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### CURRENT FDA INITIATIVES AND PRIORITIES

Food and Drug Administration Initiatives and Priorities presented by Jay Epstein, MD, director, Office of Blood Research and Review, Center for Biologics Evaluation and Research, and Leslie Kux, deputy director, Office of Compliance and Biologics Quality, included information on the FDA Transparency Initiative initiated in January 2009, guidance documents issued in 2009 and those that OBRR is giving high priority to in 2010, blood supply monitoring during the H1N1 influenza pandemic, current concerns and initiatives in addressing emerging infectious agents, an update on the OBRR Quality Program, and an introduction to new staff working in OBRR and the Division of Blood Applications. In addition, Kux provided information on FDA's agencywide initiatives related to compliance and enforcement. Of particular interest is the requirement that an establishment respond to a 483 within 15 days if the response is to be evaluated prior to any additional action by the agency.

### CURRENT AABB INITIATIVES AND PRIORITIES

Jacquelyn Fredrick, president of AABB, introduced the discussion of AABB Initiatives and Priorities and reviewed the association's Statement of Intent in support of the strategic plan that is focused on patient and donor safety through the practice of transfusion medicine and cellular therapies. Five critical focus areas for

achieving the goal of improved patient and donor health include member engagement, excellence in medicine, knowledge translation, regulatory advocacy, and standards and accreditation.

## **SPECIFIC TOPICS OF DISCUSSION WITH FDA**

### Interpretation of Recommendations Contained in Guidance Documents

Guidance documents are published with a statement that the contents are nonbinding and that alternative approaches can be used if they satisfy the regulations and statutes. Generally, the alternative approaches require validation to show they are equally effective. Blood establishments have had different experiences at the time of inspection when they have implemented a validated alternative approach, and some examples were discussed. FDA was asked to clarify its policy on review and acceptance of validated data to support implementation of an alternate approach to nonbinding recommendations contained in a guidance document.

In general, properly validated alternatives that meet the published recommendations should be acceptable. However, the alternatives should not be a deviation from manufacturer's instructions, and in the case of equipment, it is important to ensure the equipment is working properly. A discussion with the consumer safety officer, or CSO, before implementing the alternative procedure may be helpful, and Richard Davey, MD (301-827-2763), and Leslie Holness, MD (301-827-6115), made their phone numbers available to establishments that would like to call them.

### Statistical Sampling Plans for Product Quality Control

The Code of Federal Regulations written specifically for blood components (21 CFR 600 series) requires that modest numbers of blood components be tested on a monthly basis to satisfy quality control, or QC, requirements. However, current good manufacturing practices, or cGMP, that have broad applications for blood components (21 CFR 200 series) require the use of statistically significant sampling plans. In recent years, the blood community and FDA have discussed several approaches. When the December 2007 guidance for collection of Apheresis Platelets was published, a binomial sampling plan was recommended. (Prior to that, the potential use of scan statistics had been introduced but at that time was untested in the blood center environment and was essentially abandoned.) Many blood establishments continue to struggle with issues related to correct use of a statistically significant sampling plan, particularly when the institution does not collect enough platelets to use the binomial sampling plan.

Several sampling plans were reviewed, including hypergeometric distribution statistics, which are more adaptable to facilities that collect and process smaller numbers of units. The hypergeometric plan supports sample sizes of  $n < 60$  for QC purposes. However, it is not applicable to undefined population sizes and

therefore is not valid for process validation. (Tables based upon hypergeometric distribution have been prepared by the Office of Biostatistics and Epidemiology, CBER, and are expected to be made publicly available in the future.) Clusters of failures at the endpoints of the sample size may be masked when using binomial or hypergeometric approaches, but such clusters could be identified through use of scan statistics. Scan statistics is conceptually and computationally complex but software now exists to manage the use of this method. References for the various statistical approaches were provided on the final slide. When asked how an establishment would get FDA acceptance of a statistical process that has not been recommended by FDA, the committee was reminded that any sound and “known” process should be acceptable. Novel approaches might also be acceptable and a pre-implementation conversation with the CSO could answer all questions.

#### Pathogen Inactivation

The [Blood Products Advisory Committee](#) recently advised the FDA to place certain boundaries around a phase III clinical trial for pathogen inactivation of human platelets, effectively requiring a three-fold increase in the size of the trial and perhaps rendering it financially infeasible. The committee believes it is important that the inactivation processes not only be effective against disease agents but that they also result in a safe product for patient transfusion. However, there is concern following discussions at the November 2009 BPAC meeting that approval of any pathogen inactivation process or product is moving further into the future. FDA was asked about current considerations of this issue, particularly in the context of the July 2008 request from the [assistant secretary for health](#) to further the progress of pathogen reduction in the U.S.

FDA participants responded that patient safety and efficacy of the transfused product remain of paramount concern for them, and they remain very interested in the prospects for pathogen inactivation processes coming to the U.S. market. Agency representatives continue to engage the trial sponsor in conversations about possible trial designs. The Laboratory of Cellular Hematology in the Division of Hematology is conducting research on the action of UV light on platelets and red cells. Committee members reiterated concerns that pathogen inactivation appeared unlikely to be a potential tool for use with emerging infectious disease agents in the U.S. in the event that infectious disease tests become increasingly difficult to obtain or become unavailable.

#### Infectious Disease Testing

The lack of availability of tests is not restricted to those that are thought to be useful only in selective testing situations (dengue, Babesia). Universal testing volumes — such as for human T-cell lymphotropic virus I and II — alone also do not create a market incentive, and the confirmatory test market will always be small. The committee’s discussions with FDA included:

How to obtain testing (low level or universal) against a lack of market incentive — transfusion medicine infectious disease tests are a very low percentage of the in vitro diagnostic market.

