report and infection type (Scotland included from 10/1998)

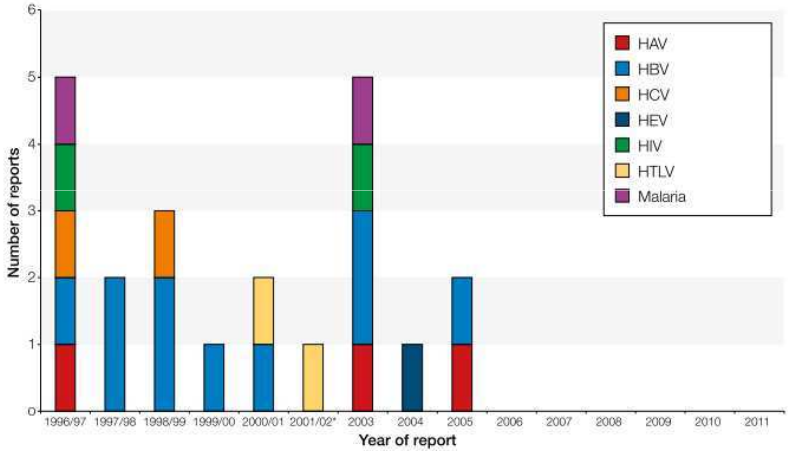


Table 20.1
Number of confirmed TTI incidents, infected recipients and outcomes (death, major morbidity, minor morbidity) in the UK between October 1996 and December 2011 (Scotland included from October 1998) NB No screening in place for the following TTIs at the time of transfusion: HAV, HEV, HTLV, vCJD/prion

Infection	Number of incidents	Number of infected recipients	Death due to, or contributed to, by TTI	Major morbidity	Minor morbidity
Bacteria	40	43	11	28	4
HAV	3	3	0	2	1
HBV	10	11	0	11	0
HCV	2	2	0	2	0
HEV	1	1	0	0	1
HIV	2	4	0	4	0
HTLV I	2	2	0	2	0
Malaria	2	2	1	1	0
Prion	1	1	0	1	0
vCJD	3	4	3	0	0
Total	**66**	**73**	**15**	**51**	**6**

Authors: Paula Bolton-Maggs and Hannah Cohen

Figure 4.1
Cases reviewed in 2011 (excluding near miss and instances where the patient received a correct component despite errors having occurred – RBRP) n=1815

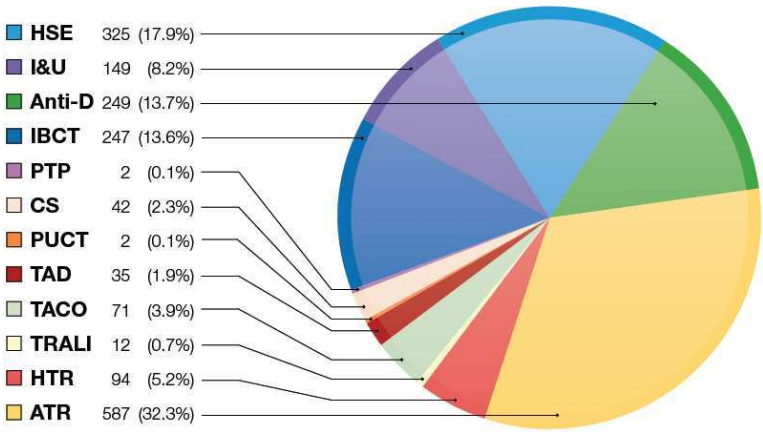
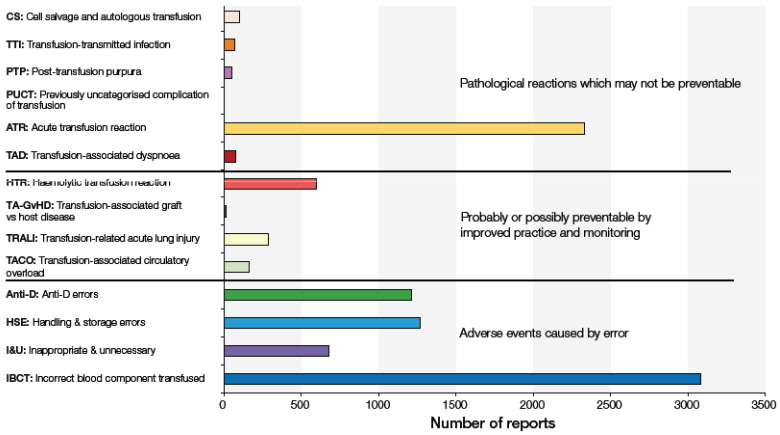


Figure 4.2
Cumulative data for SHOT categories 1996/7-2011 n=9925



Agence française de sécurité sanitaire des produits de santé

Rapport annuel de l'hémovigilance 2010

Sicurezza degli emocomponenti

Eventi avversi sui riceventi per 100.000 unità.

Incidenza EA per le Piastrine: 3 volte >> GRC / 9 volte >> Plasma

	Nombre de diagnostics pour 100.000 unités			
Diagnostics	CGR	Plaquettes	Plasmas	Total
Allo-immunisation isolée	67,1	83,4	1,3	63,5
RFNH	59,0	70,8	1,3	52,9
Allergie	13,6	280,1	49,9	42,7
TACO	9,4	5,0	1,3	8,1
Incompatibilité immunologique	5,8	34,9	0,3	7,8
Dont ABO	0,1			
Réaction hypertensive	3,4	1,8	0,0	2,8
Inefficacité transfusionnelle	0,3	22,3	0,0	2,3
TRALI	1,8	4,3	2,1	2,1
Réaction hypotensive	1,1	2,9	0,0	1,2
Hémosidérose	0,8	0,0	0,0	0,7
Infection bactérienne	0,4	2,9	0,0	0,6
Hémolyse autre	0,6	0,0	0,0	0,6
Autres	3,1	6,1	1,6	3,4
Diagnostic inconnu[21]	5,1	13,3	1,0	5,4
Total	171,5	**527.9**	58,8	193,9

bjh review

Emerging pathogens and their implications for the blood supply and transfusion transmitted infections

British Journal of Haematology doi:10.1111/bjh.12031

Roger Y. Dodd

Research and Development, American Red Cross, Holland Laboratory, Rockville, MD, USA

The threat of infection by conventional transfusion-transmitted agents has been essentially eliminated from the blood supply in developed countries**, thus focusing attention on the potential risk from emerging infections**. **Over recent years, actions have been taken to manage a number of such risks to blood safety**. **Emerging infections** are simply defined as **'those whose incidence has increased within the past two decades or threatens to increase in the near future'**. The term emergence is, however used to cover a number of situations that go beyond an increased presence of an existing or new pathogen.

INATTIVAZIONE DEI PATOGENI →PIASTRINE

questo approccio prevede il trattamento dei concentrati piastrinici per l'inattivazione/riduzione del livello di contaminazione batterica…e contemporaneamente l'inattivazione di virus, protozoi e leucociti.

