PATHOGEN INACTIVATION OF BLOOD COMPONENTS

DR ANUPAM CHHABRA
KOLKATA, NOVEMBER’04, 2016
Blood safety

**Historical measures to enhance blood safety**

- Donor screening/deferral
- Serology
- NAT
- Leukoreduction
- Gamma irradiation
- Bacterial detection

*Is it safe enough?*
Blood safety
Residual risks

**BACTERIA**
Platelet products are stored up to 5 to 7 days at room temperature.
This environment permits the growth of bacteria which could lead to sepsis in the patient.

**TESTED PATHOGENS**
Highly prevalent pathogens such as HIV, HBV and HCV are routinely tested in most countries.
A residual risk exists from the window period.

**EMERGING/UNTESTED PATHOGENS**
Emergence of new pathogens in untouched geographies.
Known pathogens that are not routinely tested or pose a threat to the blood supply.

**WHITE BLOOD CELLS**
Despite the many approaches to eliminate white blood cells, residual amounts can still cause (serious) adverse reactions in patients.
Foundation of blood safety
*Being selective versus comprehensive*

Donor screening/deferral
- Serology

Nucleic Acid Testing (NAT)
- Leukoreduction
- Gamma irradiation
- Bacterial detection

Pathogen reduction

Selective measures

Comprehensive measure
### Risk from bacterial contamination

**Worldwide experience**

- Bacterial contamination of platelet products is one of the largest threats to blood safety and estimated to be around 1:3,000\(^{1-3}\)

<table>
<thead>
<tr>
<th>Country</th>
<th>Sepsis</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UK</strong></td>
<td>1:105,000</td>
<td>1:390,000</td>
</tr>
<tr>
<td>SHOT 1996 to 2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>The Netherlands</strong></td>
<td>1:56,500</td>
<td>1:113,000</td>
</tr>
<tr>
<td>De Korte, 2006</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>USA</strong></td>
<td>1:59,071</td>
<td>1:498,711</td>
</tr>
<tr>
<td>Eder et al., 2007</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Yomtovian, et al., 1993; (2) Hillver, et al., 2003; (3) Yomtovian, et al., 2006
Risk from bacterial contamination
Experience at the Welsh Blood Service, United Kingdom

- Estimated bacterial titer up to 24 h post-collection was between 5 and 62 cfu/unit
- Eight years following the implementation of bacterial detection, the incidence of bacterial contamination was reported to be 1:1,567 (from 2003 to 2010)
- Staphylococcus sp. were the most common cause of contaminations (55%)

(1) Pearce, et al., 2011
Infection Risk in Platelets: bacterial vs viral

Risk per unit:
- HCV
- HBV
- HIV

According to Mike Busch, modified
The effectiveness of bacterial detection is reported to be between 50% and 70%.
Growth of bacteria in platelets based on one viable organism in a 400 ml unit (0.0025 organisms/ml)

Generation time of most bacteria in platelets at 22°C is 1-4 h

Detection limit using 8 ml culture volume at 24 h

Reduction of transfusion-transmitted infectious diseases

The Mirasol System further protect for the window period

- Further reduces the window period of serological and nucleic acid testing (NAT) for the detection of viruses such as HIV, HBV and HCV

- ~2 to 6 log reduction (99% to 99.9999%) demonstrated by infectivity assay (TCID_{50})

![Log Reduction Chart]

- HIV latent: 4.5
- HIV active: 5.9
- HBV model: 2.5
- HCV: ≥4.1

Viral Load (copies/mL)

- 10^3
- 10^6

Number of days post-infection

- 0
- 5
- 15
- 19

ID NAT, p24-Ag, HIV - Ag, Anti-HIV, No detection by ID-NAT

HIV-1

(1) Ruane, et al. 2004; (2) Adapted from Kleinman, et al., 2009
Emerging/re-emerging infectious diseases: 1995-2005