HTLV-I/II testing is required by regulations, and there is concern that in 2010, the one licensed test distributed in the U.S. may at some point in time not be universally available because of lot issues relating to manufacturing.

Re-entry of some donors, especially those deferred in high numbers for markers that have low prevalence in the U.S. population, is very desirable, yet there are no licensed tests available. Are there other options for re-entry?

FDA participants stated they share the same concerns and said they have interacted with several AABB task forces and manufacturers in an effort to support products coming to market.

Committee members and FDA agreed there are shared responsibilities and opportunities to resolve some of the issues. The problems have been described multiple times, and the time appears ripe for a roundtable summit.

#### Disasters and Pandemic Preparedness

The transfusion medicine community acting through the Interorganizational Task Force on Domestic Disasters and Acts of Terrorism and other working groups has met repeatedly over the past few years and engaged with government liaisons and participants in an effort to establish robust preparedness plans for unplanned events. The goal of such planning is to ensure an adequate and safe supply of blood (and tissues) for the patient community in the event of a disaster or pandemic. A final unresolved issue for blood establishments is that of pre-determining which processes and procedures may be necessary to vary from during the manufacturing process. One area of focus is relaxation of certain donor requirements in order to ameliorate catastrophic shortages. Preparation of a robust preparedness plan is hampered by a facility's inability to write the necessary standard operating procedures and train staff under acceptable cGMP conditions during an emergency.

The agenda submitted in advance to FDA asked for a discussion of options available to blood establishments including, but not limited to, alternative procedures/variances, as well as areas where enforcement discretion might be more appropriate in the event of an unexpected, sudden expansion of a pandemic. Clarification of existing regulations with regard to emergency situations would be helpful. The goal is to find concrete steps that would enable establishments to have a fully robust preparedness plan that could be initiated rapidly in the event of need.

FDA participants acknowledged their understanding of the need to preplan and have attempted to provide some additional guidance in the form of the [draft guidance](#) issued in November 2009: "Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus."

#### Blood Pressure and Pulse Measurements in the Allogeneic Blood Donor Population

At the [November BPAC](#) meeting the committee considered evidence of currently available data for blood pressure and pulse measurements as predictors of risk for adverse reactions to blood donation and whether data support specific ranges for these measurements as predictors of adverse reactions. BPAC advised that while blood pressure measurements are not predictors of adverse reactions, pulse appears to have relevance. Committee discussion further suggested that measurement of blood pressure and pulse may be of value even if FDA does not retain them as donor eligibility determinations.

Following the BPAC discussion, FDA participants were asked about their current considerations for the use of blood pressure and pulse measurements in the allogeneic blood donor setting. Several items were noted:

- BPAC's concern with the paucity of data on donors with blood pressures >180.
- Knowledge gaps creating an opportunity for surveillance.
- Establishing limits in regulations may not be ideal.
- Having no requirements may not be ideal.

#### Abbreviated Donor History Questionnaire

BPAC advised that the use of the abbreviated donor history questionnaire (DHQ) is desirable and suggested that a post-implementation study/data collection would be appropriate. The AABB Donor History Task Force developed a study (with the advice of FDA liaisons to the task force) and submitted it to FDA in April 2008. The task force has been told on several occasions that the plan submitted satisfactorily addressed all outstanding issues.

FDA was asked to explain the pathway for advancing the abbreviated donor history questionnaire and responded that the necessary guidance document is in an editing stage. The post-implementation plan will likely be referenced in the guidance. This draft guidance is on OBRR's priority list for 2010, but the time frame is not definitely known.

#### Plasma Obtained From Whole Blood Donors for Further Manufacturing Use

A regulatory framework for plasma obtained by apheresis that could be used for further manufacture was proposed for BPAC's [discussion](#) last April.

FDA was asked for an update on current considerations for these blood components and responded that staff within OBRR continue to look at the issues raised by the BPAC discussion and remain in contact with the AABB task force. There was nothing definite to report.

Requirements for Human Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use, Proposed Rule, November 2007

The committee was interested to hear an update on progress regarding reconciling comments received to the proposed rule and to know if it is likely to be reissued as a proposal or to be published as a final rule.

FDA continues to look at the many proposals put forth in the document and has engaged BPAC in discussions on some of the subjects (i.e., hemoglobin and blood pressure/pulse). It took more than 10 years to draft the document, and it likely will not be reissued as a draft. However, it is also likely that all proposals may not be issued as final at the same time.

Publication of Documents

The FDA Liaison Committee remains concerned with the significant delay in publishing documents that are of critical importance to blood establishments. Updates to several documents provided by discussions at the meeting are included in the list below. FDA participants stated that although they have assigned the documents priority for 2010, they are not in control of the overall process and timelines involved.

- Guidance document that would recognize the abbreviated DHQ – A draft is on the 2010 priority list for OBRR.
- Guidance document that would recognize v1.3 of the DHQ – A guidance with final recommendations is on the 2010 priority list for OBRR. The AABB Donor History Task Force has decided to proceed with posting v1.3 on the AABB Web site along with instructions for submitting the materials to FDA as a Prior Approval Supplement to the establishment's Biologics License Application before implementation.
- Nucleic Acid Testing (NAT) for Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV): Testing, Product Disposition, and Donor Deferral and Reentry (draft guidance, July 2005) – A guidance with final recommendations is on the 2010 priority list for OBRR.
- Pre-Storage Leukocyte Reduction of Whole Blood and Blood Components Intended for Transfusion (draft guidance, January 2001) – Reissuance as a draft guidance is a priority for OBRR in 2010.
- Revisions to Labeling and Storage Requirements for Blood and Blood Components, Including Source Plasma (proposed rule, July 2003) – No update was provided.
- Safety Reporting Requirements for Human Drug and Biological Products (proposed rule, March 2003) – No update was provided.