Due tecnologie di inattivazione/riduzione dei patogeni per i CONCENTRATI PIASTRINICI hanno ottenuto il Marchio CE e sono state introdotte in routine sul mercato:

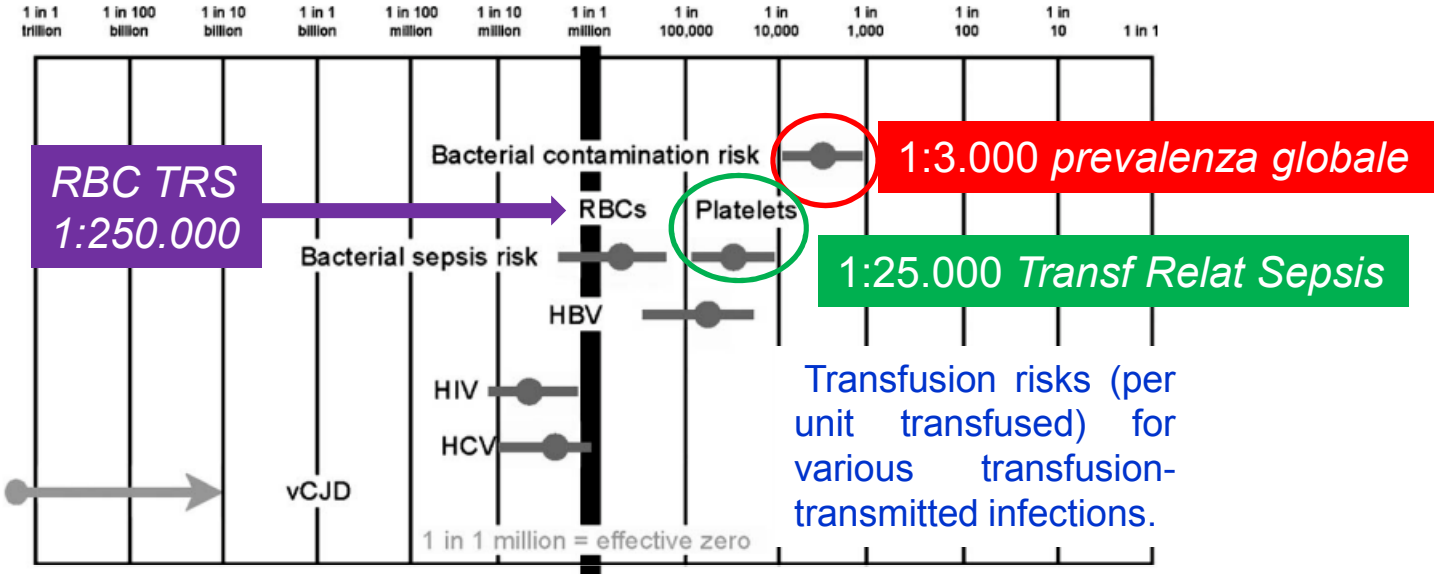
- **INTERCEPT Blood System – CERUS**
- **Mirasol – Caridian BCT.**

Entrambe utilizzano un **sistema di inattivazione foto chimica** ma i meccanismi di azione alla base delle due tecnologie sono fondamentalmente diversi.

PIASTRINE	VIRUS BATTERI PARASSITI	Cerus	Amotosalen + UV	Marchio CE 2002
		Caridian BCT	Riboflavin + UV	Marchio CE 2007

Bacterial Detection of Platelets: Current Problems and Possible Resolutions

Morris A. Blajchman et Al., Transfusion Medicina Reviews, Vol. 19, Ottobre 2005



1:3.000 *prevalenza globale*

1:25.000 *Transf Relat Sepsis*

9 studi prospettici con valutazione di 192.053 unità di Piastrine ➔ Stima approssimativa 1:3000 unità contaminate da batteri…

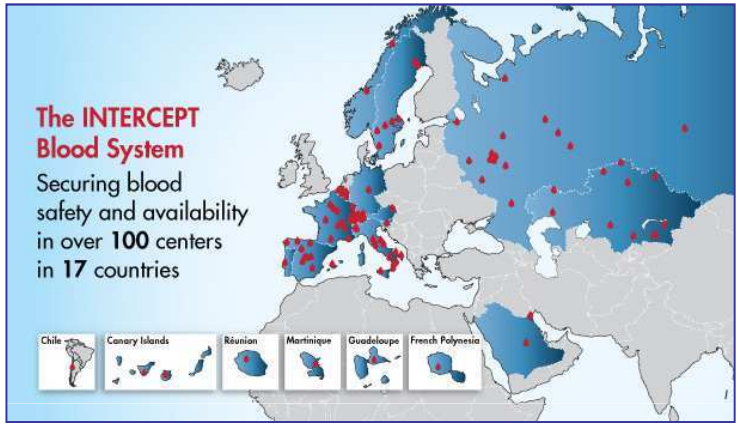
Valutazione della CB al momento della raccolta…

Valutazione della CB su unità di CP in scadenza e quindi al momento di massima proliferazione batterica

La determinazione della CB dipende dai **livelli di contaminazione** e dalla **sensibilità dei metodi** utilizzati per il rilevamento …

INTERCEPT

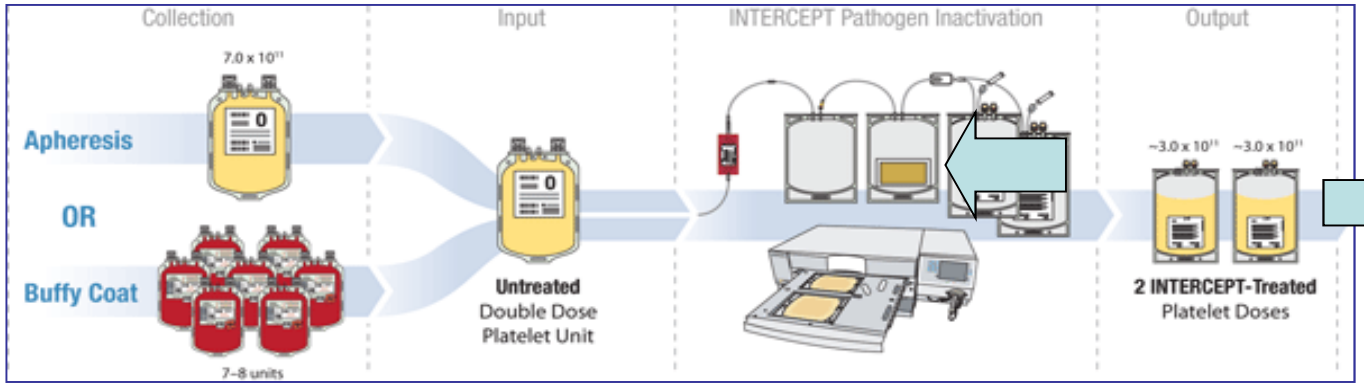
La tecnologia INTERCEPT ha ottenuto il Marchio CE nel 2002
E' registrato come Dispositivo medico in classe III.



E' in uso routinario in più di 17 paesi (100 Centri di trasfusione) con più di 700.000 trasfusioni effettuate.

I concentrati piastrinici trattati con INTERCEPT hanno ricevuto autorizzazioni specifiche in diversi paesi europei:

Regulatory Status	
Austria	The first blood center marketing authorization was approved by AGES for INTERCEPT Platelets in 2010.
Europe	The INTERCEPT Blood Systems for platelets and plasma are CE marked Class III medical devices and produced to EN ISO 13485 Quality standard.
France	INTERCEPT Platelet and Plasma product characteristics are approved by Afssaps and published in *Journal Officiel*.
Germany	The first blood center marketing authorization was approved by PEI for INTERCEPT Platelets in 2007.
Switzerland	The INTERCEPT Blood System for platelets was approved by Swissmedic in 2009.