- Vancomycin-resistant Staphylococcus aureus
- Cryptosporidiosis
- Multidrug-resistant tuberculosis
- Drug-resistant malaria
- SARS
- E. coli O157:H7
- H5N1 influenza
- Vancomycin-resistant Staphylococcus aureus
- Nipah virus
- Hendra virus
- Enterovirus 71
- Rift Valley fever
- HIV
- Anthrax bioterrorism
- Human monkeypox
- Whitewater arroyo virus
- Hantavirus pulmonary syndrome
- Dengue
- Yellow fever
- Cholera
- Marburg hemorrhagic fever
- Ebola hemorrhagic fever
- Plague
- West Nile virus
- Lyme disease
- Lyssavirus (vCJD)
- Lassa fever

Legend:
- Red: Newly emerging
- Blue: Re-emerging and/or resurging
- Black: Deliberately emerging
Recent Worldwide outbreaks

27 July 2015 at 4:20pm

Suspected MERS outbreak closes Manchester Royal Infirmary A&E

Manchester Royal Infirmary. Credit: Manchester Royal Infirmary.
Risk from residual white blood cells

Response from Donor WBC to Recipient Cells and Tissues

Donor WBCs → Release of donor cytokines → FNHTR → TA-GVHD

Response from Recipient WBC to Donor Cells (alloimmunization)

Donor WBC → Recipient WBCs → Recipient anti-donor HLA antibodies → Donor platelet

Donor antigen
Riboflavin

- Riboflavin binds and breaks nucleic acids upon light activation
  - Kuratomi and Kobayashi, 1977
  - Speck et al., 1975
  - Korycka-Dahl et al., 1980
  - J. G. Peak et al. 1983

- Riboflavin + Light Kills Virus
  - A. Tsugita et al., 1965
  - J. S. Bellin and Oster, 1960
  - M. I. Simon and H. van Vunakis, 1962

- Riboflavin (vitamin B2) is one of the essential nutrients in humans diet
  - Recommended daily allowance 1.7 mg
  - Toxicology, Mutagenicity (GRAS, USA FDA)
No long term safety concern of riboflavin photochemistry.

Riboflavin and its photoproducts are naturally present in blood; no new compounds are formed\(^1\)

Following Mirasol treatment, Riboflavin and the photoproducts formed are non-toxic\(^2\) and non-mutagenic\(^2,3\)

No safety concern detected in any of the Mirasol system clinical trials

Extensive toxicology testing has shown no safety concerns\(^4\)

The FDA classified riboflavin as a GRAS (Generally Recognized as Safe) chemical

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The Mirasol system

Overview

- Reduction of viruses, bacteria, parasites
- Inactivation of residual leukocytes

Blood product + Riboflavin (vitamin B2) solution + UV light
The Mirasol system inactivates disease-causing agents by altering their nucleic acids in two primary ways:¹,²

1. **UV light only: reversible inactivation**
   - UV light alone breaks chemical bonds in the nucleic acids of pathogens

2. **UV light + riboflavin: irreversible inactivation**
   - Riboflavin molecules form complexes with nucleic acids
   - UV light from the Mirasol Illuminator activates the riboflavin molecule in the complex
   - Photoactivated riboflavin induces a chemical alteration to nucleic acids, making pathogens unable to replicate

¹ Kumar, et al., 2004; ² Marschner, et al., 2011
The Mirasol system

Mode of action$^{1,2}$

Electron transfer from riboflavin to nucleic acid

DNA is unable to replicate

$\rightarrow$ Reduce pathogen load

$\rightarrow$ Inactivate residual WBCs

(1) Kumar, et al., 2004; (2) Marschner, et al., 2011
The Mirasol system is SIMPLE

Platelet unit treated and stored in PAS or plasma

- Immediate release: transfuse or store for up to five or seven days
- Platelet unit from component lab
- Sterile connection
- Riboflavin bag
- Sterile connection
- Mirasol disposable set
- Illumination and storage bag
The Mirasol system is SAFE
Phototherapy supplemented with riboflavin has no long-term safety concern

Long-term peace of mind for phototherapy supplemented with riboflavin

- For the treatment of hyperbilirubinemia (jaundice), neonates receive UV phototherapy supplemented with riboflavin

- A Danish retrospective study analyzed the incidence of leukemia in 55,120 infants up to 14 years of age that received riboflavin phototherapy for the treatment of jaundice

- The study revealed no increase in childhood leukemia or other childhood cancers

(1) Olsen, et al. 1996
Riboflavin and its photoproducts are naturally present in human blood; no new compounds are formed after Mirasol treatment.  

