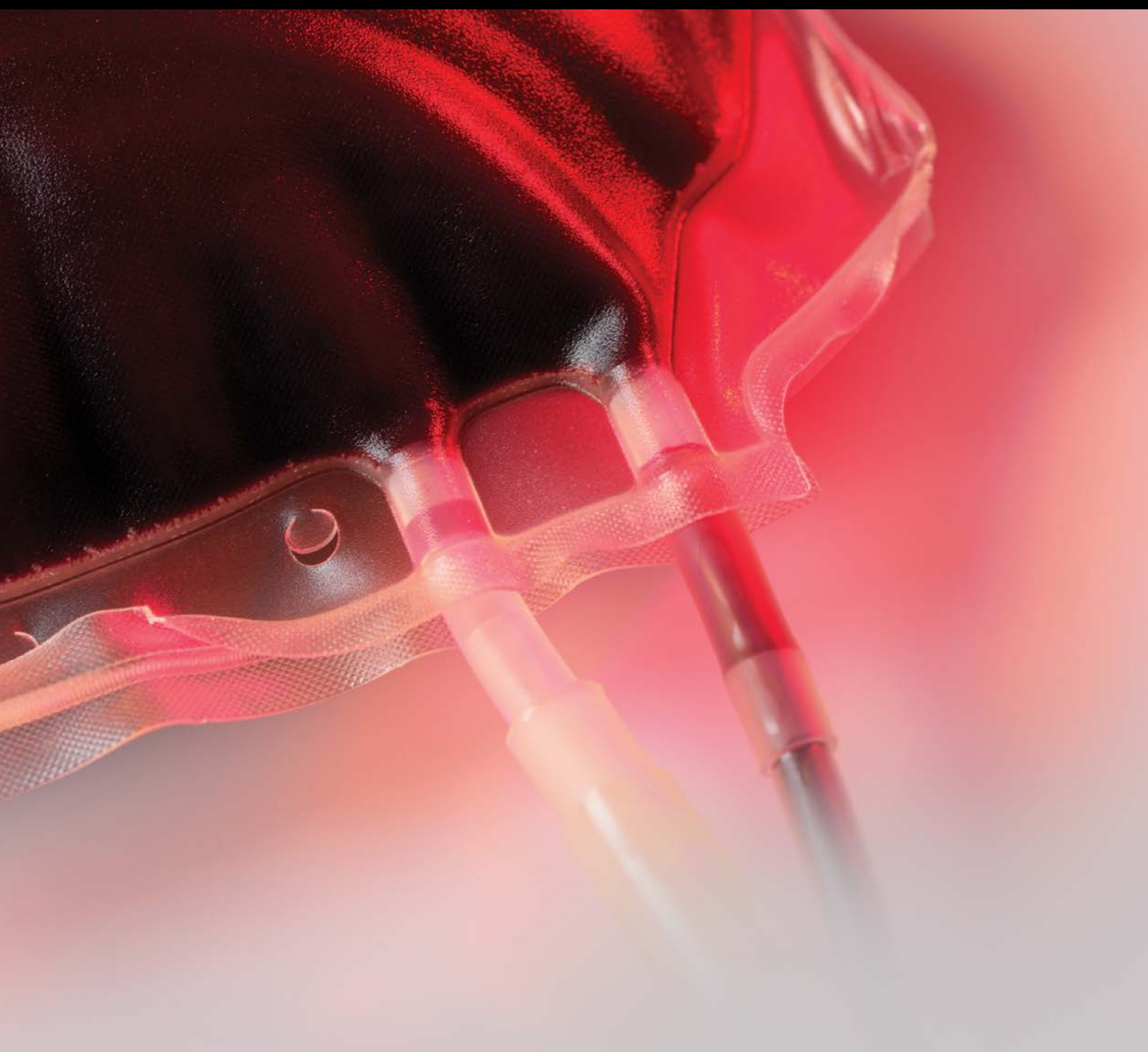
Journal of Blood Transfusion

Quality and Safety of Blood Products

Guest Editors: Sandra Ramirez-Arcos, Denese C. Marks, Jason P. Acker, and William P. Sheffield



Quality and Safety of Blood Products

Journal of Blood Transfusion

Quality and Safety of Blood Products

Guest Editors: Sandra Ramirez-Arcos, Denese C. Marks, Jason P. Acker, and William P. Sheffield

Editorial Board

Contents

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 2482157, 2 pages
http://dx.doi.org/10.1155/2016/2482157

Editorial
Quality and Safety of Blood Products

Sandra Ramirez-Arcos,[1] Denese C. Marks,[2] Jason P. Acker,[3,4] and William P. Sheffield[5,6]

[1]*Canadian Blood Services, Ottawa, ON, Canada K1G 4J5*
[2]*Research and Development, Australian Red Cross Blood Service, Sydney, NSW, Australia*
[3]*Canadian Blood Services, 8249-114 Street, Edmonton, AB, Canada T6G 2R8*
[4]*Department of Laboratory Medicine and Pathology, University of Alberta, 8249-114 Street, Edmonton, AB, Canada T6G 2R8*
[5]*Canadian Blood Services, Hamilton, ON, Canada*
[6]*Department of Pathology and Molecular Medicine, McMaster University, HSC 4N66, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5*

Correspondence should be addressed to Sandra Ramirez-Arcos; sandra.ramirez@blood.ca

Received 12 December 2016; Accepted 12 December 2016

Blood components used for transfusion therapy in developed countries include platelet concentrates (PCs), red cell concentrates (RBCs), and plasma. Current Good Manufacturing Practices (cGMP) ensure that blood operators maintain the Safety, Quality, Identity, Potency, and Purity of blood components, criteria collectively encompassed by the acronym SQuIPP. As highlighted in the review article "Quality Assessment of Established and Emerging Blood Components for Transfusion" by J. P. Acker et al. from Canada and Australia, assuring that blood components maintain high quality during storage and benefit transfusion recipients is challenging due to the variable nature of biological raw materials, different manufacturing processes, and variation in quality testing methods. Adding to this complexity is the fact that some products are subjected to nontraditional manufacturing or storage conditions. The authors concluded that, despite the development of novel quality assays, current tests including coagulation factor VIII activity for plasma, pH and platelet yield for PCs, and hemolysis of RBCs are user-friendly and remain valuable tools to determine blood product quality. It is however recognized that controversy still persists among transfusion practitioners as to the link between in vitro quality parameters and posttransfusion clinical effectiveness of blood components.

E. Maurer-Spurej and K. Chipperfield in Canada in their article "Could Microparticles be the Universal Quality Indicator for Platelet Viability and Function?" addressed the issue of lack of relatedness between in vitro quality assessment and in vivo effectiveness specifically related to PCs. The authors cited donor variation as one of the major factors affecting PC quality and clinical efficacy and suggested that this property should be considered when targeting different patient groups. While homogeneous viable platelets may be ideal for prophylactic treatment of cancer patients, trauma patients may benefit more with transfusion of heterogeneous platelets. The authors proposed that routine PC screening should include a rapid noninvasive assay to determine microparticle content in PCs as a universal indicator of platelet quality.

Quality testing should ensure similarity in the SQuIPP criteria of blood components prepared by different manufacturing methods. A. W. Shih et al. in Canada in their article "Quantification of Cell-Free DNA in Red Blood Cell Units in Different Whole Blood Processing Methods" assessed the content of cell-free DNA during RBC storage. Cell-free DNA is released by neutrophils prior to leukocyte reduction, which occurs at different times during unit preparation by the two methods used in Canada, the RBC filtration method or the whole blood filtration method. In the RBC filtration process, whole blood units are stored at room temperature for up to 20 h before production of RBCs, plasma, and PCs, with leukocyte reduction occurring at room temperature. In the whole blood filtration method, whole blood is refrigerated within 8 hours of collection, leukoreduced in the cold, and stored before production of plasma and RBCs within 72 h of phlebotomy. The authors detected lower cell-free DNA content in RBC units prepared by the RBC filtration

method compared to RBC units produced by the whole blood filtration method. The clinical significance of these findings is unknown and merits further investigation.

Variation of practice for quality testing of blood products exist not only between blood suppliers but also among related products. W. P. Sheffield et al. in Canada in their article "Stability of Thawed Apheresis Fresh-Frozen Plasma Stored for up to 120 Hours at 1°C to 6°C" have demonstrated that there is no reason to have divergent standards for storage of thawed plasma from apheresis or whole blood collections. Current Canadian standards mandate whole blood-derived plasma to be frozen within 24 h after blood collection, which can be thawed and stored for up to 5 days at 1–6°C. However, apheresis plasma should be frozen within 8 h after phlebotomy and can only be stored refrigerated for 24 h after thawing. The authors provided evidence that the activity of coagulation factors and fibrinogen was significantly higher in thawed apheresis plasma compared to whole blood-derived plasma throughout a 5-day storage period. Results of the study can therefore be used to propose an extension of the shelf-life of thawed apheresis plasma to 5 days in refrigeration, which would result in reduced wastage of this product in Canadian hospitals.

Safety of blood components considers the relative freedom from harmful effect to patients, directly or indirectly, of a prudently administered product taking into account the character of the product and the condition of the recipient at the time of the transfusion. To assure blood component safety, several measures are taken into consideration during product manufacturing and storage. Additionally, testing for infectious agents, including viruses, bacteria, and parasites, is routinely or seasonally performed by different blood operators. Variability in detection of infectious agents should be avoided to ensure valid surveillance data. A. C. Shrestha et al. from Australia in their article "A Comparative Study of Assay Performance of Commercial Hepatitis E Virus Enzyme-Linked Immunosorbent Assay Kits in Australian Blood Donor Samples" highlighted the poor agreement between the results obtained with two commercial enzyme-linked immunosorbent assays for the detection of IgM and IgG against hepatitis E virus in plasma samples. The study concluded that interpretation of serology results for hepatitis E virus should be done with caution.

Results of testing for the presence of infectious agents in blood components are one of the criteria used for donor deferral. K. Rooks et al. in Australia in their article "Mitigating the Risk of Transfusion-Transmitted Dengue in Australia" conducted a study to determine the prevalence of dengue virus RNA and nonstructural protein NS1 in plasma of donors living in high-risk areas during dengue outbreaks. Currently, donors residing in Australian dengue endemic areas are not allowed to donate fresh components and can only donate plasma for fractionation. The authors reported no detectable dengue RNA or antigen NS1 and no cases of dengue within blood donors. It was concluded that the risk of transfusion-transmitted dengue is likely low and implementing dengue detection is not necessary. Deferring high-risk donors is still the best practice to mitigate dengue transmission by transfusion in Australia.

Bacterial contamination of blood components, in particular PCs, continues to pose the highest transfusion infectious risk in industrialized countries. Measures implemented worldwide to mitigate that risk include donor screening, skin disinfection, first aliquot diversion, PC screening for the presence of bacteria, and pathogen inactivation technologies. M. Maclean et al. in the United Kingdom and the United States in their article "A New Proof of Concept in Bacterial Reduction: Antimicrobial Action of Violet-Blue Light (405 nm) in *Ex Vivo* Stored Plasma" described a method to inactivate bacteria in plasma samples based on the use of a 405 nm light emitting diode exposure system. This methodology does not require the use of photosensitizers as other currently used pathogen inactivation technologies. The authors showed 99% bacterial inactivation in low-volume plasma bags. Further validation is required to show system effectiveness in PCs, the blood component manifesting the highest risk for bacterial contamination.

Throughout this special issue, the authors described the challenges faced by blood product manufacturers to meet the highest quality and safety standards. Production of blood components is an evolving field and the development of new blood product containers, anticoagulants and additive solutions, and screening and treatment technologies require continuous optimization of procedures and consequent actualization of standards that govern the transfusion industry. Hopefully, the articles presented herein will stimulate the publication of other studies on current and underdevelopment practices related to improving the quality and safety of blood products for the benefit of transfusion patients.