È possibile inattivare una DOPPIA dose Terapeutica fino a 7 x 10E11 con il medesimo Kit

INTERCEPT

Bacteria species inactivated by the INTERCEPT™ system for platelets.	*Extent of inactivation (#) log_{10} reduction*
Gram-negative bacteria	
Escherichia coli	**>6.4**
Serratia marcescens	**>6.7**
Klebsiella pneumoniae	**>5.6**
Pseudomonas aeruginosa	**4.5**
Salmonella choleraesuis	**>6.2**
Yersinia enterocolitica	**>5.9**
Enterobacter cloacae	**5.9**
Gram-positive bacteria	
Staphylococcus epidermidis	**>6.6**
Staphylococcus aureus	**6.6**
Streptococcus pyogenes	**>6.8**
Listeria monocytogenes	**>6.3**
Corynebacterium minutissimum	**>6.3**
Bacillus cereus (includes spores)	**3.6**
Bacillus cereus (vegetative)	**>6.0**
Bifidobacterium adolescentis	**>6.5**
Propionibacterium acnes	**>6.7**
Lactobacillus species	**>6.9**
Clostridium perfringens (vegetative form)	**>7.0**
Spirochete bacteria	
Treponema pallidum	**≥6.8 to ≤7.0**
Borrelia burgdorferi	**>6.8**

Photochemical treatment of platelet concentrates with amotosalen and UVA inactivates a broad spectrum of pathogenic bacteria…

Lin L, Dikeman R, Molini B et al. Transfusion 2004

Use of additive solutions and pathogen inactivation treatment of platelet components in a regional blood center: impact on patient outcomes and component utilization during a 3-year period

TRANSFUSION 2011;51:622-629.

Jean-Pierre Cazenave, Hervé Isola, Chantal Waller, Isabelle Mendel, Daniel Kientz, Michel Laforêt, Jean-Pierre Raidot, Gérard Kandel, Marie-Louise Wiesel, and Laurence Corash

TABLE 2. Production and utilization of PCs

Variable	Period 1*	Period 2†	Period 3‡
Number of components	10,629	9,151	13,241
PLT content of transfused components§			
Mean dose/unit ($\times 10^{11}$)	5.2	4.4	4.2
Median ($\times 10^{11}$)	5.4	4.7	4.4
Range ($\times 10^{11}$)	0.6-9.2	0.8-7.8	0.5-7.3
PC use/patient (n)‖			
Patients	2,050	1,678	2,069
Mean	5.2[b]	5.5[c]	6.4[b,c]
Median	2.0	2.0	2.0
Range	1-104	1-114	1-289
Total PLT dose/patient ($\times 10^{11}$)¶			
Mean	27.1	24.1	26.9
Median	10.9	9.4	9.1
Range	0.2-543	0.2-503	0.5-1,302

* PCs were prepared in plasma. During Period 1, PLT content of whole blood–derived PC was determined in 3% of components.
† PCs were prepared in plasma with PAS. During Period 2, PLT content of whole blood–derived PC was determined in 17% of components.
‡ PCs were prepared in plasma with PAS and PI treatment.
§ ANOVA, difference if $F \geq 3.0$. The difference is significant for the three periods ($F = 4396.9$). t test for two samples assuming equal variances: [a]P1 – P2, [b]P1 – P3, [c]P2 – P3. $p < 0.05$.
‖ ANOVA, difference if $F \geq 3.0$. The difference is significant for the three periods ($F = 6.8$). t test for two samples assuming equal variances: [a]P1 – P2, $p = 0.37$; [b]P1 – P3, $p = 0.0008$; [c]P2 – P3, $p = 0.01$.
¶ ANOVA. The difference is not significant for any comparisons: $F = 1.9$.

TABLE 5. Adverse reactions after transfusion of PCs

Variable	Period 1*	Period 2†	Period 3‡
Number of patients transfused	2,050	1,678	2,069
Number of components transfused	10,629	9,151	13,241
Number of patients with adverse reactions	59	33	36
Number of adverse events§	67	41	37
Number of RBC alloantibodies detected	11	16	19
Number of RBC alloantibodies/1000 PCs	1.03	1.75	1.43
Number of transfusion reactions	56	25	18
Number of total reactions/1000 PCs	6.3	4.5	2.8
Number of PC-related reactions/1000 PCs‖	5.3[a,c]	2.7[a,b]	1.4[b,]
Patients with PC reactions (%)¶	2.9[a,c]	2.0[a]	1.7[c]

* PCs were prepared in plasma.
† PCs were prepared in plasma and PAS.
‡ PCs were prepared in plasma and PAS with PI treatment.
§ Total transfusion reactions including alloantibodies detected to RBC antigens.
‖ Transfusion reactions excluding RBC alloantibodies not associated with symptomatic reactions. Chi-square test with $\alpha = 0.05$, difference if $p < 0.05$: [a]P1 – P2, $p = 0.0053$; [b]P2 – P3, $p = 0.0214$; [c]P1 – P3, $p = 7 \times 10^{-8}$.
¶ Symptomatic reactions to PCs, excluding RBC alloantibodies. Chi-square test with $\alpha = 0.05$, difference if $p < 0.05$: [a]P1 – P2, $p = 0.0094$; [b]P2 – P3, $p = 0.0779$; [c]P1 – P3, $p = 6.7 \times 10^{-8}$.

TABLE 7. Utilization of PLT and RBC components by hematology-oncology patients

Variable	Period 1*	Period 3†	p value
Number of patients	671	699	
Number of PCs transfused			
Mean ± SD‡	8.7 ± 12	11 ± 20	0.01
Median‖	4.0	4.0	
Range‖	1-103	1-264	
Total PLT dose			
Mean ± SD‡	45.3 ± 62.8	46.1 ± 86.1	0.85
Median	21.6	18.4	
Range‖	0.8-536	1.5-1189	
Number of RBC units transfused			
Mean§	15.2 ± 16.5	13.6 ± 18.4	0.10
Median‖	9.0	8.0	
Range‖	0-93	0-272	

The conclusions regarding efficacy and safety that may be drawn from this study are potentially limited due to the lack of a blinded, randomized trial design.

…each of the PC methods reflected **routine production practices**.

1. monitoring of patient clinical outcomes utilized objective measures, such as **utilization of PCs and RBCs**,
2. and an **active hemovigilance program** with oversight by clinical monitors who were not associated with the blood center producing the blood components.

…we believe that the data reported from this study indicate that **PASs and PI treatment can be implemented into routine practice without impacting either PC or RBC utilization and with a reduction in acute transfusion reactions.**

It is also similar to the Belgian experience of universal routine use of Intercept-inactivated PCs for 3 years, that enables learning of how well the products function in broad populations.

In addition, the **PI process was used in place of gamma irradiation for prevention of TA-GVHD and in place of cytomegalovirus serology** resulting in the use of a single PLT inventory with elimination of these additional tests and procedures.

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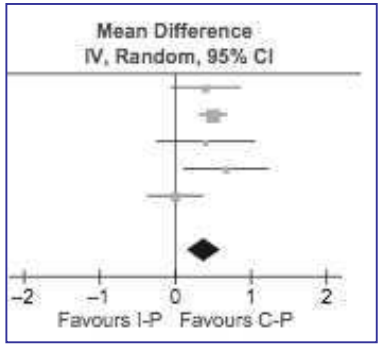
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2012)
ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2012.01614.x

Therapeutic efficacy of platelet components treated with amotosalen and ultraviolet A pathogen inactivation method: results of a meta-analysis of randomized controlled trials

J. Cid, G. Escolar & M. Lozano
Department of Hemotherapy and Hemostasis, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain

	C-P vs IP
* Mean difference in the post-transfusion CCI-1 h	95% CI, 0.11x10e3 – 2.69x10e3; P = 0.03
Mean difference in the post-transfusion CCI-24 h	95% CI, 2.32x10e3 – 3.69x10e3; P < 0.00001
* Mean difference in the transfusion interval	95% CI, 0.33 - 0.64; P < 0.00001
OR of bleeding	95% CI, 0.72 – 1.25; P = 0.71



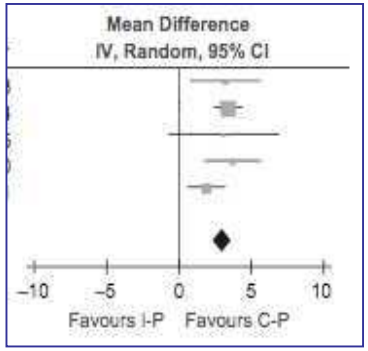


Table 1 Randomized controlled trials that compare inactivated vs. noninactivated platelet transfusion: study descriptors