(1) Hardwick, et al., 2004

Photoproduct comparison
The Mirasol system is SAFE

Toxicology studies confirm the safety profile of riboflavin

There is a strong in vivo history\(^1\) and Terumo BCT in vitro and in vivo testing supporting the safety of riboflavin and its photoproducts\(^2\)

No serious adverse events were attributed to use of Mirasol-treated platelets in a well-controlled clinical trial\(^3,4\)

in vitro\(^\dagger\) and in vivo\(^*\) toxicology\(^2\)

- Acute toxicity\(^*\) Negative
- Neoantigenicity\(^*\) Negative
- Ames mutagenicity\(^\dagger\) Negative
- CHO clastogenticity\(^\dagger\) Negative
- Cytotoxicity\(^\dagger\) Negative
- Reproductive toxicity\(^*\) Negative
- Subchronic toxicity\(^*\) Negative
- MMN genotoxicity\(^*\) Negative
- Blood compatibility\(^\dagger\) Passed
- Leachables and extractables\(^\dagger\) Passed


LD50
The Mirasol system is SAFE
*Toxicology profile LD50*

LD50 (Lethal Dose 50) is a measure of acute toxicity and represents the required dose resulting in death of half the members of a tested population (after a specified test duration).
The Mirasol system is EFFECTIVE
An alternative to bacterial detection

- The effectiveness of bacterial detection is reported to be between 50% and 70%\(^1,2,3\)
- The Mirasol system is **98%** effective in inactivating bacteria\(^4\)

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(1) Dumont, et al. 2008, (2) Benjamin, et al., 2007; (3) Foley, et al., 2007; (4) Goodrich et al., 2009
Risk from tested pathogens

The Mirasol system performance

- Further reduces the window period of serological and nucleic acid testing (NAT) for the detection of viruses such as HIV, HBV and HCV\(^1\)

- ~2 to 6 log reduction (99% to 99.9999%) demonstrated by infectivity assay (TCID\(_{50}\))

---

Viral Load (copies/mL)

- 10\(^3\)
- 10\(^6\)

Number of days post-infection\(^2\)

- 0
- 5
- 15
- 19

HIV-1

- No detection by ID-NAT

ID NAT

p24-Ag

HIV - Ag

Anti-HIV

RNA

(1) Ruane, et al.2004; (2) Adapted from Kleinman, et al., 2009

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Log Reduction

- HIV latent: 4.5
- HIV active: 5.9
- HBV model: 2.5
- HCV: ≥4.1

>LOD
The Mirasol system is EFFECTIVE
>70% of the investigated emerging/untested pathogens are reduced at LOD

- Infectious pathogen load shown to be reduced by 99.9% for the majority of pathogens investigated
- Eleven out of the fifteen (>70%) inactivated at the limit of detection (LOD)
- Reduces the infectious pathogen load of several clinically relevant non-enveloped viruses such as Hepatitis A (HAV) and Hepatitis E (HEV), a category of viruses shown to be resistant to other pathogen reduction technologies
- The Mirasol system has been shown to prevent the transmission of 6 log of CMV following transfusion in a mouse disease transmission model
- The Mirasol process, in combination with leukoreduction, can be used as an alternative to CMV testing for prevention of CMV transmission through blood

(1) Hauser, et al., 2014, (2) Roback, et al., 2010

Development effort for the reduction of Malaria transmission via whole blood transfusions
The Mirasol system is EFFECTIVE
Prevents cytokine expression and may reduce FNHTRs