Sandra Ramirez-Arcos
Denese C. Marks
Jason P. Acker
William P. Sheffield

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 4860284, 28 pages
http://dx.doi.org/10.1155/2016/4860284

Review Article

Quality Assessment of Established and Emerging Blood Components for Transfusion

Jason P. Acker,[1,2] Denese C. Marks,[3] and William P. Sheffield[4,5]

[1]*Centre for Innovation, Canadian Blood Services, Edmonton, AB, Canada*
[2]*Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada*
[3]*Research and Development, Australian Red Cross Blood Service, Sydney, NSW, Australia*
[4]*Centre for Innovation, Canadian Blood Services, Hamilton, ON, Canada*
[5]*Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada*

Correspondence should be addressed to William P. Sheffield; sheffiel@mcmaster.ca

Received 31 August 2016; Accepted 2 November 2016

Academic Editor: Maria Rios

Blood is donated either as whole blood, with subsequent component processing, or through the use of apheresis devices that extract one or more components and return the rest of the donation to the donor. Blood component therapy supplanted whole blood transfusion in industrialized countries in the middle of the twentieth century and remains the standard of care for the majority of patients receiving a transfusion. Traditionally, blood has been processed into three main blood products: red blood cell concentrates; platelet concentrates; and transfusable plasma. Ensuring that these products are of high quality and that they deliver their intended benefits to patients throughout their shelf-life is a complex task. Further complexity has been added with the development of products stored under nonstandard conditions or subjected to additional manufacturing steps (e.g., cryopreserved platelets, irradiated red cells, and lyophilized plasma). Here we review established and emerging methodologies for assessing blood product quality and address controversies and uncertainties in this thriving and active field of investigation.

1. Introduction

Blood component therapy became the standard of care in transfusion medicine throughout the industrialized world in the latter half of the twentieth century. The widespread adoption and retention of component therapy were driven by innovations in refrigeration, blood bag design, anticoagulant and preservative solution composition, infectious disease testing, and other means of donor screening [1]. The traditional trio of blood components are red cell and platelet concentrates and plasma, which may be generated either by the processing of whole blood donations or via apheresis. Whole blood is processed by centrifugation, predominantly by one of two main protocols which generate different intermediates: platelet-rich plasma (PRP) or a buffy coat (BC) [2]. White blood cells may be removed from blood components through the use of leukoreduction filters, often during blood processing and before storage [3]. Blood components require different storage conditions, with plasma being frozen, red cells being refrigerated, and platelets being maintained at ambient room temperature (RT) (see Figure 1 for an overall schematic diagram of component manufacturing). Blood component therapy remains widely practiced and widely supported for the majority of patients requiring transfusions; however in the trauma setting it has been suggested that whole blood may be superior to component therapy [4]. Although out of the scope of this review, this controversial concept is under active investigation. This article reviews issues, concepts, methodology, and challenges in assessing the quality of blood components and is not limited only to the traditional trio but also addresses emerging products such as cryopreserved platelets and lyophilized plasma. Below we explore each component in this context in detail, in no particular order.

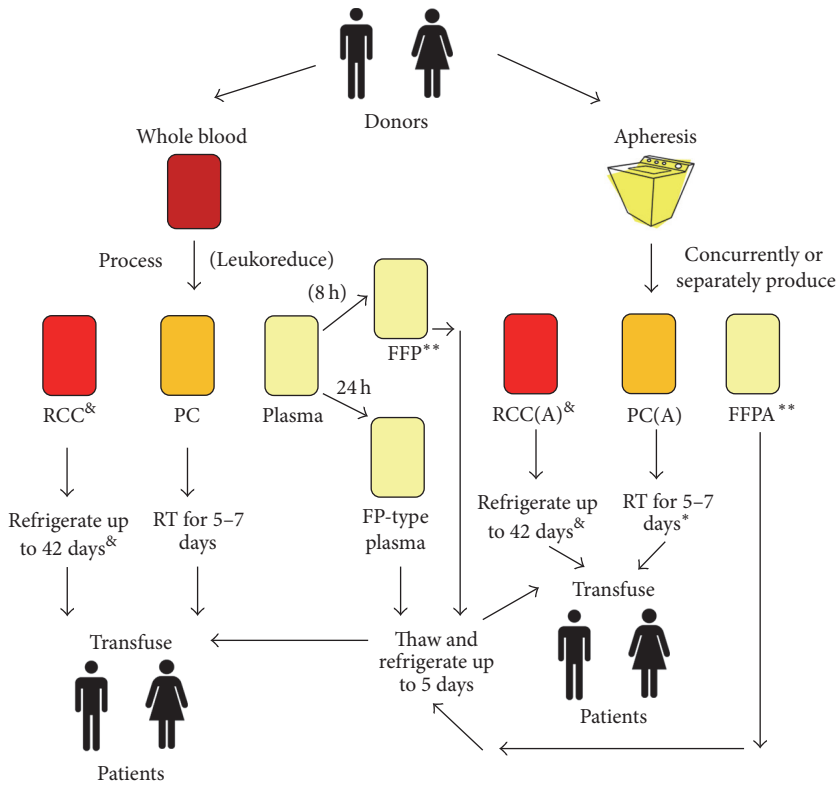


FIGURE 1: Schematic diagram of blood component manufacturing. Donations are either whole blood (left branch) or apheresis (right branch). At left, whole blood donations are processed into red cell concentrates (RCCs), platelet concentrates (PCs), or (transfusable) plasma, with or without leukoreduction by filtration. At right, apheresis donations (A) yield RCC(A), PC(A), or FFPA; some products may be made concurrently (e.g., FFPA and PC(A)). FFP is frozen within 8 hours in some jurisdictions or may be defined by quality control standards in others. FP-type plasma is frozen within 24 hours of phlebotomy. FFP or FFPA may be thawed and stored refrigerated up to 5 days prior to transfusion in some jurisdictions, while RCC or RCC(A) may be refrigerated no more than 42 days and platelets are typically stored at RT for 5–7 days, although *the FDA allows refrigeration and transfusion of cold-stored platelets for 72 hours; cryopreserved platelets are also under investigation. **FFP or FFPA may be further manipulated by drying or pathogen reduction treatment. &RCC may be further manipulated, for example, by washing, irradiation, or cryopreservation, in licensed procedures that may reduce shelf-life.

2. Overview: Assessing the Quality of Transfusable Plasma

Plasma is the liquid portion of an anticoagulated blood donation. Ideally, transfusable plasma would be manufactured under controlled conditions and assayed, prior to its release, for in vitro activities known to correlate with efficacy in each of its clinical indications. The first aspect has been consistently achieved, with impeccable control readily demonstrated by manufacturers. However, with respect to the second aspect, that of quality testing, there is currently no single test or combination of tests established to correlate with clinical efficacy of transfusable plasma. Moreover, quality testing is typically done on a portion of units selected from inventory during the product's shelf-life; prerelease testing is currently done only for transmissible disease screening [5]. Linking quality markers to outcomes of plasma transfusion has not been achieved to date, in large part due to the weakness of clinical evidence of efficacy for most indications for which plasma is transfused. Physicians nevertheless transfuse plasma frequently, relying for guidance on clinical experience and expert opinion. Manufacturers of transfusable plasma and some regulatory bodies therefore rely upon surrogate measures assumed to correlate with therapeutic efficacy. These measures typically relate to one or more labile coagulation factor whose decline might be an early indicator of decreased quality. Investigational quality assessment has evolved beyond the simple determination of regulated coagulation factor activities, towards the assessment of as many coagulation-related protein activities as is feasible, and some work has also been done on global assays of coagulation such as thrombin generation and viscoelastic testing.

2.1. Indications and Rationale for Plasma Transfusion. Most recommendations from national or professional bodies indicate therapeutic plasma transfusion for the correction of clotting factor deficiencies in patients who are bleeding or prophylactic transfusion for those judged to be at risk of bleeding [6–12]. The deficiencies may be of single clotting factors for which no appropriate concentrate is available to the treating physician, or of two or more clotting factors, in the setting of disseminated intravascular coagulation (DIC),

vitamin K antagonist reversal, liver disease, cardiopulmonary bypass (CPB), or massive transfusion. Plasma transfusion, or more specifically plasma exchange (PEX), is also indicated in the treatment of thrombotic thrombocytopenic purpura (TTP).

Most indications for plasma transfusion are tied to coagulopathy, defined by Hunt as "a condition in which the blood's ability to clot is impaired" [13]. Providing plasma by transfusion to remedy coagulopathy is biologically plausible, given that plasma contains all of the soluble coagulation factors, with the caveat that platelets and red cells also contribute to hemostasis, the balanced state in which blood loss from injury is quickly stemmed by clotting [14]. For plasma proteins for which no purified concentrate is available (e.g., Factor V or Protein S), transfusable plasma is the only replacement source available to the clinician treating such hereditary or acquired single factor deficiencies. In DIC and CPB, coagulation factor consumption and/or bypass pump fluid management procedures reduce circulating levels of multiple coagulation factors. Vitamin K antagonists such as warfarin reduce the plasma concentration of functional factors (F) II, VII, IX, and X (FII, FVII, FIX, FX) and Proteins C and S, all of which can be found in greater concentration in donor plasma than that of the warfarinized patient, if the pharmacotherapy needs to be rapidly reversed. As the liver is the site of synthesis of most coagulation factors and most coagulation inhibitors, plasma transfusion seems logical to assist the bleeding patient with liver disease, unless hemostasis has been rebalanced. In massive transfusion, usually defined based on the number of red cell units transfused, it is hard to envisage clinical care specialists being able to establish hemostasis without the provision of the coagulation factors found in plasma. Nevertheless, plasma is a relatively dilute source of many coagulation factors that can only be administered relatively slowly, and biological plausibility is no substitute for high-level clinical evidence to guide physician practice.

2.2. Evidence Base for Plasma Transfusion. High quality clinical evidence of plasma transfusion for most of its indications is sparse. In overlapping systemic reviews covering 1964–2011, Yang et al. [15] and Stanworth et al. [16] found 80 completed or near-completed randomized clinical trials (RCT) addressing the efficacy of this medical intervention. No picture of significant benefit across most clinical indications for plasma transfusion emerged, with most studies being judged to be underpowered. Murad et al. reported similar findings [17]. Prophylactic use of plasma transfusion in particular was found to be unsupported by evidence. There were sufficient trials in the area of cardiac surgery to perform a meta-analysis, but in this setting no benefit could be ascribed to plasma transfusion with respect to 24-hour postoperative blood loss [15].

Plasma exchange for TTP is arguably the indication for which the strongest evidence exists, specifically showing a survival benefit of plasma exchange (PEX) versus plasma infusion (PI) in two RCTs with appropriate randomization [18, 19]. Meta-analysis of these trials showed a significant reduction of mortality after seven PEX procedures, to about 31% of that seen with PI [20]. PEX may be effective in TTP due to removal of anti-ADAMTS13 autoantibodies, removal of pathological ultra-large von Willebrand factor (VWF) multimers, and/or via provision of transfused ADAMTS13.

In critically bleeding patients requiring massive transfusion, considerable retrospective data led many military and civilian trauma specialists to adopt formula-driven treatment protocols, in a ratio of one red cell unit (and platelet unit) to one plasma unit, over the last decade or so [21]. An RCT has now tested this approach: the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial [22]. This RCT randomized 680 patients arriving at Level 1 trauma centres in North America directly from the scene of their injury, and predicted to require massive transfusion, to either a 1 : 1 : 1 plasma : platelets : red cell unit treatment ratio or a 1 : 1 : 2 ratio. No difference in 24-hour or 30-day mortality, the primary outcomes, was found, although, in the ancillary outcome of cause of death in the first 24 hours, death by exsanguination was significantly reduced in the 1 : 1 : 1 group. The results may provide some support for a plasma-rich treatment approach.