Study descriptor	EuroSPRITE [17]	SPRINT [18]	Janetzko [19]	Kerkhoffs [20]	Lozano [21]
Design of the study	Double blinded	Double blinded	Double blinded	Open label	Double blinded
Number of days of platelet transfusion support	Up to 56	Up to 28	Up to 28	Up to 42	1
Platelet transfusion trigger ($\times 10^9$/l)	<20	<10	<20	10–40–60	10–20
ABO compatibility	All ABO compatible	Variable	Not stated	Not stated	All ABO compatible
Maximum storage duration of transfused platelets (days)	5	5	5	7	7
Methodologic quality score[a]	3	4	3	2	4
Grading of bleeding	Own scale	WHO[b] scale	WHO scale	CTCAE[c] criteria	WHO scale

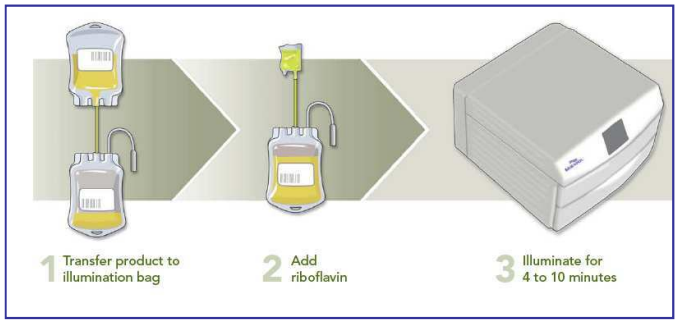
…the transfusion of C-P was associated with statistically significant higher CCI-24h when compared with the transfusion of I-P. Regarding the CCI-1h, transfusion interval and OR of bleeding, there was a high variations among the findings of RCTs…(*the hypothesis of homogeneity was rejected when all five RCTs were combined*). No statistically differences in the OR of bleeding I-P vs C-P

Riboflavina - Mirasol

La tecnologia Mirasol ha ottenuto il Marchio CE nel 2007 ed è registrato come dispositivo medico in Classe II.

I concentrati piastrinici trattati con Mirasol non hanno attualmente autorizzazioni nazionali specifiche.

Possibilità di inattivare una singola dose terapeutica (max $5x10^{11}$)
Il processo prevede l'inattivazione di una singola sacca per volta



Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Ruane PH, Edrich R, Gampp D, Keil SD, Leonard L, Goodrich RP. *Transfusion* 44, 877–885 (2004).

The Mirasol PRT system for pathogen reduction of platelets and plssma: an overview of current status and future trends. Goodrich RP, Edrich RA, Li J, Seghatchian J. *Transfus. Apheresis. Sci*. 35, 5–17 (2006).

Bacteria species inactivated by Mirasol® Pathogen Reduction Technology System for platelets.	*Extent of inactivation (#) log_{10} reduction*
Gram-negative bacteria	
Escherichia coli	≥4.4
Serratia marcescens	4.0
Pseudomonas aeruginosa	4.7
Gram-positive bacteria	
Staphylococcus epidermidis	≥4.2
Staphylococcus aureus	3.6
Staphylococcus aureus	4.8
Bacillus cereus (includes spores)	1.9
Bacillus cereus (vegetative)	2.7
Streptococcus mitis	3.7

MIRASOL vs colture

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.

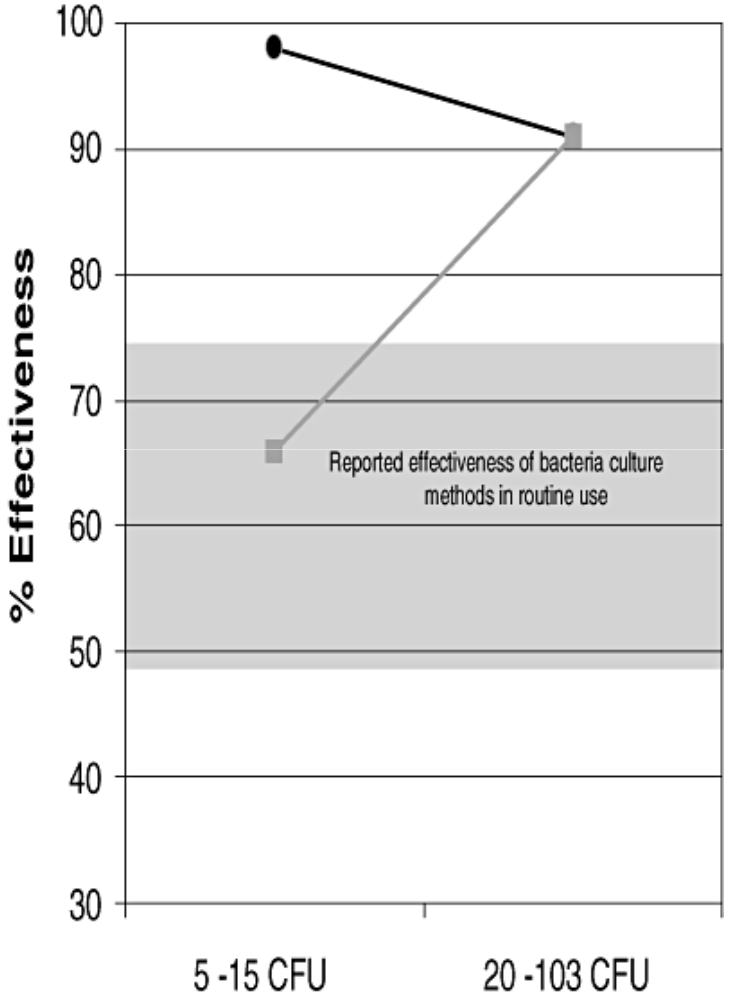
***Transfusion* 49, 1205–1216 (2009).**

L'efficacia del sistema Mirasol nell'inattivare basse cariche batteriche è stata messa a confronto con l'identificazione batterica mediante colture con aerobi ed anaerobi tenute in quarantena per 48 h.

Il sistema di inattivazione dei patogeni Mirasol è in grado di inattivare i batteri contaminanti PLT .

In particolare viene dimostrata **l'efficacia della Riboflavina – UV per l'inattivazione di prodotti contaminati con < 20 CFU/unità.**

In queste condizioni l'efficacia di Mirasol è pari al **98%** rispetto al 60% - 66% dei sistemi di coltura.



Efficacia di PRT (●) nei confronti di sistemi di coltura dei batteri (■) in base a 2 livelli di contaminazione

MIRASOL: clinical trial

"A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology"

This randomized controlled clinical trial **assessed the efficacy and safety of PRT-PLTs using the 1-hour corrected count increment (CCI1hour) as the primary outcome.**

CONCLUSION:
The study failed to show non inferiority of PRT-PLTs based on predefined CCI criteria.

PLT and RBC utilization: not significantly different ➔slightly lower CCIs (PRT-PLTs) did not increase blood product utilization.

Safety data showed similar findings in the two groups.