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**LE08 PATHOGEN INACTIVATED PLATELETS AND PREVENTION OF IMMUNOLOGICAL ADVERSE REACTIONS: THE ITALIAN PLATELET TECHNOLOGY ASSESSMENT STUDY (IPTAS)**

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**Introduction**

Although extremely safe, platelet (PLT) transfusion still carries measurable risks of pathogen transmission, allo-immunization to human leucocyte antigens (HLA) carried by viable white blood cells (WBCs) present in platelet concentrates (PCs) -in turn causing refractoriness to random donors in 10-15% of recipients- and transfusion associated graft vs host disease (TA-GvHD). Traditional approaches to prevent these untoward effects include improved donor screening procedures, bacterial detection assays, WBC removal by filtration and  $\gamma$ -irradiation. Recently, two commercial photo-chemical technologies "Intercept Blood System", by Cerus and "Mirasol Pathogen Reduction Technology", by Caridian, previously named Navigant Biotechnologies/Gambro, have been developed which not only inactivate pathogens but also prevent WBC replication, thus offering the potential additional benefit of reducing immunological side effects. These pathogen inactivation (PI) systems use UV-A light to irradiate PLTs in the presence of a photo sensitizer (amotosalen, S-59, in Intercept Blood System; or riboflavin - Vitamin B<sub>2</sub>, in Mirasol Pathogen Reduction Technology). Clinical studies<sup>1-7</sup> show safety of both procedures and non inferiority of PI treated PLTs vs standard PLTs in regard to bleeding prevention<sup>8</sup>. Data from published preclinical studies show that transfusion of pathogen inactivated PLTs can prevent presensitization to transplants<sup>9</sup>. Moreover, using PI in place of  $\gamma$ -irradiation to prevent TA-GvHD was authorised by some organizations and regulatory bodies including the Paul Ehrlich Institut.

In view of broad clinical use of expensive PI procedures in transfusion medicine, technology assessment (TA) studies including cost to benefit evaluations can provide suitable information for evidence based decisions in health care<sup>10</sup>.

Blood components are highly complex mixtures of plasma proteins and cells. During the production, inactivation, and storage of PCs there is the risk of changes in their integrity, especially at the protein level. It is therefore a major challenge to identify significant alterations of these products. At present, blood component quality control is mainly focused on standardized quantitative assessments providing relatively limited information about products. Proteomics allows a comprehensive study of protein modifications, qualitative and quantitative analysis, and high-throughput protein identification, thus potentially enabling a global assessment of processing, inactivation and storage methods, as well as of possible contaminants and neoantigens that may influence the immunogenic capacity of blood-derived therapeutics<sup>11-14</sup>.

**The Italian Platelet Technology Assessment Study (IPTAS)**

IPTAS will provide new information on the safety and efficacy of PCs prepared with standardized procedures using WBC inactivation in place of filtration to reduce anti-HLA allo-immunization and acute transfusion reactions. Incidence of both side effects and the cost impact of PI technology (net balance between added and avoided costs) will be compared for buffy coat (BC) PLTs and for leuco-reduced apheresis (AP) PLTs, which represent the prevalent types of PCs used in Italy. Data from this study will support the analysis on whether PI can be used to replace WBC filtration,  $\gamma$ -irradiation and donor CMV serology.

Proteome analysis will provide additional in vitro data on the possible mechanisms of inactivation of residual WBCs in pathogen-inactivated PCs and new insights on the impact of PI processes on the global proteome profile of PCs, including functional studies on PLT mitochondria and the potential to identify markers of PLT lesion and activation, both in treated and untreated PLTs. Therefore, proteomics will offer new approaches for PC quality control.

**IPTAS objectives**

The primary objective of the clinical arm of this study is to determine: 1) if pooled whole blood (WB) PLT components prepared by the BC method without leuco-filtration and with photochemical PI treatment and 2) if AP PLT components prepared with leuco-reduction and photochemical PI treatment are associated with similar or lower incidence of HLA-alloimmunization compared to non PI treated PLTs transfused to onco-haematology recipients.

Secondary objectives are: 1) to compare the incidence of acute transfusion reactions as a measure of safety; 2) to compare the cost of photochemical PI treated vs untreated PLT components; 3) to implement an active haemovigilance program for all types of PLT components, and to evaluate the effectiveness of this haemovigilance program to monitor transfusion safety in multiple blood transfusion services (as recommended in the EUCOMED Position Paper "Health Technology Assessment for Medical Devices in Europe"; [www.assobiomedica.it](http://www.assobiomedica.it)).

The primary objective of the proteomic study is the comprehensive assessment of protein modifications, with high coverage, through qualitative and quantitative analysis of BC versus AP pathogen-inactivated PCs. Secondary objectives are: 1) to improve understanding of PLT storage lesions; 2) to identify potential storage lesion biomarkers; 3) to study molecular mechanisms of the elimination of proliferative and functional capacities of WBCs; 4) to compare mitochondrial mechanisms of energy production in inactivated versus standard PCs.

**Clinical study design and interventions**

The clinical arm of IPTAS will be a multicenter, non randomized, prospective controlled study involving 6 large blood transfusion services (BTS). Three BTSs will use the Cerus technology (CeT) vs local standard (STD) PLTs while 3 BTSs will use the Navigant-Gambro technology (NGT) vs local STD PLTs. PI and STD laboratory procedures and protocol deviations will be recorded. The PLT dose will be determined in all PLT components. Each BTS will allocate consecutive patients (PTs) alternatively to PI treated or STD PLT components. PI treatment will be used in place of  $\gamma$ -irradiation and CMV serology following ethical approval.