Blood component therapy, including plasma transfusion, was introduced into medical practice prior to the establishment of modern evidence-based medicine. Most RCTs (excepting those in TTP) have failed to find evidence of benefit for plasma transfusion. It is not known whether this failure derives from a true lack of benefit or from limitations in trial design for those studies undertaken to date. This uncertainty and the sparseness of evidence complicate the task of assessing the quality of transfusable plasma as a biological product but have not discouraged the evolution of an extensive literature on plasma quality.

2.3. Nature and Amounts of Coagulation-Related Proteins in Plasma. Proteomics has demonstrated that human plasma contains thousands of different proteins, present over a range of concentration spanning 10–12 orders of magnitude [23, 24]. Coagulation-related proteins include fibrinogen and coagulation factors II (prothrombin), V, VII, VIII, X, XI, XII, and XIII, as well as von Willebrand Factor (VWF). ADAMTS13 is also of special interest due to its role in TTP. Major coagulation inhibitors include Proteins C and S, antithrombin, alpha-1-antitrypsin, and C1-esterase inhibitor. Among the fibrinolytic proteins, plasminogen and alpha-2-antiplasmin are the most abundant. Most of these proteins demonstrate a wide normal range in healthy blood donors. Reference ranges for coagulation factor activity levels (defined as the 95% confidence limit of the mean ± two SD) were found to vary between the smallest interval of 0.65–1.3 IU/mL for prothrombin and the largest interval of 0.40–1.9 IU/mL for FXII, in a study of over 400 normal men and women [25].

Which factors patients require, and how much of these factors need to be present in transfusable plasma, is unclear, because different plasma proteins appear to be present in excess of physiological requirements, with different reserve margins. FVIII levels between 0.05 and 0.40 IU/mL are considered to result in only a mild bleeding tendency. In contrast, fibrinogen levels only slightly below the lower limit of the normal range have been suggested as triggers for fibrinogen

supplementation in bleeding patients [26]. Apart from FIX and FVIII, most coagulation-related protein genes are not sex-linked but are instead found on autosomes. In consequence, their deficiencies are recessive and require mutation or inactivation of both gene copies; 50% levels of most coagulation factors, therefore, fail to cause clinically relevant symptoms. Rare individuals with genetic deficiencies of FV, FVII, FX, or FXIII typically do not present with a bleeding diathesis unless their circulating levels of these proteins fall below 0.05 IU/mL [27]; in contrast some individuals with prothrombin deficiency exhibit a bleeding diathesis with activity levels as high as 18.9% [28].

A threshold of coagulation factor levels of 0.30 IU/mL (30% of normal) is often cited as being sufficient to support clinical or surgical hemostasis. At least one textbook asserts this concept without attribution. Evidence in support of this estimated threshold appears to come from two sources: a 1961 review of surgical experience in patients with single factor deficiencies [29] and surgical studies from the 1980s and 1990s in which shed blood was replaced with plasma-poor red cell concentrates [30]. Aggeler found coagulation factor levels of 20–30% of normal sufficient to avoid bleeding complications during surgery in single factor deficiency patients [29]. Murray et al. replaced blood losses occurring during major elective surgery with colloid solutions and packed washed red cells to maintain a hematocrit of 30% [30]. Replacement of one blood volume did not provoke bleeding from incision edges or bleeding judged to be atypical for a specific surgery, in a small series of 12 patients. Excessive bleeding, as clinically defined, was found after greater than one calculated blood volume replacement. Hiippala et al. performed a similar study in 60 patients undergoing major urological or abdominal surgery, finding by regression analysis that persistent oozing of incision edges or surgical fields occurred after 118 ± 17% blood volumes, when prothrombin levels were 34 ± 5% of normal, similar to the expected 70% pan-factor depletion calculated on the basis of exchange of one volume of blood with equivalent packed red cells [31]. Erber summarized these studies as defining a critical level of 30% clotting factor activity required to maintain hemostasis, also defining a fibrinogen concentration of 1.0 g/L and a platelet count of 100×10^9/L as critical values [32].

The 30% threshold concept provides a potential explanation for the difficulty in demonstrating the clinical benefit of plasma transfusion in coagulopathy: the "zone of therapeutic anticoagulation" is relatively narrow [33]. Many patients feared to be at risk of bleeding on the basis of elevated laboratory clotting time values are not actually at risk of bleeding; and some patients with severely traumatic blood losses are not salvageable. In the plasma quality field, such uncertainties have given rise to competing perspectives. If the goal of plasma transfusion therapy is to restore clotting factor levels to 30%, then from first principles this goal can be achieved more rapidly, and with the infusion of lower volumes of plasma, if mean factor levels in the product are as high as possible (i.e., 100% versus lower levels). On the other hand, if some coagulation proteins are less important determinants of coagulability than others, then too great an attention to their preservation could be wasteful in terms of the efficiency of donated blood processing.

2.4. Labile Coagulation Factors. Some coagulation factors decline more rapidly during plasma processing or storage than others. In 1979, Counts et al. reported that FV and FVIII were the only clotting factors to decline to less than 50% of initial values in modified whole blood from which platelets had been removed, over 14 days of refrigerated storage [34]. In 1984, Kakaiya et al. reported that extending the refrigeration of whole blood from 6 to 18–20 hours prior to separation of plasma led to significant losses in FVIII, but not FV activity [35]. More recently, in a multicentre, Biomedical Excellence for Safer Transfusion (BEST) Collaborative study employing a pool-and-split design, whole blood was cooled to ambient temperature (18–25°C) and processed into plasma under two conditions: less than 8-hour hold or 24-hour hold. FVIII was the most affected factor, declining 23% on average relative to the shorter hold; FV, FVII, FXI, FXII, FXIII, VWF, and antithrombin were unaffected. FII, FIX, and FX declined <5%, while Proteins C and S lost 6 and 14% activity, respectively [36]. A stability study of thawed refrigerated plasma over 120 hours showed that FVIII and FV activity significantly declined, by 8.5% and 27% within 24 hours, but that fibrinogen remained stable and that FVII remained stable for 48–72 hours of refrigerated storage [37]. Overall, the results suggest that FVIII is the most consistently labile of the major coagulation-related proteins in plasma, with FV, Protein C, and Protein S showing some instability in some studies.

In spite of the reproducibility of FVIII instability, its molecular cause is not well understood. FVIII is a heterodimer stabilized in plasma by copper ions and via binding to VWF [38]. Loss of the copper ion leads to dissociation and loss of activity. Activation of FVIII to FVIIIa by controlled proteolysis by thrombin also promotes dissociation from VWF. FVIIIa loses activity spontaneously due to dissociation of its A2 domain or via proteolytic attack by Activated Protein C. It is not known whether storage in anticoagulated blood or plasma promotes loss of activity through either mechanism or whether the instability of FVIII is due to alternative causes.

2.5. Plasma versus Modified Plasma for Transfusion. Plasma for transfusion is obtained either by processing of anticoagulated whole blood or from apheresis systems. Most blood operators exploit the ability to freeze plasma to extend its shelf-life, although never-frozen liquid plasma is licensed in some locations (e.g., the USA) [39]. Thawed plasma may be stored in a refrigerated state for lengths of time varying from hours to days, depending on the jurisdiction and the specific details of how it was handled and frozen prior to thawing. Several modified versions of plasma also offer enhancements over the traditional products with respect to ease of administration or safety. Dehydrated plasma is stable at room temperature and can therefore be more easily transported into austere environments remote from hospitals, rapidly rehydrated, and administered in the field [40]. Specifically, lyophilized plasma is available for military and/or civilian use

in some countries, and spray-dried plasma is under investigation. Pathogen-reduced plasma is also in routine clinical use in many countries; this product comprises plasma to which a nucleic acid-damaging additive that can be photochemically activated has been added. Quality comparisons of modified plasma to standard plasmas are readily available, but they suffer from the same difficulties in terms of extrapolation to predicting clinical efficacy of the standard plasmas, due to the unsatisfactory evidence base [16].

2.6. "Standard" Forms of Transfusable Plasma and Regulations: FFP and FP-Type Plasma. Transfusable plasma units vary with respect to the time from phlebotomy to the frozen state and the methods of preparation. Fresh-frozen plasma (FFP) may be prepared by apheresis (FFP apheresis, FFPA, or apheresis FFP, AFFP) or whole blood processing and must be frozen within 8 hours of phlebotomy in Canada [41] and the United States [42]; in Australia, freezing of FFPA must commence within 6 hours of collection and that of FFP within 18 hours of collection [43]. In Europe, plasma may be labeled as FFP if it is separated from whole blood and frozen within 6–18 hours of phlebotomy and provided that it contains ≥0.70 IU FVIII activity/mL; whole blood may also be used for FFP manufacture for up to 24 hours after phlebotomy, provided that it is rapidly cooled and held at 20 to 24°C and that it satisfies the FVIII activity requirement [10]. Frozen plasma (FP, also known as FP24 or PF24) must be frozen within 24 hours of phlebotomy and is employed in Canada and the United States. Canada specifies that 75% of units tested must contain ≥0.52 IU FVIII activity/mL and that 1% of monthly site production must be tested [41]; neither American government regulations nor AABB standards specify required activity levels. The United States Food and Drug Administration (FDA) recognizes two forms of FP: PF24 and PF24RT24 (plasma frozen within 24 hours after phlebotomy held at room temperature up to 24 hours after phlebotomy). PF24 must be refrigerated or be made from whole blood refrigerated within 8 hours of phlebotomy and frozen within 24 hours of phlebotomy. PF24RT24 must be made from apheresis devices, whereas PF24 may be manufactured using either whole blood processing or apheresis.

In general, FP, PF24, and PF24RT24 (collectively called FP-type plasma in this review) may show diminished levels of some labile coagulation proteins compared to FFP. Most recommendations concerning plasma transfusion do not stipulate a difference in indications between FFP and FP-type plasma, except where these products are transfused for the treatment of individual deficiencies of labile clotting factors such as FV, FVIII, Protein C, and Protein S. In such instances, FFP is favoured over FP-type plasmas as they have been shown to have lower levels of labile factors. Given the widespread availability of FVIII concentrates and the availability of Protein C concentrates in some jurisdictions, operational differences between FFP and FP-type plasmas reduce to a theoretical preference for FFP in FV and Protein S deficiencies alone.

Those regulatory bodies that focus on FVIII activities presumably do so because FVIII is the most labile of the coagulation factors. However, the connection between FVIII activity levels and the ability of transfused plasma to restore hemostasis is dubious. Firstly, FVIII appears to be present in considerable excess in healthy individuals; reductions below 0.4 IU/mL FVIII activity are needed on a chronic basis to show any clinical signs of pathology [44]. Secondly, FVIII is an acute phase reactant [45] whose levels are expected to rise in injury or disease. Thirdly, FVIII levels in massively transfused surgical patients decline less rapidly than other factors as the number of transfused red cell units increases [34].

Cryoprecipitate is a blood product derived from frozen plasma by slow thawing at refrigerated temperatures. Under these conditions large adhesive proteins reversibly precipitate and are captured in a small volume following expression of cryosupernatant. Historically cryoprecipitate served as a source of FVIII/VWF, but it is now employed primarily as a source of fibrinogen where fibrinogen concentrates are not available or not nationally indicated. Plasma quality indirectly affects the quality of cryoprecipitate, which remains in use in the USA, UK, Canada, and Australia, but which has been replaced by fibrinogen concentrates in much of Western Europe [46]. UK regulations require that 75% of units tested contain >140 mg fibrinogen and 70 IU/mL FVIII [47], Canada mandates at least 150 mg of fibrinogen per unit [48], the FDA stipulates a minimum of 150 mg fibrinogen and 80 IU FVIII per bag [46], and Australia specifies the same content of fibrinogen and FVIII as the UK, as well as a minimum of 100 IU VWF per unit [43].