Cazenave JP, Folléa G, Bardiaux L, Boiron JM, Lafeuillade B, Debost M, Lioure B, Harousseau JL, Tabrizi R, Cahn JY, Michallet M, Ambruso,D, Schots R, Tissot JD, Sensebé L, Kondo T, Mc-Cullough J, Rebulla P, Escolar G, Mintz P, Heddle,NM, Goodrich RP, Bruhwyler J, Le C, Cook RJ, Stouch B; for members of the ***The Mirasol Clinical Evaluation Study Group***: - **Transfusion 2010;50:2362–2375.**

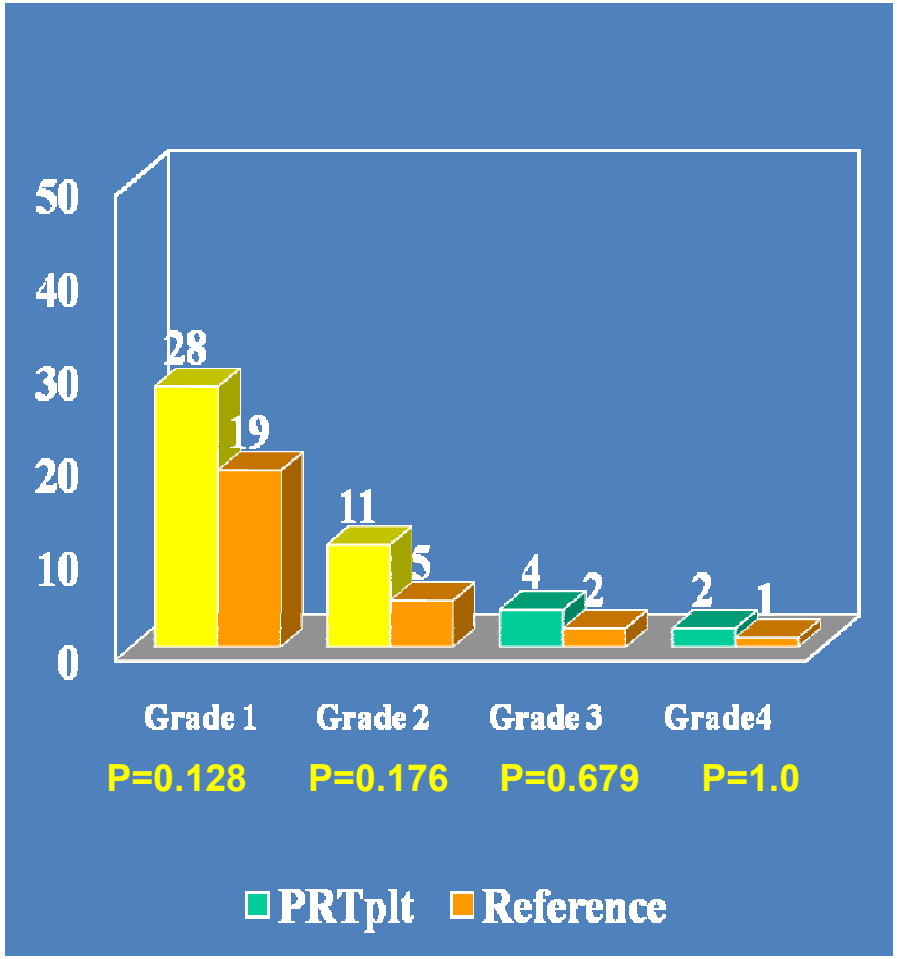
MIRASOL: clinical evaluation – Trial results

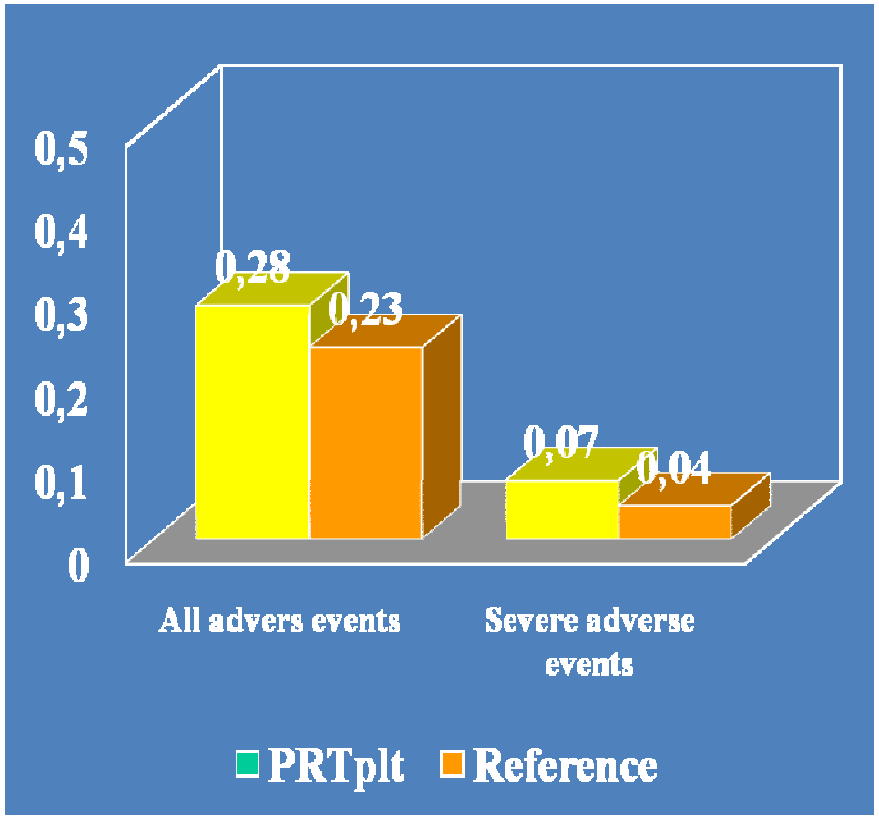
	PCT	CONTROL	p	PCT	CONTROL	p
	First eight transfusions within the 28 day			within the 28 day		
Platelet transfusion	258	209		303	238	
N°transf/patients.	4.0	3.0	0.09	4.5	3.0	0.001
Platelet dose $x10^{11}$	5.37	5.38	0.96	5.23	5.22	0.98
Storage day	2.8	2.6	0.08	2.7	2.6	0.22
1h C.C. Increment 10^9/L	11.7	16.9	0.0001	11.0	16.6	0.001
24h C.C. Increment 10^9/L	6.6	9.8	0.0014	7.1	10.07	0.001
Transfusion interval	2.32	2.72	0.0107	2.16	2.3	0.290

Transfusion 2010; 50:2362–2373

MIRASOL: clinical evaluation – Trial results

Bleeding by WHO Grade and adverse events





P=0.144 P=0.171

CONCLUSIONI

*Studi europei hanno dimostrato che l'**inattivazione dei patogeni**:*

- *Riduce le reazioni avverse*
- *Rappresenta un'alternativa all'irradiazione delle PLT (intercept)*
- *Rappresenta una valida "alternativa" allo screening per la contaminazione batterica*

*Dati relativi all'**emovigilanza** in Europa su un elevato numero di pazienti, hanno dimostrato un'**ottima tollerabilità** dei concentrati piastrinici inattivati, con un'evidente di **riduzione delle reazioni trasfusionali acute** (Intercept).*

CONCLUSIONI - 2

Alcuni studi hanno dimostrato una diminuzione degli ***incrementi post-trasfusionali*** *(CI/CCI - 1h/24h)…ma i dati sono contrastanti.*

È stato evidenziato un significativo ***effetto-dose*** *…*

Non sono state confermate differenze in termini di "complicanze emorragiche" (complicanze emorragiche grado 2-3: n.s.) nei pazienti trasfusi con PLT inattivate.

INTERVALLO TRASFUSIONALE*: non sono state dimostrate variazioni significative rispetto al gruppo di controllo*

CONCLUSIONI - 3

L’inattivazione dei patogeni può sicuramente contribuire a:

*Eliminare il **rilascio** di PC contaminate*

*Evitare il **recall** di PC*

Sostituire i test per la ricerca della contaminazione batterica

Trasfondere piastrine entro 24 dalla raccolta e con un minor storage

Aumentare la disponibilità di emocomponenti (scompare l’ostacolo dei falsi positivi)

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2011) 100, 169–178
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DOI: 10.1111/j.1423-0410.2010.01374.x

A comparison of methods of pathogen inactivation of FFP

G. Rock
University of Ottawa, Ottawa, Ontario, Canada

Current methods for pathogen inactivation of plasma involve four major processes using **solvent–detergent (SD)**, **methylene blue (MB)**, **amotosalen** and **riboflavin** as additives.