(1) Fast, et al., 2011; *Peripheral blood mononuclear cells
### Effective Inactivation of (WBCs)

*Reducing Immunological Complications of Transfusion*

<table>
<thead>
<tr>
<th>White Blood Cell Inactivation Study</th>
<th>Study Type</th>
<th>Performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC inactivation</td>
<td>In vitro</td>
<td>≥ 6 log (99.9999%)</td>
<td>Fast, et al., 2011, 2013</td>
</tr>
<tr>
<td>Cytokine production and expression</td>
<td>In vitro</td>
<td>prevented</td>
<td>Fast, et al., 2006a; Fast, et al., 2011</td>
</tr>
<tr>
<td>Graft-versus-Host Disease (GVHD)</td>
<td>In vivo animal study</td>
<td>prevented</td>
<td>Fast, et al., 2006b</td>
</tr>
<tr>
<td>Alloimmunization and transplant rejection</td>
<td>In vivo animal study</td>
<td>prevented</td>
<td>Asano, et al., 2007</td>
</tr>
</tbody>
</table>

- Mirasol system is approved as an **alternative to gamma irradiation** for the prevention of GVHD
- Mirasol system offers **significant additional safety benefits** not offered by gamma irradiation or leukoreduction (i.e. added protection against infectious agents)
The Mirasol system is SIMPLE

Processes and disposables

1 platform

4 processes

5 commercially available disposables

- Platelets in plasma
- Hyperconcentrated platelets (Apheresis PPC)
- Platelet in PAS
- Plasma (FFP)
- Whole Blood

- Single-bag kit for platelets in plasma
- Double-bag kit for platelets in plasma (allows PAS addition post-treatment)
- Single-bag kit for platelets in PAS
- Double-bag kit for platelets in PAS
- Plasma (FFP) kit

September 2015
Transfer product to illumination bag
Transfer product to illumination bag
Add riboflavin solution
Add riboflavin solution
Illuminate 4 to 10 minutes
Illuminate 4 to 10 minutes

**Product Requirements**

<table>
<thead>
<tr>
<th>Source, Apheresis Collection</th>
<th>Apheresis platelets</th>
<th>Whole blood derived platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>170 mL to 360 mL</td>
<td></td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>0.8 to 2.1 x 10^6/µl</td>
<td></td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td>Up to 7.5 x 10^11</td>
<td></td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>0.7 to 2.1 x 10^6 platelets/µL</td>
<td>Max. 5.1 x 10^11/bag,</td>
</tr>
</tbody>
</table>

If platelets per bag >5.1 x 10^11, treated product must be divided within 24 h of treatment;

**Process Requirements**

- Apheresis platelets: treat within 2 h to 22 h of collection
- Whole blood-derived platelets: treat within 8 h of pooling, but no more than 32 h after collection

#DOUBLE+ kit with second storage bag available

*As determined by the upper limits for platelet concentration and volume; different limits may apply in a given blood center*
Platelet concentrates treated with the Mirasol system demonstrated minimal platelet loss due to treatment and acceptable cell quality through 7 days of storage.

Fig. 3. Platelet count as a function of storage time; error bars indicate 1 standard deviation. (■) Control platelets, (□) Mirasol-treated platelets.

\(^1\)Castrillo A et al, Transf Med Hemother 2013, 40: 44-48
The Mirasol system is SIMPLE
*Specifications for the treatment of plasma*

**Incoming Product Specifications**

<table>
<thead>
<tr>
<th>Source</th>
<th>Apheresis Whole blood-derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>170 mL to 360 mL</td>
</tr>
</tbody>
</table>

**Processing Windows**

<table>
<thead>
<tr>
<th>Plasma separation from whole blood</th>
<th>≤18 h from whole blood collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time until start of freezing</td>
<td>Apheresis: ≤8 h from collection</td>
</tr>
<tr>
<td></td>
<td>WB: ≤6 h from separation</td>
</tr>
</tbody>
</table>