2.7. Measures of Plasma Quality. Plasma quality is currently assessed in vitro, using coagulation factor and coagulation-related protein assays. Investigators have employed single factor assays, more global hemostasis tests, thrombin generation, or viscoelastic approaches to characterize different forms of plasma.

2.7.1. Coagulation Factor Assays. Most investigators addressing plasma quality issues have employed one-stage coagulation tests. In these assays, anticoagulated plasma samples are recalcified in the presence of factor-depleted plasmas, with coagulation being initiated by the addition of either tissue factor and anionic phospholipids (prothrombin time- (PT-) based assay) or a silicate or other negatively charged polymers combined with anionic phospholipids (activated partial thromboplastin time- (APTT-) based assay). The time to form a clot in such assays is then measured, and factor activity levels in the test plasma are correlated to standard plasma sample clotting times. Most investigators in this field employ automated coagulation analyzers.

2.7.2. Hemostasis Screening Tests. Two in vitro clotting tests are widely used by hematologists to screen patients for bleeding disorders or to monitor drug therapy: the PT and the APTT [49]. These tests use the initiators of coagulation defined above for the coagulation factor assays, but simply on recalcified plasma. Both have also been employed in investigations of plasma quality. However, these tests are typically insensitive to reductions in single coagulation factor activity of less than 50%. The APTT is best suited to the initial

investigation of suspected hemophilia or the monitoring of heparin or heparinoid drugs used to counter thrombosis. The PT is best suited to monitoring drugs like warfarin that disrupt vitamin K antagonism and reduce the functionality of vitamin K dependent proteins, for antithrombotic benefit. The PT has been standardized as the international normalized ratio (INR), which compares patient PT clotting times to the geometric mean of a group of healthy controls of both genders and which includes a factor related to the potency of the tissue factor preparation used to initiate the test. Both APTT and PT may be performed rapidly as they can be completed in less than one minute per sample, but several commentators have noted that the use of these tests to guide transfusion practice constitutes a use for which neither was designed [50].

2.7.3. Thrombin Generation. The thrombin generation assay (TGA) relies on thrombin cleavage of a fluorogenic substrate to follow changes in thrombin levels in test plasma during a 30- to 60-minute period. It is initiated with either low concentrations of tissue factor for extrinsic pathway activation or negatively charged biopolymers such as ellagic acid or kaolin for intrinsic pathway activation and is accelerated through the inclusion of anionic phospholipids. TGA features a lag phase, a period of increasing thrombin generation, and a period during which the amount of thrombin being generated declines back to baseline. Time to peak, peak thrombin concentration, and endogenous thrombin potential (ETP, the area under the thrombin versus time curve) are typical calculated parameters. For plasma quality determination, the test is performed in plasma rather than whole blood. Although useful for relative within study comparisons, such as before and after manipulations such as pathogen reduction, TGA is currently viewed as being too variable for routine clinical use, a criticism that may have implications for use in plasma quality investigations [51]. It is certainly a much more time-consuming test than "time to clot" assays.

2.7.4. Viscoelastic Testing. Viscoelastic testing refers to thromboelastography (TEG) or rotational thromboelastometry (ROTEM) [51, 52]. Both technologies record viscoelastic changes during blood or platelet-rich or platelet-poor plasma clotting by following the changes in oscillation of a pin or wire immersed in the test fluid; in TEG the cup is mobile and the pin or wire immobile, while in ROTEM the mobilities are reversed. Either assay can be initiated using kaolin and phospholipids or tissue factor. Test runs can require up to 30 minutes for completion. Both technologies have been used as point-of-care tests to guide hemostatic therapy and have been employed in pivotal trials of fibrinogen concentrates [53].

2.8. Manipulations of Donated Blood or Plasma with the Potential to Affect Clotting Activity. Plasma samples for reference range determinations are taken under ideal conditions, in which a small volume of blood is taken from a volunteer into vacutainer tubes and immediately centrifuged, and the plasma is aspirated and snap-frozen for subsequent assay. Transfusable plasma, in contrast, is collected on a larger scale and subjected to additional manipulations, all of which have the potential to decrease one or more coagulation factor activities. Many investigations of plasma quality have focused on the effect of such manipulations, to address the question of whether or not a perceived benefit, such as leukoreduction to reduce transfusion reactions, extended holds of whole blood prior to processing to improve blood operator efficiency, or extended storage of thawed plasma to reduce wastage, is worth the cost of reduced coagulation-related protein activity.

2.8.1. Leukoreduction. Prestorage leukoreduction of whole blood has been implemented for all blood donations in various European countries, in Canada, and by the majority of American blood operators. Benefits include reductions in the frequency and severity of febrile nonhemolytic transfusion reactions (FNHTRs); in the risk of cytomegalovirus transmission; and in the risk of alloimmunization and platelet refractoriness [3]. Cardigan et al. examined five different whole blood filters, using a pool-and-split design, and processed filtered or nonfiltered whole blood into plasma. Some filters had no effect on any tested coagulation factor activity, including fibrinogen or prothrombin; others caused a modest increase in median PT or APTT of less than one second. Filters associated with a reduction in coagulation factor activity decreased median FV, FXI, and FXII activity by 13–20%, with no effect on VWF and only a 5% reduction in median FVIII activity [54]. Heiden et al. reported similar findings, along with the observation that elastase released from neutrophils during leukoreduction was fully neutralized by alpha-1-antitrypsin and that complement-related C3a was found in plasma from filtered blood as inactive C3a-desArg [55]. Most recently Chan and Sparrow split whole blood into pediatric-sized packs and applied 6- or 24-hour holds with or without leukoreduction prior to plasma production [56]. These authors found that the leukoreduction filter they employed partially trapped microparticles and its use was associated with 15–20% reductions in fibrinogen, FVIII, and FXII activities. Leukoreduction slowed clot formation and reduced clot strength as judged by kaolin-initiated thromboelastography; whole blood hold time did not affect these parameters despite the reductions in clotting factor activities.

2.8.2. Holding Effects. Holding blood at either refrigerated or ambient temperatures provides considerable logistical advantages to blood operators, with respect to the ability to collect at remote clinics and to rationalize shift work by processing staff. Effects of hold times on component quality have been reviewed by van der Meer and de Korte [57]. Pietersz et al. reported mean losses of 20% of FVIII activity in whole blood units ($n = 10$) rapidly cooled to 20–24°C and held for 24 hours prior to plasma production via the buffy coat method without leukoreduction, less than that reported in refrigerated whole blood held for 26 hours in a smaller study ($n = 5$) [58]. O'Neill et al. split whole blood units ($n = 10$) into half-units and stored them at 4°C or 22°C for 8 hours and then refrigerated all half-units for the next 16 hours, making plasma from all units at 8 or 24 hours via the platelet-rich plasma (PRP) method. FV, FVII, FX, fibrinogen, and Proteins C and S activities in plasma were unchanged relative to baseline, at collection values [59]. Eight-hour storage reduced

mean FVIII activity 13% relative to baseline values, and twenty-four-hour storage reduced it by an additional 20% [59]. Wilsher et al. allocated 80 whole blood donations into four equal groups to assess FFP production within 8 hours or after 24-hour holds at refrigerated or ambient temperatures, finding no effect on fibrinogen activities of any condition, and only a modest loss of FV activity associated with 24-hour ambient temperature hold. FVIII activity losses were limited to 21% relative to <8-hour processing with or without active cooling and were significantly less than the 36% mean reduction in activity seen in 24-hour refrigerated hold units [60]. van der Meer and de Korte also reported no effect of active cooling of whole blood held overnight on FVIII activity in generated plasma [61]. These results on holding effects have in general been replicated by investigators using different whole blood processing methods and different anticoagulants in different nations and transfusion services [36, 62–66]. There is no evidence that the declines in coagulation factor activity associated with holding effects compromise clinical efficacy, and therefore economic and strategic considerations have prompted greater use of FP-type plasmas over FFP [42, 67].

2.8.3. Speed of Freezing. Initial studies on the effect of the speed of freezing of plasma focused on FVIII activity, since efficient recovery of FVIII was of paramount importance in the fractionation industry prior to the advent of recombinant FVIII. Carlebjork et al. noted a positive correlation between the rate of freezing of plasma and the recovery of FVIII activity [68]. Swärd-Nilsson et al. examined 100 mL aliquots of plasma in plastic bottles. FVIII activity in plasma was found to be stable to prefreezing room temperature holds of up to 4 hours. Rapid freezing to a core temperature of −30°C within 60 minutes, as compared to slow freezing over 24 hours, resulted in mean FVIII recoveries of 93.6 versus 68.2% [69]. Runkel et al. pooled and split whole blood donations, generating plasma within 8 hours of phlebotomy that was frozen at the 8-hour time point or held at either ambient or refrigerated temperatures for an additional 16 hours prior to freezing [70]. Freezing was conducted either in slow (transfer to −20°C) or in fast (to −30°C core temperature) modes to imitate FDA or European requirements, respectively. Prolonging the "time to freezer" reduced median FVIII levels ~10%, while combining prolonged time to freezer with slow freezing together reduced median FVIII levels 18.5%. Room temperature storage reduced median Protein S activity by 27%, and prolonged storage at room temperature correlated with a 7% decline in FVII activity. No changes were noted in FV, FXI, fibrinogen, VWF, or Protein C activities. APTT and PT values were modestly increased by prolonged time to freezer and slow freezing. In general, available data suggests that rapid freezing and limiting the time from phlebotomy to freezer best preserve coagulation activity in transfusable plasma, but it does not seem that such steps give any clinical advantage to FFP over FP-type plasmas. Where permitted by regulations, the selection of lower cost slow freezing modalities for transfusable plasma has therefore not been impeded.

2.8.4. Stability of Thawed Plasma for Transfusion. Plasma takes time to thaw and time to infuse. Dose recommendations in the 10–15 mL/kg range translate into 700–1050 mL of plasma for a 70 kg individual or 3-4 units [33]. Two factors spurred interest in the question of how long thawed plasma may be refrigerated prior to use in an effective and safe transfusion: efforts to reduce transfusion-related acute long injury (TRALI) and efforts to facilitate early administration of plasma to trauma patients. Shifts to predominantly male transfusable plasma were made by several blood operators to reduce one factor contributing to TRALI, anti-HLA antibodies in previously pregnant donors [71]. This move put supply pressure on FFP and increased interest in prolonged use of thawed FP-type plasmas. Similarly, concern over the development of trauma-induced coagulopathy and a perceived need to start plasma transfusion as soon as possible led many trauma centres in the industrialized world to adopt massive transfusion protocols, involving, in part, the maintenance of thawed inventory of AB-universal donor plasma [72].