MB, Amotosalen and Riboflavin, are designed for use in a blood bank.

SD method is generally applied at a centralized manufacturing centre and involves large plasma pools.

INATTIVAZIONE PATOGENI

- PLASMA
- PIASTRINE
- *(RBC)*

Table 1 A comparison of four methods of pathogen inactivation

	Solvent and detergent	MB and white light	Amotosalen and UVA light	Riboflavin and UV light
Product Source	Made from apheresis plasma frozen within 4 h of collection	Whole blood-derived plasma as single unit and apheresis plasma (apheresis must be split if > 315 ml)	Apheresis jumbo collections up to 635 mL treatable as single unit; whole blood derived, at least two plasma units have to be pooled together	Whole blood-derived plasma as single unit and apheresis plasma (apheresis plasma must be split if > 360 ml)
Shelf life	4 years at ≤ −18°C	2 years at −30°	2 years below −25°C. I year between −18 and −25°C.	2 years at −30°C
Illumination time	N/A	15 min new Macotronic B2 20 min with older equipment	3–6 min for 2 bags	5–8 min for 1 bag
Primary target	Lipid membranes	Nucleic acids	Nucleic acids	Nucleic acids
Compound Toxicity	Low	Low	High	None
Removal of active compounds	Yes, oil extraction and hydrophobic Chromatography	Yes, by special filter (not universal)	Yes, by compound absorbing device	Not required
Residual level of active compounds.	Undetectable or trace amounts far below toxicity levels	Very low levels of MB and photoproducts. Mutagenic effects improbable, but not excluded	Very low levels of free S-59 and photoproducts. Mutagenic effects improbable, but not excluded.	Low levels of riboflavin and photoproducts are normally present in blood Active compounds not removed
Permanent binding to lipids or proteins in plasma	No binding to proteins or residual lipids	Proteins intercalated with phenothiazine-like dyes	Majority of amotosalen bound to lipids and 2% to proteins[74]	Does not appear to bind

MB, methylene blue; UVA, ultraviolet A.

Effectiveness of pathogen inactivation

VoxSanguinis
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A comparison of methods of pathogen inactivation of FFP
G. Rock
University of Ottawa, Ottawa, Ontario, Canada

Viruses (models for)	SD plasma	MB plasma (Springe)	MB plasma (Theraflex)	Amotosalen	Riboflavin
Human hepatitis A	0 [21,22]	0 [21]	N/A	0 [23]	1·6[a]
Human immunodeficiency virus, active or extra-cellular	> 5 [50]	≥ 6·3 [46]	≥ 4·0 [75] 4·9 [48]	> 6·2 [74]	5·9 [49]
Human immunodeficiency virus, latent or cellular	N/A	≥ 0 [21] ≥ 3·8 [46]	N/A	6·4 [5]	4·5 [49]
Influenza A	N/A	5·1 [46]	N/A	≥ 3·4[b]	≥ 5·0[a]
Porcine parvovirus	0 [22,52][c]	0 [21,46]	N/A	0 [23]	≥ 5·0 [49]
Vesicular stomatitis virus	> 8 [50]	≥ 4·9 [46,76]	N/A	N/A	≥ 6·3 [77]
West nile virus (HCV)	≥ 6·0 [51]	N/A	5·75 [48]	≥ 6·8 [5]	≥ 5·2 [49]
Chikungunya virus	N/A	N/A	N/A	≥ 7·6[b]	2·1 [58]
Encephalomyocarditis	013	0 [46]	N/A	N/A	3·2[a]
Sindbis virus (HCV)	> 6·0 [50]	≥ 9·73 [46]	N/A	N/A	3·2[a]
Bovine viral diarrhoea Virus (HCV)	N/A	N/A	≥ 5·4 [75]	≥ 5·4[b]	N/A
Pseudorabies virus (HBV)	> 7·0 [50]	N/A	≥ 5·5 [75]	N/A	2·5[a]
Duck hepatitis B virus (HBV)	N/A	3·9 [21]	N/A	4·4–4·5 [5]	N/A
Parasites	SD plasma	MB Plasma (Springe)	MB plasma (Theraflex)	Amotosalen	Riboflavin
Trypanosoma cruzi (Chagas'disease)	N/A	N/A	> 3·4 [66][d]	> 5·0 [5]	≥ 6·0 [65]
Babesia microti	N/A	N/A	N/A	≥ 5·3 [5]	≥ 5·0 [62]
Plasmodium falciparum (malaria)	N/A	N/A	N/A	≥ 6·9 [5]	≥ 3·4 [63]
Orientia tsutsugamushi	N/A	N/A	N/A	N/A	≥ 5·0 [67]
Leishmania donovani infantum	N/A	N/A	N/A	N/A	≥ 5·0 [68]

N/A, data not available; HCV, hepatitis C; HBV, hepatitis B; MB, methylene blue; SD, solvent–detergent.
a Mirasol product literature - b Intercept website.
c References do not state directly that PPV is not inactivated by SDP but emphasize that SD plasma does not inactivate non-enveloped viruses, such as human hepatitis A virus, parvovirus B-19, reovirus and adenovirus.
d MB action primarily because of filtration of the product.

All of them are widely effective against lipid-enveloped viruses, including HIV and hepatitis C.

- All of the methods are effective against lipid-enveloped pathogens
- SD and MB treatments are not effective against the non-enveloped viruses such as hepatitis A virus (HAV) and parvovirus, retrovirus and adenovirus
- Amotosalen and Riboflavin have been found to be active against many, non-lipid-enveloped viruses.
- Amotosalen and Riboflavin are effective against parvovirus B19 with
- Amotosalen and Riboflavin techniques also have a broad range of activity against bacteria, parasites and leucocytes
- Amotosalen and Riboflavin are effective against *Dengue virus, Chikungunya virus*, *Babesia microti* and *Plasmodium falciparum*.