**Patient population, analytical procedures and outcome indicators**

The PT population will be haematology-oncology adult PTs expected to require 2 or more PLT transfusions (TXs). The study will include both new and previously transfused PLT recipients, but transfused PTs with historical documented record of 2 or more 1-hour post-TX PLT count increments  $<5,000/\mu\text{L}$  will be excluded. Presence of HLA antibodies (Abs) on admission will not be an exclusion

criteria. PTs will be transfused with the PLT component assigned by the BTS until: 1) PLT TX independent; 2) detection of refractoriness; 3) discharge or death, for a maximum of 8 weeks.

PLTs will be transfused according to the American Society of Clinical Oncology guidelines<sup>15</sup> and to local policies. While this study will not be stratified with respect to primary diagnoses or therapy, these data will be collected to permit analysis of the distribution of primary diagnoses and therapies among the treatment groups.

For each PLT component administered, the response to TX will be evaluated using standardized case report forms recording PT demographics, primary diagnosis and therapy. For each TX the type of PLT transfused will be indicated together with the requirement for: 1) TA-GVHD prevention; 2) CMV Ab negative components; 3) HLA-matched or cross-matched PLT components. Following each PLT TX, PTs will be evaluated for adverse events (AEs) for a period of 24 h. AEs will be recorded using a standardized form, assessed for relation to the PLT TX and graded for clinical severity. TXs with AEs possibly, probably, or definitely related to PLT TX will be defined as TXs with an acute reaction. PTs will be assessed for HLA allo-immunization using conventional assays at each study site. As a minimum, each PT will be evaluated for anti-HLA Ab status on a serum sample collected at enrolment and within 2 days of the conclusion of participation in the study. HLA matched or cross-matched PLT TXs will be specifically recorded.

A PT daily record will be used including morning PLT count, haemoglobin, no. of RBC and PLT TXs, PLT age, type and dose, post-TX PLT count, body temperature, presence/absence of sepsis, WHO bleeding grade, bleeding type/organ/system and outcome, occurrence and type of post-TX reactions, drugs negatively impacting on PLT transfusion.

### Sample size and statistical analysis

In the TRAP study it was estimated that 570 PTs were necessary for a statistically significant reduction of HLA allo-immunization from 40% to 20% with type I error = 0.05 and power = 0.92<sup>16</sup>. In the IPTAS study, according to the formula described by AR Feinstein<sup>17</sup>, the required sample size, for type I error = 0.05 and power = 0.80 of detecting a reduction of HLA-alloimmunization from 40% (control) to 20% (treatment), is 162 PTs per each comparison, i.e. 162 for the comparison of CeT treated PLTs (81 PTs) vs STD PLTs (81 PTs) in 3 BTSs and another 162 PTs for the comparison of NGT treated PLTs (81 PTs) versus STD PLTs (81 PTs) in the other 3 BTSs. To take into account PT drop out, we aim at collecting 100 PTs per arm (total: 400 PTs). We expect that IPTAS will include about 500-1,000 CeT PLT TXs, 500-1,000 "local" control TXs in the 3 BTSs using CeT, 500-1,000 NGT PLT TXs, 500-1,000 "local" control TXs in the 3 BTSs using NGT, for a total of 2,000-4,000 PLT TXs.

All data will be analysed according to an intention to treat model. The proportions of PTs who develop HLA Abs and their 95% CI will be determined. Comparisons between CeT treated PLTs vs local STD PLTs and comparisons between NGT treated PLTs vs local STD PLTs will be made using the  $\chi^2$  or the Fisher's exact test with  $p = 0.05$ . Similar comparisons will be made for the incidence of acute TX reactions on a per TX basis and a per PT basis. Descriptive statistics will be used to report number of TXs, PLT doses, pre- and post-TX PLT counts.

### Cost analysis

Direct costs of collection and preparation of WB PLTs prepared without WBC filtration and PI treated will be compared with those of PI treated AP PLTs and those of conventional PLTs prepared with WBC reduction,  $\gamma$ -irradiation, and CMV serology. The cost per PT for HLA matched or cross-matched PLTs will be compared between treatment arms. The costs of  $\gamma$ -irradiation and CMV serology per PLT component transfused and per PT in each treatment arm will be compared between treatment groups. The cost impact related to treatment of acute TX reactions will be determined and compared between treatment groups.

### Proteomic study

Proteomic analysis will be used to compare the effects of CeT and NGT on the proteome profile of AP and BC PCs and of WBCs. To this aim, 10 AP and 10 BC non inactivated units and, for each PI method, 10 AP and 10 BC inactivated units will be studied. Moreover, up to 18 WBC samples will be studied: 9 harvested from pooled BCs and 9 from Gambro Trima AP devices. Non treated WBCs and WBCs treated with both PI methods will be compared using procedures based on published methods<sup>18,19</sup>.

### Discussion

The results from this study will be applicable to estimates related to the routine use of PI treatment for all platelet components on a national basis. These data will provide a balanced economic rationale to regional healthcare administrators and hospital managers to evaluate whether or not to introduce blood component pathogen inactivation, extensively or for selected categories of patients. Moreover, additional scientific data will be made available about outcomes of low cost (BC) vs high cost (AP) PCs, thus allowing suitable clinical governance of this specific aspect of blood therapeutics. The implementation of an active haemovigilance system to monitor the safety of transfusion of platelet components in this study will provide a system for monitoring safety of all platelet components and ultimately all other labile blood components. This study will provide clinical and technical staff at study centres with training and experience on active haemovigilance. This experience could be easily transferred to other blood centres not participating in this study.

Proteomic technologies will provide new comprehensive information about changes occurring during processing and storage of blood products, introducing a novel approach to qualitative and quantitative assessment of blood components.