Downes et al. carried out a small study ($n = 5$ for each of 3 ABO blood groups) of thawed FFP stability after AABB standards expanded its shelf-life from 24 to 120 hours. No significant differences from day 1 to day 5 activity values were noted for FII, FV, FVII, FX, or fibrinogen. FVIII values dropped 35–41% during the storage period, with the majority of the decline occurring in the first 24 hours of storage [73]. Scott et al. examined 20 FFP units at thaw or after 120 hours of refrigerated storage, noting a 47% drop in FVIII activity as well as losses of FV and FVII activity of 21 and 33% and smaller losses of 3–8% in FII, FX, VWF, and Protein S activities [74]. Scott et al. also examined the stability of 14 FP24 units, noting similar patterns; the largest losses in this product were for FV, FVIII, VWF, and Protein S (31, 28, 17, and 15%, resp.) [74]. Sheffield et al. probed the stability of Canadian thawed FP ($n = 54$), produced using the buffy coat method, finding it noninferior in residual FVIII activity at 120 hours to the 0.48 ± 0.12 IU FVIII/mL reported by Sheffield et al. from PRP method FP24; mean losses of 20, 14, and 41%, in FV, FVII, and FVIII, respectively were observed, with no alteration in fibrinogen activity and a 9% prolongation of PT by 120 hours [37]. Similar results were obtained in stability studies of FFPA: Sidhu et al. found FV and FVIII decreases of 9 and 14% after 120 hours of refrigerated storage and no change in FII, FVII, FX, FXI, and fibrinogen ($n = 20$, sodium citrate anticoagulant) [75]; and Von Heymann et al. noted losses of FII, FV, FVII, FVIII, FIX, FX, and FXI ranging from −8% (FII) to −47% (FVIII) ($n = 20$, acid citrate dextrose [ACD] anticoagulant) after 144 hours of refrigerated storage [76]. Cookson et al. observed mean losses of 11% FV and 33% FVIII activities relative to baseline values and lesser declines of FII, FVII, FIX, and FXII after 144 hours of refrigerated storage of thawed FP made from whole blood held at room temperature for 24 hours. These factor losses failed to affect thrombin generation and produced only marginal lengthening of clot time, but no change in clot strength, in viscoelastic testing [77].

These data have been interpreted in different ways in different jurisdictions, likely due to the lack of clinical data with which to anchor them to outcome measurements.

American and Canadian regulators permit the transfusion of refrigerated thawed plasma stored for up to 120 hours, whereas the Council of Europe requires transfusionists to administer the product as soon as possible after thaw, and in the United Kingdom 24-hour refrigerated storage is permitted [78].

2.9. Pathogen-Reduced Plasma. Plasma may also be subjected to additional manipulations designed to increase the considerable protection already afforded to patients by its prerelease immunological and nucleic acid testing for transfusion-transmitted pathogens [5]. These include treatments of pooled plasma with solvent-detergent mixtures [79] and of individual plasma units with agents such as methylene blue or amotosalen, which must be removed prior to infusion after illumination of units with ultraviolet or visible light, or riboflavin (vitamin B2), for which there is no removal requirement [80]. Quality assessment of these products has been largely limited to comparisons to the FFP from which they are derived. Efficacy determinations have included clinical studies, including those leading to licensure on the basis of similar performance to FFP.

2.9.1. Pathogen Reduction of Pooled Plasma. Solvent-detergent plasma (SDP) is a pooled plasma product subjected to solvent (tri(n-butyl)phosphate) and detergent (Triton X-100) treatment to destroy enveloped viruses; prior to filtration, bagging, and freezing, the solvent and detergents are removed by oil and solid phase extraction steps [79]. The process was originally invented by scientists at the New York Blood Center [81]. Although several SDP are licensed in different countries, most of the information concerning SDP in the public domain relates to Octaplas, the SDP product manufactured by Octapharma GmbH (Vienna, Austria), which has been in continuous clinical use in Europe since 1992, and elsewhere for lesser periods of time. Unpaired comparisons of 12 consecutive batches of Octaplas and 12 random quarantine FFP units revealed no significant differences in FII, FV, FVIII, FIX, FX, FXII, FXIII, VWF, antithrombin, or Protein C activities [82]. Minor reductions in fibrinogen and FVII were noted, and substantial reductions in mean Protein S activity (38%) and alpha-2 antiplasmin (78%) were also observed. In 2009 the manufacturing process for Octaplas was modified to include a prion reduction affinity chromatography ("liquid gel") step (Octaplas LG). After implementation of this change, no substantial differences were noted between batches of the first or second generation product by coagulation factor assays, thrombin generation, or viscoelastic testing [83].

2.9.2. Pathogen Reduction of Single Plasma Units. Three technologies, licensed in at least some parts of the world, are available for pathogen reduction (PR) of single plasma units [80]. All rely on photoactivation by visible or ultraviolet light and the addition of chemicals to plasma or PRP: methylene blue (MB); amotosalen (AS); or riboflavin (RF). All attack pathogen nucleic acids in different ways, reducing infectivity by many orders of magnitude. All lead to some reductions in coagulation factor content as a potential cost of increased safety. These effects have been most clearly demonstrated in paired comparisons of FFP, MB-FFP, and AS-FFP. Osselaer et al. found that losses of fibrinogen, FV, FVIII, FXI, and Protein S activities were greater for MB-FFP than AS-FFP, although the most affected proteins, fibrinogen and FVIII, still retained 70–80% of their FFP activity levels after treatment and freezing and thawing of MB-FFP. The authors noted that the mean values for all 18 plasma protein activities tested fell within normal references ranges in all cases and that ADAMTS-13 was unaffected by either PR treatment [84]. Backholer et al. reported similar findings in another paired comparison in which FV, FXI, FXIII, and fibrinogen losses were significant for MB-FFP, but not for AS-FFP, by analysis of variation (ANOVA) of FFP, MB-FFP, and AS-FFP; six other coagulation factors were unaffected by either treatment [85]. Cardigan et al. noted reductions of 10% in ETP and 30% in peak thrombin in thrombin generation assays of paired MB-FFP versus FFP, but no effect on clot formation rate and a surprising 20% increase in clot firmness by ROTEM [86]. Hubbard et al. noted alterations in fibrin clot structure for both AS-FFP and RF-FFP [87].

Hornsey et al. examined the recovery of coagulation-related proteins before and after production of RF-FFP (n = 20) [88]. While Protein S and antithrombin were unaffected by riboflavin treatment, ten other proteins exhibited diminished recoveries, with the lowest recoveries being noted for fibrinogen, FVIII, FXI, and ADAMTS13 (68–79%). FVIII activity levels nonetheless met Council of Europe requirements, at 0.76 IU/mL in the study, as did all other factors.

2.10. Dehydrated Plasma. Plasma was originally introduced into clinical practice, during World War II, as a lyophilized product appropriate for battlefield use [40]. Such dehydrated plasma formulations attempt to replace conventional plasma with a product that does not require a cold-storage chain and which can be reconstituted more rapidly than frozen plasma can be thawed. Plasma may be dried either in pools (with or without pathogen inactivation) or as single units. The most common approach is freeze-drying, or lyophilization, a process by which plasma is rapidly frozen and maintained at low temperatures under partial vacuum. Sufficient heat is then introduced such that frozen water in the product is driven off via sublimation. An alternative approach involves spray drying, in which atomization and heat are used to evaporate microdispersed water droplets. Both technologies provide stable, reversibly dried products in which >95% of the original water content is removed [89].

The two best characterized freeze-dried plasma (FDP) products licensed for use in Western countries are French lyophilized plasma (PLyo) [90] and LyoPlas N-w, produced by the German Red Cross Blood Service West [91]. The former product is made from a pool of A, B, and AB pathogen-inactivated FFPA plasmas from no more than 10 donors, with each unit containing ≥0.9 IU/mL FVIII activity; the latter is made from a single unit of quarantined plasma. Quarantined plasma is not released for manufacturing until the donor has returned to the blood or plasma centre in good health and tested negative for infectious disease markers a second time.

Martinaud et al. tested aliquots of 24 batches of PLyo before and after lyophilization [92]. Fibrinogen, FXI, FXIII,

Proteins C and S, antithrombin, and alpha-2 antiplasmin were unaffected by the process. Factors V and VIII exhibited lyophilization-dependent losses of 20–25%. No differences were found between the two kinds of plasma in thrombin generation assays initiated with 5 pM tissue factor and no differences were found in viscoelastic testing.

Bux et al. described aspects of the quality of LyoPlas N-w, which is the single donor, quarantined plasma-derived German FDP [91]. Compared to the thawed FFP from which the product was made, lyophilization had no effect on fibrinogen, Protein S, or antithrombin activities. Ten to 25% losses in activity were noted for factors V, VIII, XI, vWF, and plasminogen, as well as a 10% prolongation in aPTT. This product has been extensively used in both military and civilian settings; from 2007 to 2011 more than 230,000 units were delivered to hospitals and doctors' offices and provided to the Germany Army. However, no true measures of clinical efficacy appear to have been reported in the public literature.

2.11. Fractionation versus Transfusion of Plasma. Plasma does not have to be transfused to be medically useful. Recovered or source plasma can be fractionated into purified plasma protein products such as immunoglobulin or coagulation factor concentrates [93]. Immunoglobulin therapy clearly benefits some patients, such as those with primary or secondary immune deficiency, immune thrombocytopenia, and chronic inflammatory demyelinating polyradiculoneuropathy [94]. vWF [95] or fibrinogen concentrates benefit patients with deficiencies of these factors [96], and prothrombin complex concentrates reverse warfarin therapy more rapidly than plasma transfusion with respect to laboratory values and more effectively with respect to restoration of hemostasis [97]. Fibrinogen concentrates and PCCs may provide superior therapy compared to plasma in bleeding patients without genetic deficits due to the possibility of rapid restoration of hemostasis and are under active investigation. Even in TTP, a recombinant ADAMTS13 is in preclinical development, a product that may in future supplement the most evidence-supported indication for plasma transfusion [98]. Given the paucity of evidence of benefit for the medical use of transfusable plasma and the existence of high-level clinical evidence of efficacy for some of the indications for which fractionated products are employed, it is likely that an increasing proportion of plasma will be fractionated rather than transfused.

2.12. Appropriateness of Plasma Transfusion and Utilization Trends. The amount of plasma transfused in several countries has declined in recent years, including Canada, the United Kingdom, and the United States [99, 100]. The decline has been linked to efforts to increase appropriateness of plasma transfusion, to the increased availability of plasma protein products such as PCCs, and to improvements in surgical techniques. It seems likely that this trend will continue, barring new clinical data supporting plasma transfusion.

Although there is disagreement among physicians as to the value of plasma transfusion and some indications are supported by weaker evidence than others, there is reasonable expert consensus as to uses of transfusable plasma which are inappropriate [33]. Multiple audits of transfusable plasma utilization have shown that over 30% of the time, plasma is transfused inappropriately, typically in efforts to alter a mildly elevated INR and to nonbleeding patients [101–104]. Although most studies of plasma quality end with an exhortation to trialists to obtain better RCT data, it is likely that improvements in plasma utilization could be achieved more readily by diminishing inappropriate transfusion of plasma.

2.13. Future Prospects for Quality Assessment of Transfusable Plasma. An extensive literature exists regarding the effect of different manipulations or process changes on coagulation factor activities in transfusable plasma or its derivatives. However, linking these changes to differences in overall hemostatic function of plasma has not been extensively attempted. Available data using more global tests of hemostasis such as thrombin generation and viscoelastic testing has started to suggest that many of the observed alterations in one or more coagulation factors are not particularly relevant to hemostasis, given its complexity and the number of mechanisms that can combine to adapt to changes in procoagulant and/or anticoagulant protein profiles within the large functional reserve of this biological fluid. If thrombin generation and viscoelastic tests can be better correlated with patient clinical status and adapted to more rapid execution, such assays may supplant coagulation factor assays in the effort to answer the elusive question, "is this unit of transfusable plasma of high quality?"

3. Quality of Platelet Concentrates

3.1. New Modes of Platelet Storage: What Should We Be Measuring? Platelet transfusions are essential for the treatment of patients with acute bleeding or hemorrhage and for prevention of bleeding in severe thrombocytopenia [105–107]. When the vasculature is damaged, platelets adhere, aggregate, and become activated, eventually forming a platelet plug, as well as providing a catalytic surface for thrombin generation. Platelet concentrates (PCs) for transfusion are typically prepared either from whole blood or by apheresis and are stored at RT (20–24°C) for up to 7 days. The shelf-life of platelets stored at RT is limited due to the risk of bacterial growth and contamination, which can cause life-threatening transfusion-related infections [108]. Additionally, platelets stored at RT gradually deteriorate and undergo a decline in hemostatic and metabolic function, which is known collectively as the platelet storage lesion [109–112].