**Storage Specifications**

<table>
<thead>
<tr>
<th>Mirasol-treated FFP</th>
<th>Protein quality demonstrated for up to 2 years at ≤−30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thawed FFP</td>
<td>Treat and refreeze ≤2 h or transfuse ≤6 h</td>
</tr>
</tbody>
</table>
Average age of platelets in days
Study performed at Centro de Sangre e Tejidos del Principado de Asturias

Intercept: 5.8 days, P<.001 – highly significant
Mirasol: 3.9 days

Garcia-Gala JM et al, Vox Sang *2015, 109 (suppl.1), 184
## Benefits of Working with Mirasol PRT

**Dr. T. Jimenez-Marco, 4th Mirasol User’s Meeting, Estoril 2014**

### TABLE 7. Outdated PLT units before and after PI implementation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of outdated PLT units</td>
<td>2,226</td>
<td>397</td>
<td>-17.8</td>
</tr>
<tr>
<td>Outdated PLT units (%)</td>
<td>16.8</td>
<td>2.7</td>
<td>-83.9</td>
</tr>
<tr>
<td>Outdated PLT units cost</td>
<td>€310,860.90</td>
<td>€94,021.50</td>
<td>-69.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Production period</th>
<th>Pre-PRT period</th>
<th>Post-PRT period</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outdated PLT units</td>
<td>2,226</td>
<td>10</td>
<td>-99.55%</td>
</tr>
<tr>
<td>% Outdated PLT units</td>
<td>16.8%</td>
<td>0.18%</td>
<td>-98.93%</td>
</tr>
<tr>
<td>Outdated PLT units cost</td>
<td>€310,860.90</td>
<td>€2,368</td>
<td>-99.24%</td>
</tr>
</tbody>
</table>
Quality of Mirasol-treated blood products

Plasma

Platelets
Quality of Mirasol-treated plasma

Mirasol-treated FFP maintains adequate protein quality

- Mirasol-treated FFP meets the Council of Europe Guidelines for protein content of PRT-treated and untreated FFP\(^1-4\)
  - Data from multiple external validation studies, plus internal data representing varied processing conditions:\(^1-4\)
    - Apheresis and whole blood-derived
    - Different anticoagulants
    - Held for varying amounts of time at 22 \(^\circ\)C prior to freezing
  - Average retention of FVIII and Fibrinogen is \(~75\)%\(^4\)
- Mirasol-treated FFP retains qualitative and functional activity of immunoglobulins\(^5\)

(1) Smith and Rock, 2010; (2) Hornsey, et al., 2009; (3) Larrea, et al., 2009; (4) Miklauz, et al., 2011; (5) Kumar, et al., 2004
## Quality of Mirasol-treated plasma

*Mirasol-treated FFP protein quality meets international guidelines*

### Mirasol-Treated Protein Quality Compared to Council of Europe Guidelines (EDQM)

<table>
<thead>
<tr>
<th>Coagulation factors</th>
<th>Council of Europe Guidelines (17th Edition)</th>
<th>Mirasol-treated Average (± SD)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated plasma</td>
<td>PRT-treated plasma</td>
</tr>
<tr>
<td>Factor VIIIc (IU per 100 mL)</td>
<td>≥70 IU per 100 mL (≥0.70 IU/mL)</td>
<td>≥50 to 70 IU per 100 mL (≥0.50 to 0.70 IU/mL)</td>
</tr>
<tr>
<td>Fibrinogen (% Retention)</td>
<td>None specified</td>
<td>≥60% of the potency of fresh plasma</td>
</tr>
</tbody>
</table>

---

\(^1\) Miklauz, et al., 2011
The Warsaw Institute of Hematology in Poland, followed up seven patients with Thrombotic thrombo-cytopenic purpura (TTP) who received a total of 1,508 Mirasol-treated FFP units in 163 therapeutic plasma exchange (TPE) procedures:

- All patients recovered and their platelet count improved
- No transfusion-related adverse reactions (> grade 1) were observed in any of the patients receiving prophylactic transfusions of Mirasol-treated FFP
- Mirasol-treated FFP was found to be safe and effective when used for TPE in the treatment of TTP

<table>
<thead>
<tr>
<th>Acquired TTP Patient</th>
<th>ADAMTS 13 activity (%)</th>
<th>Anti-ADAMTS 13 (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before TPE</td>
<td>after TPE</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 2</td>
<td>42; 28</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 2</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>&lt; 2</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 2</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>3,9</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>&lt; 1</td>
<td>28, 49, 46</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>71</td>
</tr>
</tbody>
</table>

After TPE the ADAMTS 13 activity increased and antibody titers decreased in all patients.