The proteomic study will potentially provide data to optimize PLT production processes and/or pathogen inactivation systems, thus increasing present safety and standard of care in PLT transfusion therapy.

The combination of data from the in vivo and the in vitro parts of the study will hopefully allow an original and useful impact on the comprehensive clinical setting of blood transfusion.

### Acknowledgments

The authors gratefully acknowledge Larry Corash from Cerus and Ray Goodrich from Caridian for significant contributions in the development of IPTAS design and Giusy Baldocchi for secretarial assistance.

### Disclosures

*Paolo Rebullà has received fees for scientific advice to Cerus and to Caridian.*

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## 血小板製剤への初流血除去導入の効果について

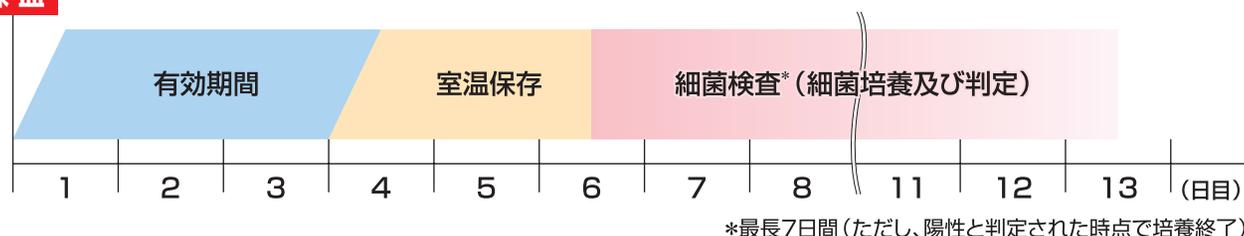
日本赤十字社では2006年10月26日採血分から細菌混入を低減する目的で、血小板製剤の初流血除去を開始しました。採血時の皮膚消毒では殺菌効果が届きにくい毛嚢等を含む皮膚片の製剤への混入を防ぐことが目的です。この細菌混入低減効果を評価するため、期限切れ血小板製剤を用い、初流血除去導入前後で細菌陽性率\*を比較しました。その結果、初流血除去導入により細菌陽性率\* (*Propionibacterium acnes*以外の細菌)が0.06%から0.02%に減少していることが分かりました。

\*細菌陽性率：細菌検査の結果、細菌が検出され陽性と判定された割合。

### 【評価方法】

期限切れとなった血小板製剤を採血後6日目まで室温(20~25℃)で保存しました。細菌検査には全自動血液培養装置(BacT/ALERT)を用い、好氣的、嫌氣的条件それぞれで10mLの製剤を最長7日間培養し、細菌の有無を判定しました。

### 採血



### 【評価結果】

	初流血除去導入前 2005年5月~2006年4月	初流血除去導入後 2006年12月~2008年3月
培養実施数	21,786	21,783
陽性数(陽性率)	36* (0.17%)	11 (0.05%)
<i>P. acnes</i>	24 (0.11%)	7 (0.03%)
<i>P. acnes</i> 以外の細菌	13 (0.06%)	4 (0.02%)

\*1例は *P. acnes* と non-*P. acnes* の重複混入

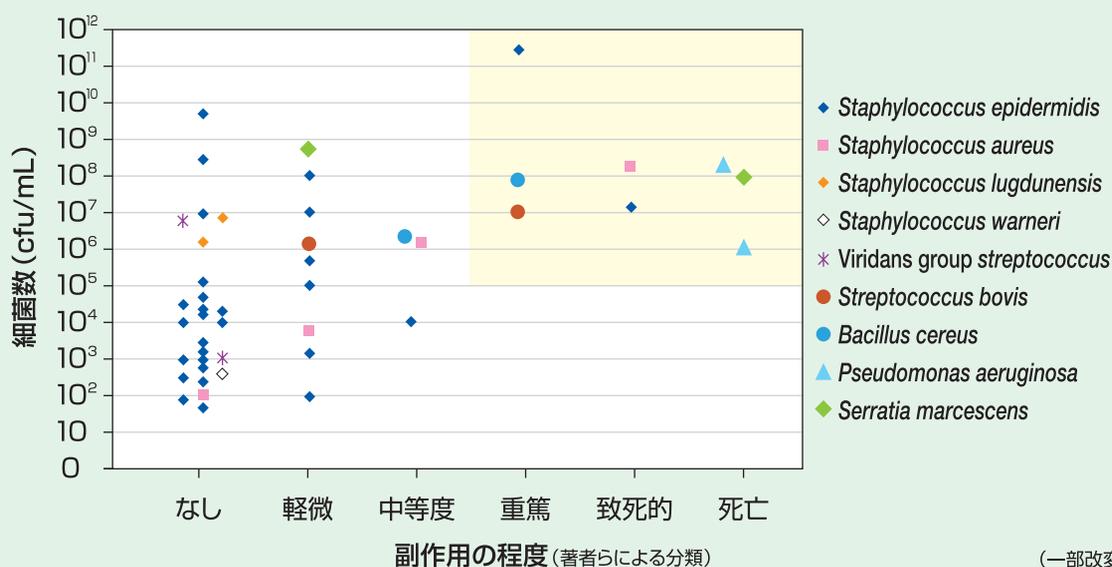
### ● 検出された細菌 (*P.acnes*以外)