Due to the short shelf-life of PCs, providing platelets to remote, rural, and austere environments is often challenging. Alternative storage modalities such as cryopreservation and cold or refrigerated storage are currently being explored to overcome the challenges associated with the short shelf-life of PCs. It is clear that platelets stored under these conditions appear to be very different from standard, RT-stored platelets, when assessed using in vitro assays previously applied to RT-stored platelets alone [113–115]. Further, cryopreservation of platelets is a relatively new field, and while cold storage of platelets has been studied for many decades, there is a

renewed interest in this storage mode, and the in vivo efficacy of these novel platelet components is only now being investigated. Standard measures of activation and metabolism are not always sufficient for cold or cryopreserved platelets and generally do not correlate well with in vivo transfusion outcomes. A well-defined panel of assays to measure the in vitro quality of these components has not yet been established. This section of the review will explore the differences between platelets stored frozen, refrigerated, or at RT and will address the techniques that may be most appropriate to measure their in vitro function and quality.

3.2. Assays for In Vitro Assessment of Room Temperature-Stored Platelets. Many techniques can be used to measure platelet function, metabolism, and quality in vitro. The simplest measurement is platelet swirl, whereby platelets with a discoid shape refract light and hence appear to swirl when exposed to a light source, whereas platelets that have lost their discoid morphology do not swirl [116]. Hypotonic shock response (HSR) is based on the ability of platelets to extrude water when placed in a hypotonic solution, is reflective of an intact membrane, and typically declines during ex vivo platelet storage [117–119]. Extent of shape change (ESC) is a photometric measurement of platelet shape change in response to agonists such as ADP and an indicator of the ability of platelets to maintain their discoid morphology. As is the case with HSR, the ESC response of platelets declines during storage [117, 120, 121]. Lactate dehydrogenase (LDH) release into the supernatant can be measured as an indicator of platelet lysis and loss of viability [122, 123], often accompanied by a reduction in platelet count and swelling, measured by an increase in mean platelet volume (MPV).

Platelets produce energy through two major metabolic pathways: anaerobic glycolysis in the cytoplasm and oxidative phosphorylation in the mitochondria, which contributes approximately 80% of platelet ATP [124]. Therefore, measurement of glucose consumption, lactate production, and changes in pH during platelet storage are widely used as indicators of active metabolism, as are measurements of pO_2, pCO_2, and HCO_3 [123, 125–128]. Platelet surface markers are also indicative of platelet activation, including CD62P (P-selectin) and exposure of phosphatidylserine (PS) measured by annexin V binding, and are typically measured by flow cytometry [129, 130]. As platelets become activated, the contents of their granules, such as RANTES, CD40L, soluble CD62P, and platelet factor 4 (PF4), are released and accumulate in PCs during storage [122, 123, 129–131].

As part of the platelet storage lesion, platelets also lose their ability to respond to agonists such as ADP, collagen, and thrombin, which can be measured photometrically using light transmission aggregometry [126, 132–134]. However, the loss of ability to respond to agonists is reversible, and platelets have been shown to recover their capacity to respond to agonists following transfusion [135]. Similar instrumentation can be used to measure HSR and extent of shape change ESC. Assays that measure platelet hemostatic function and procoagulant capacity can also be applied to RT-stored platelets. These will be discussed in more detail in subsequent sections.

Despite the wide variety of parameters that can be measured in vitro, they do not always correlate with in vivo measurements. The most commonly used in vivo measurements are platelet recovery and survival following transfusion, as mandated by the FDA (66.7% recovery of the same subjects fresh platelets after 24 hours and 58% recovery of the same subject's fresh platelets) [136]. However, platelet components in routine clinical use can fail to meet these criteria, highlighting a need for alternative criteria that provide information regarding platelet function following transfusion [137–140]. Nonetheless, there are strong correlations between some in vitro measurements and platelet recovery and survival in vivo. For example, lactate concentration has been shown to correlate strongly with reduced in vivo recovery and survival following transfusion. A similar correlation was observed for pH, which itself is influenced by lactate production [141]. Increases in markers of activation, such as expression of CD62P (P-selectin) and annexin V binding, have also been strongly correlated with reduced in vivo recovery [141, 142]. More recently, metabolomics has been used to better understand interdonor variations and identify metabolites that may be markers of high or low in vivo platelet recovery and survival [143]. However, the outputs of such studies are complex, and interpretation relies on powerful bioinformatics algorithms, rendering metabolomic testing not yet applicable for routine testing laboratories assessing platelet quality.

3.3. Alternative Modes of Platelet Storage. To circumvent problems associated with the short shelf-life of platelets, alternative modes of platelet storage have been investigated. These include platelet cryopreservation, cold storage, and lyophilization. Here we focus on cryopreservation and cold platelet storage, as these are being most actively pursued by several groups. Platelet cryopreservation typically involves addition of DMSO to a final concentration of 4–6% (vol/vol) [138, 144, 145] and storage at −80°C for between 2 and 4 years [146–148]. Platelet cryopreservation was pioneered by the US military and later the Netherlands military, who have successfully transfused over 1000 cryopreserved platelets during military deployments to Bosnia, Iraq, and Afghanistan [149, 150]. This technology is now being adopted by many countries. Platelet cryopreservation and the subsequent thawing processes are, however, time consuming and more expensive than standard RT storage, and other alternatives have been sought. One such alternative is cold storage of platelets, which is extremely simple logistically, as such platelet products can be stored in a refrigerator as per red cells and do not require agitation.

3.4. Platelet Cryopreservation. Methods used today for platelet cryopreservation were pioneered by Handin and Valeri for the US military and first published in the 1970s [151]. The original protocol described addition of 5 to 6% DMSO to hyperconcentrated platelets prepared from a single unit of whole blood using the PRP method [151, 152]. The DMSO was not removed prior to freezing, and after thawing the platelets were washed and resuspended in autologous

plasma. The platelets were found to be hemostatically effective upon transfusion [153]. Subsequent protocols described removal of DMSO prior to freezing, as this allowed platelets to be thawed and reconstituted immediately after thawing with reduction of DMSO content [144]. This process was adopted by the Netherlands Military Blood Bank [149] and many countries including the USA, Brazil, France, Turkey, and Singapore now have programs to implement cryopreserved platelets for civilian and/or military use, with similar cryopreservation protocols [138, 154, 155].

In vitro data from early studies showed changes in platelet shape and structure following cryopreservation [156]. Further studies have subsequently found that cryopreserved platelets have impaired HSR and aggregation responses to ADP, epinephrine, and collagen, as well as reduced oxygen consumption; moreover platelet activation of a high proportion of cryopreserved platelets was suggested by increased P-selectin and elevated annexin V binding to PS on a high proportion of platelets, indicative of poor in vitro quality and function [115, 138, 144, 157, 158].

Despite these impairments, cryopreserved platelets were shown to correct bleeding time in patients with hematological disorders when transfused together with other blood products [159] and to increase posttransfusion platelet counts and correct bleeding times when cryopreserved HLA-matched allogeneic or autologous platelets were used for treatment of thrombocytopenic alloimmunized patients [160, 161]. More recent investigations have demonstrated similar efficacy [162].

A randomized controlled comparison of cryopreserved and liquid-stored platelets demonstrated that cryopreserved platelets were potentially more effective than fresh, liquid-stored platelets for controlling surgical bleeding. Although the platelet count increment was lower in patients receiving cryopreserved platelets, blood loss and the number of postoperative blood products transfused were lower in this group, suggesting that despite increased clearance, cryopreserved platelets may in fact be superior to conventional liquid-stored platelets for controlling bleeding [163]. Despite in vitro deficiencies and increased activation, the platelets were still effective upon transfusion, supporting a hypothesis that despite being already activated, other changes in cryopreserved platelets rendered them more procoagulant and potentially more hemostatically active. The findings from this study also highlight the lack of correlation between in vitro and in vivo function in cryopreserved platelets, and hence the need for characterization with an extended suite of platelet assays.

More recent studies using techniques to measure the hemostatic function of platelets in vitro have expanded upon these earlier studies, suggesting they may be more appropriate measurements for cryopreserved platelets. These include techniques such as thrombin generation (see Section 2.7.3 above) and viscoelastic testing (see Section 2.7.4 above), which permit measurement of the procoagulant activity of both platelets and platelet microparticles. Consideration must also be given to enumeration of microparticles, as they contribute to the procoagulant function of cryopreserved platelets and are abundant in high numbers after thawing [115, 164, 165]. It should also be noted that no one test addresses all aspects of platelet quality and function, and several should be used in combination.

Thromboelastography (TEG) was developed to measure the clot formation and strength in whole blood and is now used routinely for evaluation of coagulation dysfunction in patients. TEG provides information regarding time to clot formation, clot strength, and fibrinolysis through continuous measurement of viscoelasticity during all stage of clot development and dissolution [166]. Rotational thromboelastography (ROTEM) provides similar information. TEG and ROTEM can be used to assess platelet function and storage-associated changes in liquid-stored PCs, as R-time (clot reaction time) and K-time (time for clot amplitude to increase from 2 to 20 mm) decrease during storage [167, 168]. TEG and ROTEM measurements are also suited to evaluation of cryopreserved platelets, which have a reduced R-time compared to liquid-stored platelet, with reduced clot strength [115]. Similar findings have been observed with ROTEM and are supported by perfusion studies under shear stress, whereby cryopreserved platelets adhere to surfaces and become activated to form stable aggregates with fibrin deposition [169].

Thrombin is a critical component of the coagulation cascade, and generation of thrombin is reflective of hemostatic function. Thrombin generation in platelet concentrates can be assessed using calibrated automated thrombinography (CAT) [170]. PS and tissue factor, both of which are present in high amounts on the membrane of cryopreserved platelets and their microparticles, have the potential to trigger thrombin generation [115, 171, 172]. Thus thrombin generation is useful for measuring the procoagulant activity of cryopreserved platelets and reveals that cryopreserved platelets and platelet microparticles are also more procoagulant than fresh, liquid-stored platelets in terms of the amount of thrombin they generate and the lag time for thrombin generation [115, 171]. However, the influence of the solution used for reconstitution after thawing must be taken into consideration, as non-plasma-based reconstitution solutions may not provide sufficient tissue factor for detectable thrombin generation [115].

CD62P (also known as P-selectin) is a well-characterized marker of platelet activation, which increases when platelets are stored at room temperature [122, 123, 173]. Given that cryopreserved platelets are known to be activated, it would be expected that CD62P expression would be high on cryopreserved platelets. However, there are variations in what has been observed by different groups examining cryopreserved platelets, whereby some have observed high expression (>60%) [138, 171], some have observed partial activation with 20–40% CD62P expression [162, 165], and others have observed low CD62P expression [145, 174, 175]. This variation may arise due to differences in the platelets themselves, differences arising from use of different antibody clones, staining protocols, or instrument settings on different flow cytometers. Exposure of PS on the outer leaflet of the platelet membrane indicates platelet activation and increases the procoagulant response of platelets [176]. Cryopreserved platelets also express high levels of PS and shed more PS-positive platelet microparticles when compared to fresh liquid-stored

platelets. Annexin V binding to PS may therefore be a better indicator of platelet activation in cryopreserved platelets, as it is consistently reported as high by many groups [115, 145, 158, 171].

PS on microparticles acts as a catalytic site for the FXa/F Va complex, accelerating thrombin formation, giving them procoagulant activity [115, 177]. The contribution of PS to procoagulant activity can be measured in clot-based or chromogenic assays that provide functional measurements of procoagulant phospholipids [178].