(1) Letowska, presentation at the Bi-Annual Polish Transfusiology Congress. Poznan, Poland. 26 September, 2013.
Quality of Mirasol-treated blood products

Plasma

Platelets
Quality of Mirasol-treated platelets

**Investigated parameters**

Platelet cell quality parameters measured in various in vitro studies:

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>pH (22 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate production rate</td>
</tr>
<tr>
<td></td>
<td>Glucose consumption rate</td>
</tr>
<tr>
<td></td>
<td>pO2</td>
</tr>
<tr>
<td></td>
<td>pCO2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activation markers</th>
<th>P-selectin expression</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Morphology and membrane integrity</th>
<th>Swirl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypotonic shock response (HSR)</td>
</tr>
<tr>
<td></td>
<td>Annexin V release (indicates loss of membrane integrity and cellular injury)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitochondrial structure and function parameters</th>
<th>JC-1 signal (measures mitochondrial transmembrane potential)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTT reduction assay (measures mitochondrial enzymatic activity)</td>
</tr>
<tr>
<td></td>
<td>ATP concentration in platelets</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelet adhesion and aggregation parameters</th>
<th>Platelet aggregation velocity upon platelet activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shear-induced platelet adhesion and aggregation as measured by Impact-R (DiaMed)</td>
</tr>
<tr>
<td></td>
<td>Thromboelastography (TEG), or ex vivo perfusion model</td>
</tr>
</tbody>
</table>
Quality of Mirasol-treated platelets

Cell concentration and pH

*Platelet Mirasol-treated in plasma and stored in additive solution; results are on file

pH requirement >6.4 (EDQM) >6.2 (AABB)
Quality of Mirasol-treated platelets

In vitro conclusion

Mirasol-treated platelets:

- Meet pH requirements throughout storage (>6.4)
- Are viable and contain functional mitochondria
- Continue to consume oxygen
- Have intact membranes and intact pumps to prevent osmotic stress as shown by hypotonic shock responses
- Show good adhesion and aggregation properties throughout storage
- May be stored up to seven days*

*If stored in additive solution containing a phosphate buffer; results are on file
### MIRASOL SYSTEM IN USE

*Decrease in Adverse Reactions (AR)*

**Spain, BST Baleares- ISBT 2015**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Period 2007-2008 N=4,893</th>
<th>Riboflavin and UV-light PLT Period N=5,711</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile reactions in Patients receiving PLTs (%)</td>
<td>2.50%</td>
<td>1%</td>
<td>0.023</td>
</tr>
<tr>
<td>Allergic reactions in patients receiving PLTs (%)</td>
<td>1.02%</td>
<td>0.75%</td>
<td>0.005</td>
</tr>
</tbody>
</table>

“In addition, there have been no documented cases of platelet transfusion related sepsis or any other transfusion transmitted infections since the implementation of PRT.” T.Jimenez-Marco, Vox Sang (2015) 109 (Suppl. 1), 192
**Institute of Hematology and Transfusion Medicine, Warsaw**

Period 2011-2013

<table>
<thead>
<tr>
<th>PC</th>
<th>M-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component (n)</td>
<td>AR (n)</td>
</tr>
<tr>
<td>4,731</td>
<td>33</td>
</tr>
</tbody>
</table>

Rate of AR

- PC: 0.70%
- M-PC: 0.33%

p < 0.05

Letowska M et al., Transfusion accepted
## Mirasol PRT Status to Date
### Registered, Routine Use, Research Use