細菌の由来(推定)	初流血除去導入前	初流血除去導入後
皮膚	<i>Staphylococcus epidermidis</i> (4) <i>Staphylococcus</i> sp. (1) CNS (Coagulase-negative <i>Staphylococcus</i> ) (1) <i>Staphylococcus saccharolyticus</i> (1) Gram(+) bacillus, 非芽胞菌 (1)	<i>Staphylococcus epidermidis</i> (1)
皮膚または血液	<i>Staphylococcus aureus</i> (2)	<i>Staphylococcus aureus</i> (1)
血液	<i>Streptococcus constellatus</i> (1) <i>Salmonella choleraesuis</i> (1) <i>Eikenella corrodens</i> (1)	<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> (1) <i>Escherichia coli</i> (1)

初流血除去導入前後でそれぞれ2万本を越す期限切れ血小板製剤について細菌検査をした結果、陽性と判定されたのは、初流血除去導入前は0.17%、初流血除去導入後は0.05%でした。P.acnesを除くと、細菌陽性率は導入前が0.06%、導入後は0.02%でした。P.acnesは、血小板製剤中では増殖が極めて遅く、また陽性判定された製剤を輸血しても副作用がなかったと報告されています<sup>1)</sup>。P.acnesも含め、検出された菌種はこれまでに論文等で報告されているものでした。また、細菌陽性率も海外での報告とほぼ同程度です<sup>2)</sup>。今回の評価では、細菌の検出率を高めるために、血小板製剤を6日目まで室温で保存しました。細菌陽性率は保存期間と共に高くなるため<sup>2)</sup>、有効期間の4日間での細菌陽性率は今回の結果よりも低いと予想されます。

臨床的に重篤な副作用は細菌が $10^5$ CFU/mL以上含まれる製剤が原因だとされています<sup>3)</sup>（下記「細菌数と輸血副作用」参照）。初流血除去により、最も細菌濃度が高い皮膚片が除かれる<sup>4)</sup>ので、初流血除去した製剤は細菌陽性率だけでなく、細菌数も少なくなると推測され、副作用の危険性は細菌陽性率の減少以上に軽減されていると考えています。

### 細菌数と輸血副作用<sup>3)</sup>



日本赤十字社では、血小板製剤の有効期間を諸外国より短く設定し、さらに初流血除去により皮膚常在菌の混入数をできる限り少なくして、細菌汚染に対する安全性を高めています。しかしながら、細菌の混入が全くなくなるわけではありませんので、輸血前の血液製剤の外観検査と輸血中、輸血後の患者さんの注意深い観察をお願いします。

### 【参考文献】

- 1) Störmer M, et al.; *Propionibacterium acnes* lacks the capability to proliferate in platelet concentrates. Vox Sanguinis 94: 193-201, 2008.
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- 3) Jacobs MR, et al.; Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. Clinical Infectious Diseases 46:1214-1220, 2008.
- 4) 松田好美 他; 初流血除去回路つき採血バッグによる皮膚常在菌及び皮膚片の混入の防止. 日本輸血学会雑誌 49: 761-766, 2003.

輸血による細菌感染が疑われる症例が発生した場合には、直ちに輸血を中止して適切な処置をするとともに、使用された製剤バッグを適切に（衛生的かつ冷所）\*に保管し、赤十字血液センター医薬情報担当者までご連絡ください。

また、原因究明のために、使用された製剤バッグ、患者さんの検体、さらに血液培養試験をはじめとする臨床検査関連情報等の提供をお願いします。

\*輸血セットのクランプを固く閉め、輸血部門に返却願います。その後、点滴筒の上下をチューブシーラーでシール（チューブシーラーがない場合は鉗子等で確実に結紮）し、ビニール袋に入れて冷蔵保存してください。

### 医療関係者向け製品情報サイト

URL <http://www.jrc.or.jp/mr/top.html>

### 《発行元》

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## Protein quality in Mirasol pathogen reduction technology-treated, apheresis-derived fresh-frozen plasma

James Smith and Gail Rock

**BACKGROUND:** The Mirasol pathogen reduction technology (PRT) system for plasma is based on a riboflavin (vitamin B<sub>2</sub>) and ultraviolet (UV) light treatment process resulting in pathogen inactivation due to irreversible photo-oxidative damage of nucleic acids. The purpose of this study was to evaluate the in vitro protein quality of apheresis-derived plasma treated with riboflavin and UV light in comparison with untreated fresh-frozen plasma (FFP).

**STUDY DESIGN AND METHODS:** Twenty apheresis plasma samples (270 ± 10 mL) were combined with 35 ± 5 mL of riboflavin solution (500 μM), yielding a mean 60 μM final riboflavin concentration, and then exposed to UV light (6.24 J/mL). Riboflavin and UV light-treated plasma was then flash frozen, within 8 hours of collection, generating treated FFP. Treated FFP was thawed and analyzed using standard coagulation assays, and the percent retention of protein activity was reported, relative to untreated, paired controls.

**RESULTS:** Plasma proteins demonstrated different sensitivities to riboflavin and UV treatment. The amount of total protein remained unchanged. After treatment, fibrinogen (antigen) showed 99% retention; Factor (F)XII, FXIII, ADAMTS-13, and von Willebrand factor (ristocetin cofactor) 96% to 100%. Fibrinogen retained 77% activity, FII 80%, FVIIIc 75%, and FV 73% after treatment. Antithrombin, protein S, plasminogen, and α<sub>2</sub>-antiplasmin retained between 91 and 100% activity.

**CONCLUSION:** The results from this study demonstrate that coagulant and anticoagulant proteins in riboflavin and UV light-treated (PRT) apheresis plasma are well preserved.

**B**lood transfusion is not a risk-free procedure even though concerns have been reduced dramatically with recent improvements in blood donor selection procedures and implementation of sensitive screening tests for known blood-borne pathogens. However, tests are not available for all known pathogens, and additional risks come with newly emerging agents.<sup>1-5</sup> Detection is also complicated because of a latent period between actual infection and detectable antibody formation in the donor. Increases in the amount of world travel are a rising concern for disease transmission through transfusions, due to migration of agents from one geography to another. As a result there is a growing interest in the implementation of pathogen reduction technologies (PRTs) to increase the safety of blood components used in transfusion.