Platelet microparticles are best measured by flow cytometry, so that expression of a platelet-specific antigen such as CD61 may be combined with annexin V for confirmation of PS externalization, as part of the membrane remodelling that occurs during microparticle generation [179, 180]. The choice of surface markers for quantitation and characterization of platelet microparticles generated during cryopreservation is also of importance, as the proportionate expression of platelet surface markers is very different from marker expression on microparticles found in liquid-stored platelet concentrates [177]. The resolution of the flow cytometer is often a limitation in accurately identifying microparticle populations, as resolution is usually limited to 400–500 nm. However, development of newer flow cytometry instrumentation allows quantification and characterization of microparticle populations with high resolution ranging from 1 μm to 20 nm [181]. Dynamic light scattering can also be used to measure platelet particle size and the relative proportions of particles, although it does not provide information regarding the phenotypic characteristics of the microparticle populations [177].

There is still a paucity of published clinical data on patient outcomes following transfusion of cryopreserved platelets, rendering it difficult to correlate in vitro activity with efficacy or safety. Furthermore, recovery of autologous platelets at 24-hour posttransfusion may not be an ideal measure for cryopreserved platelets, as it does not take into consideration their procoagulant phenotype and their potential to stem active bleeding or hemorrhage more effectively. Rather, inclusion of outcomes such as posttransfusion blood loss, bleeding time, volume, and number of transfusions in clinical studies may be more informative, and subsequent correlation of in vitro function with these outcomes may advance this field. Animal studies using wound or hemorrhage models may also help to bridge this knowledge gap.

3.5. Cold Storage of Platelets. Platelets were stored in the cold (at ~4°C) until 1969, when transfused platelets were shown to be more rapidly cleared from the circulation than platelets stored at RT [182]. The majority of platelet transfusions are given prophylactically to thrombocytopenic hematooncology patients [183]. As such, a longer lifespan in the circulation is desirable, and cold storage of platelets ceased, with adoption of storage at RT (20–24°C). However, the advantages of cold storage include prolonged shelf-life due to reduced metabolism, enhanced hemostatic activity, improved bacteriologic safety, and ease of storage and transport, as the same infrastructure for storage and transport of red cells could be utilized.

Platelets stored at 4°C undergo many ultrastructural and metabolic changes, known as the cold-storage lesion. Most notably, cold-stored platelets undergo a shape change from disc to sphere, with an increase in MPV and loss of swirl, mediated by changes in localization of cytoskeletal proteins [113, 184]. Other changes include clustering of the GPIbα subunit of the von Willebrand receptor complex on the platelet surface [185, 186] and changes in glycosylation patterns with loss of sialylation and exposure of galactose residues that ultimately leads to clearance by hepatic macrophages through Ashwell Morell receptors [187, 188].

A renewed interest in cold storage of platelets has shown that, as with cryopreserved platelets, cold storage leads to increased activation and more procoagulant function, with increased expression of activation markers such as CD62P and exposure of PS, improved in vitro TEG and ROTEM responses, more vigorous aggregation responses, and increased thrombin generation in comparison to room temperature-stored platelets [113, 114, 189–191]. As such, assays for measurement of in vitro function that are suitable for characterization of cryopreserved platelets, including TEG or ROTEM, CAT, microparticle enumeration, and flow cytometry, are also very useful for characterization of cold-stored platelets.

Some measurements that are less informative for cryopreserved platelets, such as glucose metabolism, are, however, more appropriate for examining differences between cold-stored and RT-stored platelets. For example, cold storage of platelets slows glycolysis as well as attenuating acetate metabolism, possibly through inhibition of oxidative phosphorylation. Given that cold storage causes a change from discoid to spherical shape, measurements such as MPV, swirl, HSR, and ESC are also informative [113, 114, 192].

A shelf-life for cold-stored platelets has not yet been rigorously defined, although FDA approval has recently been given for the narrowly defined instance of administration of cold-stored apheresis platelets to actively bleeding patients, provided the product has been refrigerated for less than 72 hours after phlebotomy [193]. Further investigations with cold-stored platelets under different storage conditions, such as in additive solutions versus plasma, are necessary to generate data that will help establish optimal storage conditions and determine whether a shelf-life beyond that of room temperature-stored platelets is feasible.

Cryopreservation and cold storage lead to many changes in platelet metabolism, surface receptor expression profiles, membrane and cytoskeletal structure, and importantly hemostatic function, some of which may be considered adverse and some advantageous. Measurement of these changes as they occur in vitro and the ability to correlate them with appropriate transfusion outcomes are essential for defining the appropriate indications for these novel platelet components and embedding them into routine use.

4. Overview: Quality Assessment of Stored Red Cell Concentrates

Transfusion of red blood cell concentrates (RCCs) is a necessary, lifesaving clinical therapy. RCCs are given to increase

oxygen delivery to tissues in clinical situations where the circulating red blood cell (RBC) level is low (anemia) due to RBC loss (trauma/surgical hemorrhage), decreased bone marrow production (chemotherapy, aplastic anemias), defective hemoglobin (hemaglobinopathies, thalassemias), or decreased RBC survival (hemolytic anemias). Approximately 1.2 million RCCs are collected and transfused each year in Canada [194, 195], and more than 90 million units are transfused globally [196]. RCCs are used across a variety of medical and surgical situations with approximately 30% of critical care patients and more than 50% of cardiac surgery patients receiving blood products during their hospital stay [197, 198].

To ensure that RCCs are produced in a consistent and controlled manner, blood collection agencies routinely test products as part of their quality assurance programmes and as part of their continuous improvement activities prior to making changes to equipment or processes. Acceptable standards for product safety and quality are outlined in government regulations and by standard-setting organizations. Despite these stringent control mechanisms, a great deal of variability still exists within the blood components transfused which may affect patient outcomes. Motivated by a need for more concerted efforts to understand the factors affecting RCC quality, this section will review the changes that occur to RBC during blood banking, current quality testing practices, and the factors which affect the quality of stored RCCs.

4.1. RBC Physiology and the Storage Injury. The primary function of RBCs is to transport oxygen from the lungs to the body tissues, where the exchange for carbon dioxide is facilitated through the synergistic effects of hemoglobin, carbonic anhydrase, and band 3 protein, followed by carbon dioxide delivery to the lungs for release. Successful oxygen transport is dependent on the efficacy of three critical elements: the RBC membrane, hemoglobin, and the cellular energetics.

The RBC membrane is a fluid structure composed of a semipermeable lipid bilayer with an asymmetrically organized mosaic of proteins. Membrane lipids compromise approximately 40% of the RBC membrane mass, with equimolar quantities of unesterified cholesterol and phospholipids, and small amounts of free fatty acids and glycolipids [199]. Membrane proteins comprise approximately 52% of the RBC membrane mass and can be categorized into integral and peripheral proteins according to their location relative to the lipid bilayer [199]. Integral membrane proteins, such as glycophorin and band 3 protein, transverse the membrane and function as receptors and transporters. In contrast, peripheral proteins are only found on the cytoplasmic surface of the membrane and form the RBC cytoskeleton. The major components of the RBC cytoskeleton are spectrin, ankyrin, protein 4.1, actin, and adducin, which form a mesh-like network of microfilaments that strengthens the RBC membrane while maintaining RBC shape and stability [200]. The unique characteristics of the RBC membrane and cytoskeleton afford the cells the ability to reversibly deform as they traverse the microvasculature and thereby deliver oxygen from the lungs to the tissues.

The second element that has to be maintained for the cells to function normally is hemoglobin. Hemoglobin is a conjugated protein consisting of two pairs of globin chains and four heme groups, each containing a protoporphyrin group and an iron molecule in the ferrous form [201]. The uptake and release of oxygen by the hemoglobin molecule are controlled by the RBC organic phosphate 2,3-disphosphoglycerate (2,3-DPG), which binds to the cleft between globin chains, resulting in a deoxyhemoglobin conformation that facilitates the release of oxygen. Therefore, increased 2,3-DPG levels triggered by tissue hypoxia will shift the hemoglobin oxygen dissociation curve to the right, increasing oxygen delivery to the tissues.

Maintenance of the RBC membrane system and hemoglobin function is dependent on energy generation through RBC metabolic pathways. There are four major RBC metabolic pathways: the Embden-Mayerhof pathway, in which most RBC adenosine triphosphate (ATP) is generated through the anaerobic breakdown of glucose; the hexose monophosphate shunt, which produces NADPH to protect RBCs from oxidative injury; the Rapoport-Luebering shunt, responsible for the production of 2,3-DPG for the control of hemoglobin oxygen affinity; and finally, the methemoglobin reduction pathway, which reduces ferric heme iron to the ferrous form to prevent hemoglobin denaturation. All four metabolic pathways are critical to RBC function.

Defects associated with any of the above described elements of RBC structure or function are related to the development and pathogenesis of the many forms of inherited and acquired RBC abnormalities that result in increased RBC destruction through intra- or extravascular hemolysis and therefore an in vivo survival of less than the normal 120 days. During a normal life span, circulating RBCs undergo metabolic and physical changes associated with the process of senescence, such as membrane vesiculation, decreased cell size, increased cell density, cytoskeletal alterations, enzymatic desialylation, and PS exposure. At the end of their life span, RBCs are recognized and removed by the macrophages in the reticuloendothelial system (RES). It has been estimated that 5 million RBCs are endocytosed by RES macrophages per second each day [202]. These RBCs are replaced by RBC reticulocytes which are released daily from the bone marrow storage pool.

Because hemoglobin and its constituent iron are highly reactive molecules that can be toxic to cells and tissues, the body has a number of protective mechanisms to avoid their accumulation in circulation. Excessive intravascular damage to red cells or transfusion of fragile or nonfunctional RBCs can result in the release of large amounts of hemoglobin into the plasma. Haptoglobin can rapidly bind free hemoglobin in circulation and clear it through the reticuloendothelial macrophages; however excessive hemolysis will exceed the haptoglobin scavenging capacity of the RES [10–13]. Conversion of excess free hemoglobin to methemoglobin is facilitated by endothelial-derived nitric oxide (NO) allowing for the rapid clearance of the oxidized hemoglobin from the circulation by the binding of hemoglobin to hemopexin and albumin [10–13]. This scavenging of the toxic free hemoglobin in circulation results in a reduced NO bioavailability, which

can in turn lead to endothelial dysfunction, platelet aggregation, and oxidative injury [203–205]. Hemoglobin will accumulate within the macrophages that phagocytose senescent red cells and remove hemoglobin-laden haptoglobins and hemopexins from circulation. Through the action of cytoplasmic heme-oxygenase-1, the macrophages convert the hemoglobin into carbon monoxide, biliverdin, and free iron [206]. While some iron bound to transferrin is released into circulation, the majority of free iron accumulates within the macrophages in the form of ferritin. Under conditions where there is excessive intra- or extravascular hemolysis, the iron-sequestering capacity of ferritin and transferrin can be overwhelmed, resulting in the accumulation of free iron within the macrophages and in the circulation [207]. As free iron is highly reactive and able to generate hydroxyl radicals from hydrogen peroxide it can cause significant oxidative injury to the macrophages and tissues [208–210].

While the ability to collect, process, and store RBCs for extended periods of time has facilitated the widespread adoption of RCC transfusion as a clinical therapy, the storage of RBCs outside of the body (ex vivo) in an artificial environment impairs many natural processes required for cell viability and function. The "storage lesion" is the name given to all of the progressive changes that occur to blood components during conventional blood bank storage. Refrigerated storage of RBCs is based on the principle that biochemical events and molecular reactions can be suppressed by a reduction in temperature. Although refrigerated storage minimizes RBC injury, cellular metabolism is not completely suppressed at hypothermic temperatures and the residual metabolic activity eventually results in nutrient depletion and accumulation of cell wastes [211, 212]. The progressive biochemical and biomechanical effects of the storage lesion have been well documented [211, 213–216].