The effect of pathogen inactivation on the plasma proteins


VoxSanguinis

ORIGINAL PAPER

A comparison of methods of pathogen inactivation of FFP

G. Rock

Proteins	SD Post/ pretreatment	Retention, %	MB Post/ pretreatment	Retention, %	Amotosalen Post/ pretreatment	Retention, %	Riboflavin Post/ pretreatment	Retention, %
Fibrinogen (mg/dl)	232/277 [8,15]	84	180/277 [8]	65	209/290 [5]	72	267/345 [7]	77
F II (IU/ml)	0·92/.1·13 [8]	81	1·05 /.1·13 [8]	93	0·85/0·96 [5]	88	0·9/1·1 [7]	82
F V (IU/ml)	0·60/0·95 [8]	63	0·73/0·95 [8]	77	1·19/1·30 [5]	92	0·8/1·1 [7]	73
F VII (IU/ml)	0·78/0·99 [8]	79	0·9/1·0 [8]	90	0·95/1·23 [5]	77	1·05/1·24 [24]	85
F VIII (IU/ml)	0·65/0·87 [8]	78	0·6/.9 [8]	67	1·15/1·57 [5]	73	1·0/1·3 [7]	77
F IX (IU/ml)	0·95/1·00 [11]	95	1·8/2·37 [36]	77	0·88/1·08 [5]	82	1·0/1·4 [7]	71
F X (IU/ml)	0·80/0·90 [22]	89	2·3/2·5 [36]	92	0·86/1·0 [5]	86	0·9/1·1 [7]	82
F XI (IU/ml)	1·0/.1·05 [22]	95	1·9/2·6 [36]	73	0·87/1·0 [5]	86	0·8/1·2 [7]	67
F XII (IU/ml)	0·85/1·0 [22]	85	1·02/1·1 [21]	93			1·3/1·3 [7]	100
F XIII (IU/ml)	1·1/1·15 [22]	96	2·6/2·8 [36]	93	1·02/1·1 [5]	93	1·0/0·9 [39]	113
Protein C (IU/ml)	1·05/.1·08 [8]	97	1·08/1·03 [8]	95	1·02/1·09 [5]	94	1·1/1·4 [7]	79
Protein S (IU/ml)	0·62/1·10,[8,12]	56	1·1/1·1·0 [8]	100	1·07/1·09 [5]	98	1·0/1·1 [7]	91
AT III (IU/ml)	0·89/0·93 [8]	96	0·95/0·93 [8]	102	0·91/0·94 [5]	97	1·0/1·0 [7]	100
α_2 AP (IU/ml)	0·21/1·0 [26]	21	0·96/1·0 [32]	96	0·75/0·93 [5]	80	0·9/1·0 [7]	90
vWF Ag IU/ml)	97/107 [12]	91	2·4/2·6 [34]	92	1·22/1·25 [36]	98	1·1/1·1 [39]	99
vWF:RCo IU/ml)	0·76/0·81 [35]	76	2·4/2·6 [34]	92	0·73/0·73 [36]	100	0·8/0·7 [7]	114
vWF multimers	Reduced[37]		Normal[16]		Normal[38]		Some loss[39] HMW	
Total protein (g/l)	55/57 [28]	96	56/57 [31]	98	NA	NA	58/58 [7]	100
SDS–PAGE results	N/A		Fibrinogen changes,[9,10,33]		NA	NA	No changes[7]	
Plasminogen IU/ml)	0·99/.99 [8]	100	0·98/0·99 [8]	99	1·02/1·09 [36]	94	0·75/0·80 [25]	94
ADAMTS-13 (%)	No changes[37,40]	100	No changes[36,37]	100	1·19/1·22 [36]	96	1·03/1·07 [39]	96

All technologies employed to date, show some level of **loss or degradation of protein factors** and a **decrease in plasma component quality following treatment**. …data from the literature show **decreases in Factor VIII** and **fibrinogen** following all forms of pathogen reduction treatment; however, for other parameters, the changes seem to be somewhat specific to the method.

Certainly, in the apheresis environment, the availability of pathogen-inactivated plasma would be most welcome because a small number of patients, i.e. those with thrombotic thrombocytopenic purpura who represent a large percentage of apheresis procedures, receive a considerable amount of untreated plasma with the cumulative risks of donor exposure. This is in contrast to the situation for other apheresis patients who receive albumin which has been treated to inactivate pathogens. Perhaps it is time for us to move forward.

Emerging Agents Present Transfusion Risk Today

INATTIVAZIONE PATOGENI

- PLASMA
- PIASTRINE
- *(**RBC**)*

Modeling the risk of an emerging pathogen entering the Canadian blood supply

Transfusion 2010;50:2592

Steven Kleinman, Claire Cameron, Brian Custer, Michael Busch, Louis Katz, Boris Kralj, Ian Matheson, Ken Murphy, Jutta Preiksaitis, and Dana Devine

BACKGROUND: As part of its risk management process, Canadian Blood Services (CBS) constructed mathematical models of how newly emerging pathogens might affect blood transfusion recipients.
STUDY DESIGN AND METHODS: CBS convened an expert panel including medical, health economics, analytical, risk management, and insurance professionals to examine multiple data sources. The model for emerging pathogen risk included separate modules to calculate the frequency and severity of infections from transfusion-transmitted agents that could cause either acute transient or chronic persistent infection. Important model input variables were annual number of components transfused, the presumed incidence and prevalence of a new agent, the time interval of recipient risk, recipient age and sex, projected recipient survival, rate of secondary infection, pathogen-induced morbidity, and the associated medical costs of such morbidity.
RESULTS: In the 5-year time frame considered in the model, it was estimated that approximately 3500 recipi-

In the past several years, blood services in developed countries have focused considerable attention on the possible introduction of emerging pathogens into the blood supply. Indeed, the past decade has demonstrated that pathogens will continue to emerge and featured the recognition of several such transfusion-transmitted infections: West Nile virus (WNV) in North America, variant Creutzfeldt-Jakob disease (CJD) in the United Kingdom, and an upsurge of babesiosis in parts of the United States.[1-3] In addition, other agents have also resulted in at least temporary, if not recurring, concern in specific geographic locations: these include dengue virus in Australia (and elsewhere), chikungunya virus in La Reunion; and SARS, avian flu, and influenza H1N1 internationally.[4-9]

In addition to these recent occurrences, blood services in some countries bear the legacy of previous emerging pathogens, specifically human immunodeficiency virus (HIV) and hepatitis C virus (HCV), which resulted in

Estimated number of primary infections transmitted by component type from an acute and chronic emerging pathogen.

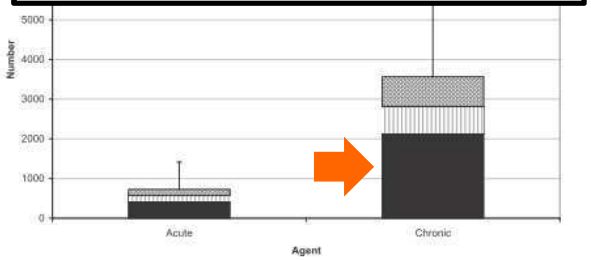


Fig. 1. Estimated number of primary infections transmitted by component type from an acute and chronic emerging pathogen. The error bars represent one SD from the mean expected value for the model. For acute infections, the expected value was 727 with a SD of 688 (two-SD range of 0 to 2103 infections), and for a chronic infection, the expected value was 3564 with a SD of 3903 (two-SD range of 0 to 11,370 infections). (▨) Infections from plasma; (□) infections from PLTs; (■) infections from RBCs.

Estimated number of primary infections transmitted by component type from an acute and chronic emerging pathogen depending on the availability of pathogen reduction procedures for specific blood components.

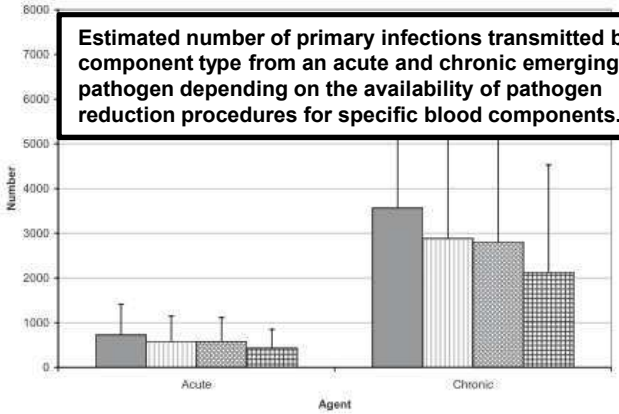


Fig. 3. Estimated number of primary infections transmitted by component type from an acute and chronic emerging pathogen depending on the availability of pathogen reduction procedures for specific blood components. The error bars represent one SD from the mean for the model. (□) No reduction; (▨) PLTs reduction; (▤) plasma reduction; (▩) PLTs and plasma reduction.