All PRTs have advantages and disadvantages. The important goal is to simultaneously improve transfusion safety while maintaining efficacy after treatment. Among the methods that have been employed successfully are solvent/detergent (S/D) and methylene blue (MB) treatment, both of which are used to treat plasma and to manufacture fractionated and purified blood-derived products. These treatments are primarily effective against enveloped viruses. They also yield some reduction in protein quality due to the treatment.<sup>6,7</sup> A psoralen compound (amotosalen, S-59) with ultraviolet (UV) light-based technology has also been developed for the reduction of pathogens

**ABBREVIATIONS:** HMW = high molecular weight; MB = methylene blue; PRT(s) = pathogen reduction technology(-ies).

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Received for publication July 14, 2009; revision received September 17, 2009, and accepted September 21, 2009.

doi: 10.1111/j.1537-2995.2009.02517.x

TRANSFUSION \*\*,\*\*:\*\*\*-\*\*.

and inactivation of white blood cells (WBCs) in platelet (PLT) preparations and in plasma.<sup>8-10</sup>

Another method is riboflavin and UV light treatment, which inactivates viruses, bacteria, parasites, and WBCs.<sup>11-14</sup> Riboflavin, a naturally occurring vitamin (vitamin B<sub>2</sub>), has the advantage of a well-characterized safety profile.<sup>15-17</sup> Exposure to UV light activates the riboflavin and causes a chemical alteration of guanine residues of the nucleic acids causing irreversible damage and making the pathogens unable to replicate.<sup>14,18,19</sup> This chemistry involves oxygen-dependent and oxygen-independent processes. The Mirasol PRT system is designed to minimize the effect of reactive oxygen by specific light wavelength selection and by minimizing residual air in the component bag.

This study evaluated the protein quality of apheresis-derived plasma products treated with riboflavin and UV light. Results were compared to paired, untreated products handled under the same conditions.

## MATERIALS AND METHODS

### Collection and preparation of plasma samples

A total of 20 apheresis-derived plasma products were collected in ACD from the following blood types: 35% group O, 45% group A, 10% group B, and 10% group AB. The blood types were selected to be representative of typical donor populations present in the United States and Western Europe. All blood products were collected at an accredited blood banking facility. Units were held at 22 ± 2°C before processing. Each apheresis unit was split into one control unit (nontreated) and one test unit (riboflavin and UV light treated).

### Product treatment

A total of 270 ± 10 mL of plasma was transferred to an illumination bag and 35 ± 5 mL of 500 μM sterile riboflavin solution was added using a sterile connector (Terumo TSCD sterile tubing welder, Terumo Medical Corp., Elkton, MD) yielding a mean 60 μM final concentration. The set was placed into the illuminator and exposed to UV light with a linear agitation of 120 cpm. Product temperature and light dose delivered were monitored continuously throughout processing. The bag was removed after the illuminator delivered the necessary dose of UV light (6.24 J/mL) based on product volume and measured flux rate.

### Sampling and storage preparation

Treated and control aliquots were frozen concurrently, within 8 hours of collection and stored at -70°C, for at least 24 hours before plasma protein quality evaluation.

### Plasma proteins assays

Treated fresh-frozen plasma (FFP) was thawed and analyzed using standard coagulation assays. Fibrinogen activity, Factor (F)II, FV, F IX, FX, FXI, FXII, FXIII, antithrombin, protein C, protein S, and total protein were evaluated in Regional Medical Laboratory (Tulsa, OK). Fibrinogen antigen, FVIIIc, von Willebrand factor (VWF) activity, and α<sub>2</sub>-antiplasmin were measured at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA). ADAMTS-13 activity was determined at Montefiore Medical Center/Albert Einstein College of Medicine (Bronx, NY) and VWF multimers at McMaster University (Hamilton, Ontario, Canada). Details are as follows:

The majority of clotting and chromogenic assays were performed on the Diagnostica Stago STA-R Evolution (Stago, France). FVIIIc was measured using a FVIII chromogenic assay from Siemens on the Behring coagulation system, extra performance (Siemens Healthcare Diagnostics, Newark, DE).

Antithrombin, α<sub>2</sub>-antiplasmin, and FXIII analyses were done using a colorimetric assay of antithrombin III activity (Stachrom ATIII, Diagnostica Stago-US, Parsippany, NJ), with Berichrom α<sub>2</sub>-antiplasmin and Berichrom FXIII (Siemens Healthcare Diagnostics), respectively.

Plasma levels of FII, FV, and FX were determined using functional assays based on the prothrombin time with human FII-, FV-, and FX-immunodepleted plasma samples. Similarly, plasma levels of F IX and FXI were determined using human F IX- and FXI-immunodepleted plasma samples, based on the activated partial thromboplastin time. Proteins C and S were determined using clotting assays (StaClot protein C and StaClot protein S, STA-Compact, Diagnostica Stago, France).

Fibrinogen antigen was determined by nephelometry. Fibrinogen activity was determined with the Clauss kinetic clotting method. The fibrinogen in plasma before and after riboflavin and UV treatment was characterized using 4% to 20% Tris-HCl criterion gels (Bio-Rad, Hercules, CA). Human fibrinogen (Calbiochem, San Diego, CA) was applied on gels as a control for plasma samples. Untreated control plasma and fibrinogen samples contained an amount of saline equal to the volume and amount of riboflavin in the treated samples. Gel images were obtained using a commercial system (GelDoc XR, Bio-Rad). Area (mm<sup>2</sup>) analyses of fibrinogen in plasma samples before and after riboflavin and UV treatment were performed on a high resolution gel imaging and documentation system (Molecular Imager GelDoc XR, Bio-Rad).