<table>
<thead>
<tr>
<th>Country</th>
<th>Registered</th>
<th>Routine Use</th>
<th>Research Sites</th>
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Mirasol System Surveillance Data on >162,000 Transfusions
(>81,000 Platelet and >82,000 FFP transfusions)

- Over 500,000 Mirasol disposable sets sold since 2007
- Hemovigilance data on almost 1/3 of all units treated
- 0% serious adverse events related to use of Mirasol-treated platelet and plasma products
- No reports of increased bleeding or increased platelet product utilization after introduction
- No reports of bacterial contamination or sepsis
- Data collected at voluntary basis from users from Luxemburg, Italy, Poland, Lithuania, Spain, Greece and Serbia
Extended customer base
Overview of the Mirasol’s footprint

Over half a million kits used in routine
In routine use in >70 centers worldwide across 18 countries (Europe, Middle East, LATAM, APAC)
The Mirasol whole blood system

*The AIMS study*

- **AIMS:** African Investigation of Mirasol System

The Mirasol whole blood system is under development and is not commercially available.
The Mirasol whole blood system

The AIMS study (ongoing)

Donors

Whole blood donation

Mirasol-treated Arm

Control Arm

Whole blood Transfusion

Whole blood Transfusion

Reduction of probability to transmit *Plasmodium* to non-malaria patients?

Increased probability of having a new individual infected with Malaria
The Mirasol whole blood System
The AIMS study (ongoing)

- **AIMS:** African Investigation of the Mirasol system
- **Number of patients to be enrolled:** 250

**Objectives:**
- **Primary endpoint:**
  To determine biologically and clinically the efficacy of Mirasol-treated Fresh Whole Blood *in preventing bacterial and malaria transmission by transfusion*
- **Secondary endpoints:**
  To compare hematological, biochemical and coagulation parameters between Mirasol-treated and standard Fresh whole blood
  To compare any immediate side effects between the Mirasol-treated whole blood and standard fresh whole blood

- This is the first clinical trial that investigates the effectiveness of a pathogen reduction technology in reducing the transmission rate of a blood-contaminating agent
# Quality of Mirasol-treated platelets

## Clinical study activities

<table>
<thead>
<tr>
<th>Trial name and location</th>
<th>Product</th>
<th>Target no. of patients</th>
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<tbody>
<tr>
<td>The Miracle trial, France (6 sites)</td>
<td>Platelets in plasma</td>
<td>118</td>
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<tr>
<td>PREPARReS trial, Sanquin, the Netherlands; Bergen, Norway; Canadian Blood Services, Canada*</td>
<td>Platelets in plasma</td>
<td>618</td>
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<tr>
<td>IPTAS trial, Italy (8 sites)*</td>
<td>Platelets in PAS</td>
<td>820 (410 in Mirasol system study sites)</td>
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</tbody>
</table>

*PREPARReS and IPTAS trials also collect WBC alloimmunization data*
Whole Blood PRT: Long Term Objective

1. Transfer WB unit to Illumination bag
2. Add Riboflavin
3. Illuminate
4. Separate into Components

- RBC
- Platelet
- Plasma
Why is Pathogen Reduction Important?

- Reduced risk of bacterially contaminated platelet transfusion
- Further closing of window period for screened viruses
- Added protection against untested pathogens
- Proactive protection against emerging pathogens (e.g. Chikungunya, West Nile virus)
- Possible reduction in adverse transfusion events caused by residual white cells
- Potential to extend Platelet shelf-life to 7 days
- Potential to replace or revisit existing blood safety measures (e.g. bacterial testing, gamma-irradiation)
- Public expectation of “zero risk”
Terumo BCT Vision for Blood Safety

We are committed to advancing global blood safety by enabling safer blood transfusions for patients worldwide and cost efficiencies for the blood center.

We achieve this through the Mirasol PRT System, which offers a complete combination of benefits:

- **safe**
- **simple**
- **effective**
- **integrated**


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