Transfusion safety and transfusion blood component exposure

INATTIVAZIONE PATOGENI→RBCs

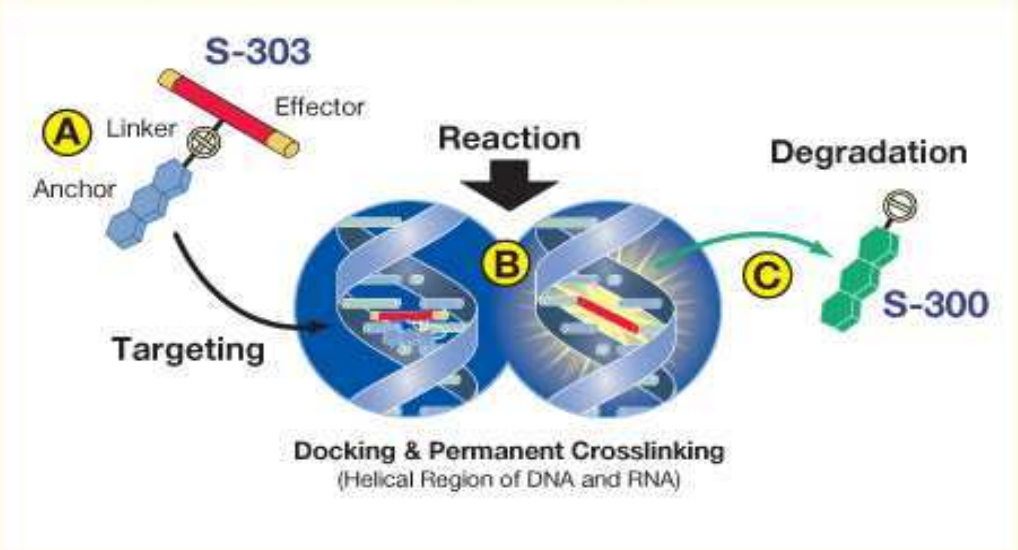
	Transfused blood components	Duration of exposure
Elective surgery	Few	Brief
Significant trauma or organ transplantation	Moderate	Brief
Cancer chemotherapy or stem cell transplantation	Moderate	Months to years
Thalassemia major	Hundreds or thousands	Lifetime

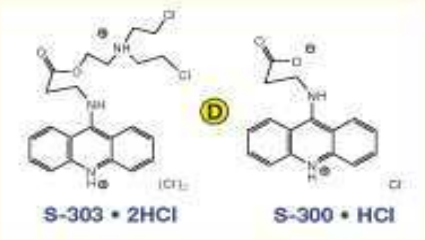
Development Background
S-303 Treated PI Red Blood Cells

INTERCEPT RBC: Mechanism of Action

- S-303 is a nucleic acid-targeted alkylator that quickly diffuses into viruses, bacteria, parasites and blood cells and is designed to react quickly and decompose
- Glutathione (GSH) is used to quench side reactions of the effector with other biological materials

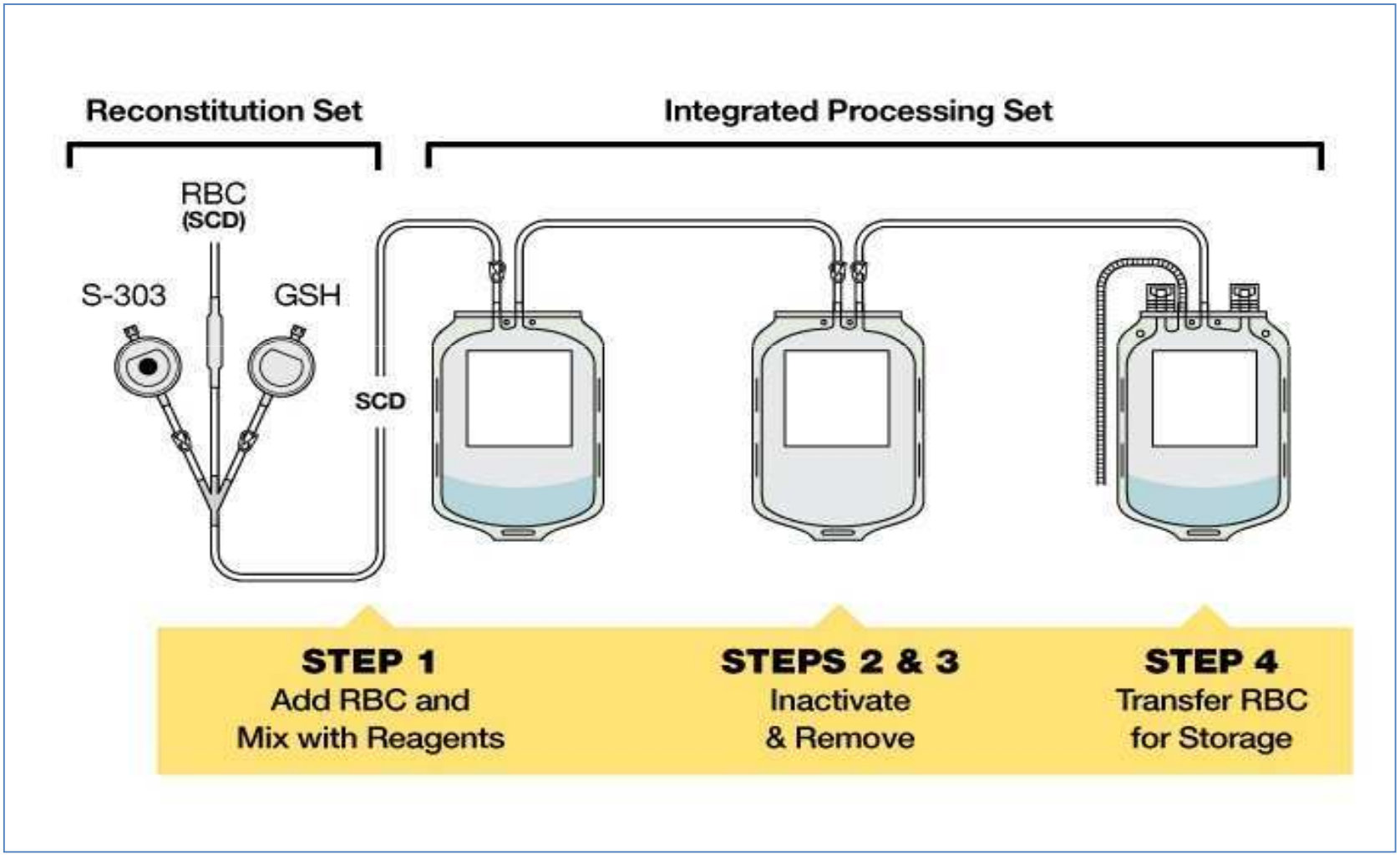
Figure 1: S-303 Treatment Process Mechanism of Action





- Anchor selectively targets nucleic acids
- Effector crosslinks nucleic acids
- Linker temporarily joins anchor and effector
- Cross-linking reaction is faster than linker degradation
- Degradation yields unreactive by-products

Second Generation INTERCEPT RBC Clinical Process



Clinical Studies

- Europe: Acute and chronic anemia separately
  - Chronic anemia – thalassemia major
  - Acute anemia – cardiac surgery

Study in Chronic Anemia

Provide clinical data for efficacy and safety in the setting of chronic transfusion

Chronic Anemia Study Design

- **P**opulation: Transfusion dependent **thalassemia major** patients
- **I**ntervention: **Transfusion of S-303 treated RBC**, stored in SAG-M, administered according to local standard clinical practice
- **Comparator**: **Conventional RBC stored in SAG-M** administered according to local standard clinical practice
- **O**utcome: **S-303 RBCs are non-inferior to conventional RBCs** with respect to mean **Hgb transfused per kg body weight** per day of support
- **T**imeframe: RBC support up to **4** transfusion cycles per product (approximately **12** months on study)

Primary and Secondary Endpoints based on RBC units (device)

- Primary Endpoint
  - Hemoglobin content per RBC unit

- Secondary Endpoints
  - Proportion of RBC units that meet local guidelines as an acceptable products (Hb/unit, Hematocrit, post storage hemolysis)
  - Cell free hemoglobin post storage (used to calculate hemolysis)
  - ATP levels post storage

- Clinical endpoints are exploratory:
  - Organ failure due to failure of tissue oxygenation
  - Functional capacity (Six Minute Walk Test – 6MWT)

grazie