The total protein content was measured by the biuret assay using a chemistry analyzer (Olympus, Center Valley, PA). Ristocetin cofactor activity of VWF was measured using a von Willebrand reagent containing human stabilized PLTs from Siemens Healthcare Diagnostics.

The VWF multimers were analyzed using sodium dodecyl sulfate (SDS)-multimer gels containing 1.25% agarose (SeaKem LGT (p), FMC Bioproducts, Rockland, ME) and 1% acrylamide.<sup>20</sup> After electrophoresis (20 hr at 35 V), the proteins were transferred onto nitrocellulose membranes and incubated with peroxidase-conjugated rabbit anti-human VWF antibodies (Dako D/S, Glostrup, Denmark). The VWF multimer patterns were developed using Western blotting chemiluminescent substrate (ECL, GE Healthcare, Piscataway, NJ).<sup>21</sup>

Internal standards on each gel included normal pooled plasma and plasma deficient in large-molecular-weight multimers of VWF (Type 2A VWD). The multimer migration of each sample was compared to the normal pool. Intermediate bands were considered to be 5 to 10 subunits and high-molecular-weight (HMW) bands were considered to be greater than 10 subunits.

ADAMTS-13 activity level was measured using guanidine-HCl-treated VWF multimers as the substrate as previously described<sup>22</sup> with the proteolytic fragments generated after incubation analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting.

### Statistical analysis

Arithmetic means and standard deviations (SDs) were calculated for each of the variables listed and reported in the final analysis. To analyze the effect of photodynamic treatment on the coagulation factors, the control coagulation factor levels (mean  $\pm$  SD) and percent retention (mean  $\pm$  SD) for riboflavin and UV light-treated plasma are reported.

## RESULTS

Plasma proteins had different sensitivities to riboflavin and UV treatment as shown in Table 1. The amount of total protein remained unchanged. Fibrinogen had 99% antigen and 77% activity retention after treatment. Results from gel electrophoresis (Fig. 1) confirmed that after riboflavin and UV treatment the electrophoretic patterns of fibrinogen were unchanged.

FXII retained 96% activity and FXIII remained normal. FII showed 80%, FVIIIc 75%, and FV 73% activity retention, respectively, after riboflavin and UV treatment. ADAMTS-13 and VWF fully retained activity. The data demonstrated a variable decrease in the HMW multimers of VWF in the postillumination samples, with some samples having normal patterns (Fig. 2).

All anticoagulant and inhibitor proteins, antithrombin, protein S, plasminogen, and  $\alpha_2$ -antiplasmin showed retention of activity between 91 and 100% (Table 1). Protein C was more sensitive and retained 81% activity after treatment.

## DISCUSSION

### Plasma proteins

The existing PRTs show different levels of loss or degradation of plasma proteins after treatment.<sup>6,7,23-30</sup> The level of these reductions and the particular factors that are affected vary from method to method. For example, S/D-treated plasma shows an overall reduction of the activity and concentration of coagulation factors, inhibitors, immunoglobulins, and other plasma proteins of approximately 5% to 20%; IgM antigen isoagglutinins are reduced

TABLE 1. Protein retention for riboflavin and UV light-treated apheresis plasma\*

Plasma protein quality variable	Before illumination	After illumination†	Percent recovery, before illumination vs. after illumination‡
Fibrinogen activity (mg/dL)	345 $\pm$ 80	267 $\pm$ 64	77 $\pm$ 4
Fibrinogen antigen (mg/dL)	344 $\pm$ 90	339 $\pm$ 87	99 $\pm$ 4
FII (IU/mL)	1.1 $\pm$ 0.2	0.9 $\pm$ 0.1	80 $\pm$ 6
FV (IU/mL)	1.1 $\pm$ 0.2	0.8 $\pm$ 0.1	73 $\pm$ 8
FVIIIc (IU/mL)	1.3 $\pm$ 0.5	1.0 $\pm$ 0.3	75 $\pm$ 16
F IX (IU/mL)	1.4 $\pm$ 0.4	1.0 $\pm$ 0.2	76 $\pm$ 6
FX (IU/mL)	1.1 $\pm$ 0.2	0.9 $\pm$ 0.2	80 $\pm$ 7
FXI (IU/mL)	1.2 $\pm$ 0.2	0.8 $\pm$ 0.1	67 $\pm$ 5
FXII (IU/mL)	1.3 $\pm$ 0.3	1.3 $\pm$ 0.3	96 $\pm$ 14
FXIII (IU/mL)	All normal	All normal	NA
VWF activity (U/mL)	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	117 $\pm$ 10
ADAMTS-13 (% activity)	107 $\pm$ 41	103 $\pm$ 36	100 $\pm$ 25
Protein C (U/mL)	1.4 $\pm$ 0.5	1.1 $\pm$ 0.3	81 $\pm$ 11
Protein S (U/mL)	1.1 $\pm$ 0.2	1.0 $\pm$ 0.2	91 $\pm$ 9
Antithrombin (U/mL)	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	99 $\pm$ 4
$\alpha_2$ -Antiplasmin (U/mL)	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	93 $\pm$ 4
Total protein (g/L)	58 $\pm$ 3	58 $\pm$ 3	99 $\pm$ 1

\* Protein retention in treated plasma is reported as the percent retention relative to the paired, untreated control products at the same storage interval. All values are mean  $\pm$  1 SD. Values are reported for a total of 20 apheresis-derived paired plasma units.

† Corrected for dilution.

‡ Calculated as mean percent retention of individual paired units.