A major element of the storage lesion that has been gaining attention recently is RBC membrane injury [215–219]. Indeed, strong evidence in the current literature suggests that RBC viability—defined as posttransfusion survival of RBCs—is closely related to the structural and metabolic status of the poststorage RBC membrane [215–219]. The membrane components implicated in the hypothermic storage lesion include (i) lipid loss through microvesiculation, which leads to decreased critical hemolytic volume, increased internal RBC viscosity, and progressive spheroechinocytosis; (ii) altered RBC rheological properties such as deformability, mechanical stability, and adhesiveness; (iii) PS exposure on the membrane surface; and (iv) decreased expression of the CD47 antigen on the membrane surface [211, 215, 220–225]. Collectively, these changes in RBC membrane structure manifest as severe changes in the deformability of the stored RBCs, alterations that result in accelerated clearance of the cells from circulation [226–229].

The effect that the biochemical changes that occur to RBCs during storage have on the safety and efficacy of transfused RCCs remains unclear. Numerous studies have shown an association of RCC transfusion with increased length of stay in the hospital, impaired tissue oxygen use, proinflammatory and immunomodulatory effects, increased infections, multiple organ system failure, and ultimately increased morbidity and mortality (reviewed in [230–233]). Based on our current knowledge of the RBC physiology and the storage lesion one may propose a number of different biological pathways that may be responsible for these adverse events including the role of microvesicles (also known as microparticles [234]) in stored blood in the pathogenesis of thrombosis, inflammation, and responses to pathogens [235]; activation of platelets in the stored RCC product that could cause platelet-white blood cell aggregate complexes with procoagulant activity [236]; deformability changes that result in red cells becoming entrapped in the spleen [237]; decreased blood flow because of rigidity of transfused red cells; and decreased oxygen delivery as microvesicle-entrapped hemoglobin and free plasma hemoglobin serve as potent scavengers of nitric oxide once a patient is transfused [203]. While prospective clinical trials have failed to show a clear relationship between the duration of RCC storage and patient outcome [10, 238, 239], there continues to be a strong interest in understanding how the obvious physiological changes that occur to RBCs during ex vivo storage affect our perceptions of RCC quality.

4.2. Impact of Manufacturing Method on RCC Quality. Blood components are produced using methods that depend on the blood manufacturer's established procedures and on the desired products. Differences in production processes can exist across different jurisdictions and even within a single organization. Regulatory standards are applied, in part to address these concerns, but current standards are loose in terms of product standardization and focus instead, not unreasonably, on donor and recipient safety. As noted by Högman and Meryman [240] using current standards the hemoglobin content of a RCC unit may range between 30 and 90 g, a fact that transfusing clinicians are generally unaware of.

One element that is emerging as an important mediator of RCC quality is the manufacturing methods that are used to separate the RBCs, platelets, and plasma from whole blood. The method used to separate blood components from whole blood [241–244], the additive solutions used [216, 245], and other factors such as prestorage leukoreduction [246] have all been shown to affect the quality characteristics of transfused products. Therefore, it has been very difficult to achieve any level of global, or even national, standardization of blood products, which has confounded current clinical and laboratory-based studies aimed at examining adverse transfusion reactions [247].

By examining the characteristics of whole blood-derived and apheresis RCCs produced in Canada and the United States it has been shown that RCCs distributed for transfusion are not equivalent [248–251]. Similar studies in the US have evaluated RCCs prepared from whole blood donations using the PRP method for separation and RBCs collected through an automated apheresis process [242]. Collectively, these studies show that there are significant differences in the levels of hemolysis, potassium, cytokine and microparticle levels, oxidative stress, oxygen carrying capacity, deformability, and residual plasma, platelet, and leukocyte concentrations across the different methods used to manufacture an RCC.

Studies examining the link between blood component manufacturing and adverse clinical outcomes are extremely limited. This is due to a number of factors. Firstly, multicentre, randomized clinical trials examining blood component quality do not account for component manufacturing differences amongst study sites in their study designs. Participating hospitals assume that the blood components received by the blood bank are the same as those used at other sites. Only recently has there been awareness that differences in blood component manufacturing across international sites may need to be considered in secondary analysis of data. Secondly, RCCs prepared using different methods are intermixed within the inventory of a blood center and have the same product label and product code. This makes awareness of potential differences difficult for clinicians or hemovigilance programs to consider when evaluating transfusion outcomes. Thirdly, a lack of coordination between blood manufacturers, blood banks, and clinical research groups makes the randomization of specific blood components for clinical trials difficult. Fourthly, access to products manufactured using different methods can be difficult for investigators due to institutional, regional, or national purchasing agreements and regulations. Finally, limited pre- and postmarketing studies are performed to assess the impact of new manufacturing processes on patient outcomes. For example, comparison studies between apheresis and manual methods of RCC production have looked exclusively at conventional in vitro quality control parameters or radiolabelling survival studies and rarely patient outcomes [242, 252, 253]. For these reasons, the impact of blood component manufacturing and adverse transfusion outcomes has not been adequately evaluated in well-designed prospective clinical studies.

In a recently published retrospective study, the method of whole blood processing was shown to be associated with in-hospital mortality of transfused adults [254]. Patients who received fresh RCCs (≤7 days of storage) that were prepared by a whole blood filtration, top/top manufacturing method, were associated with a higher risk of in-hospital mortality than was transfusion with mid-age RBCs (stored 8–35 days) prepared by the red cell filtration, top/bottom method. This work is significant in that it suggests that adverse transfusion outcomes might be reduced by making minor changes to blood processing methods and inventory management practices.

4.3. Impact of Donor Factors on RCC Quality. Donor factors have long been associated with clinical outcomes in blood transfusions, ranging from fundamental transfusion practices such as ABO/Rh blood group matching and pathogen screening to more current concerns such as the increased risk of TRALI associated with plasma products from female donors [255]. Recently it has come to light that donor factors might also influence the in vitro quality of stored red blood cell products [256–259]. Analysis of quality control data from national blood banking agencies suggests that storage-associated hemolysis in stored RCCs is dependent on donor characteristics such as sex, age, ethnicity, and heritable genetic traits [256, 260–264].

Multiple groups have reported significantly lower hemolysis in units from premenopausal female donors when compared to donations from other donor populations [256, 261, 262, 265]. A number of theories have been suggested to explain this trend, based on known physiological differences between aging male and female blood donors and their circulating RBCs [261, 266, 267]. It has been suggested that a decrease in the surface area to cell volume ratio of circulating RBCs results in an increase in the osmotic fragility of circulating RBCs in older individuals [259]. Due to the association between storage hemolysis and female menopausal status, several hypotheses attribute hemolysis to the action of sex hormones [261, 266]. Testosterone has been shown to promote erythropoiesis [268], which could explain the increased hematocrit and hemoglobin levels observed in male donors compared to female donors [269]. Alternatively, it has been suggested that monthly blood loss in premenopausal female donors results in a younger population of circulating RBCs, which are less susceptible to stress and hemolysis during storage [267]. As evidenced by this list of contrasting observations and ideas, we are far from reaching a consensus on the mechanisms behind the influence of donor factors on RBC storage.

Retrospective studies have shown that there is an association between RCC donor factors and transfusion recipient outcomes. Donor-recipient sex mismatch [270–273] and/or age [271, 273] of the blood donor has been shown to be associated with an increased risk of mortality. For example, receiving a red cell product from young (<45 years) female blood donor has been associated with up to an 8% increase in the risk of death for each unit transfused compared to receiving an RCC transfusion from a male door [271]. As this observation has not been seen in retrospective analysis of French or Scandinavian patient cohorts [274, 275], more detailed retrospective data linkage studies and prospective clinical studies are needed to help further understanding the role that donor factors have on patient outcomes.

4.4. Quality of Irradiated RBCs. Gamma-irradiation of red blood cell concentrates is used to prevent transfusion-associated graft versus host disease (GVHD), a rare but usually fatal complication in recipients at risk. Although there is limited evidence for GVHD after transfusion of leukodepleted RCCs containing $<5 \times 10^6$ leukocytes/unit, irradiation of RCC is the standard of care for immune deficient patients. Irradiation is also used for transfusions from 1st- or 2nd-degree relatives, for all intrauterine transfusions and for allogeneic stem cell recipients, even if the recipient is immunocompetent [276]. Gamma-irradiation targets and inactivates residual white blood cells that may be present in RCCs [277].

It is generally known that irradiation of RCCs causes harm to the erythrocytes, with the most prominent being biochemical changes, including lipid peroxidation and membrane damage [278, 279], reducing the function and viability of RBCs which may impact their posttransfusion recovery [280]. Irradiation exacerbates many of the classical hallmarks of the red cell storage lesion [281], causing red cell swelling, decreasing deformability [282], affecting metabolism [283],

increasing the release of potassium [284, 285], and increasing hemolysis [286]. Some of these changes (e.g., increased hemolysis and potassium loss) could cause harm to certain recipients [287, 288]. For these reasons, the timing of irradiation during RCC storage and the length of the postirradiation storage period are thought to be critical to the quality of gamma-irradiated RCCs [289, 290].

4.5. Quality of Cryopreserved RBCs. Cryopreservation is the process of preserving the biologic structure and/or function of living systems by freezing to and storage at ultralow temperatures. As with refrigerated storage, cryopreservation uses the beneficial effect of decreased temperature to suppress molecular motion and arrest metabolic and biochemical reactions. Below −150°C, a state of "suspended animation" can be achieved in samples where there are no cryoprotectants added as there are very few biologically significant reactions or changes to the physicochemical properties of the system that occur below this temperature [291]. In contrast to hypothermic storage (at 1–6°C), RBC physiology, including hemoglobin structure and membrane and cellular energetics, is unaffected by extended storage in the frozen state [292, 293]. Cryopreservation is the only current technology that maintains ex vivo biologic function and provides long-term product storage. It has been recently suggested that cryopreserved RCCs are as safe and effective as liquid-stored products [294] and for certain patient groups may in fact be a superior product for transfusion [295].

To take advantage of the protective effects of low temperature and to successfully store RBCs for extended periods using cryopreservation techniques, damage to the cells during freezing and thawing must be minimized. The successful cryopreservation of human red blood cells became possible after the development of techniques using glycerol as a cryoprotectant to minimize both rapid and slow cooling injury [296, 297]. Over the last century, enormous progress has been made in understanding the basic elements responsible for low temperature injury in RBCs and in the development of effective techniques to protect RBCs from this cryoinjury.

Currently, there are two methods used clinically for the cryopreservation of RCCs: low glycerol/rapid cooling [298–300] and high glycerol/slow cooling [301, 302]. Low concentrations (15–20%) of glycerol, rapid cooling (>100°C/min), storage in liquid nitrogen (−196°C), or nitrogen vapour (−165°C), with rapid thawing in a 42–45°C water bath, is less commonly used method for clinical cryopreservation of RCCs. The more common cryopreservation method found in most international blood centres is the use of a high concentration of glycerol (40%) in conjunction with slow cooling (~1°C/min), storage at <−65°C, and rapid thawing in a 37°C water bath. In each case, controlled addition and removal of glycerol are required to prevent osmotic lysis of the RBC and to minimize the recipient's exposure to the chemical cryoprotectant. Both methods meet the standards set by the regulatory agencies [223, 303] requiring the removal of the cryoprotecting agent (CPA), minimal hemolysis, and recovery of at least 80% of the original red blood cell volume following deglycerolization. Deglycerolized RBCs must be stored at 1–6°C and achieve a 75% in vivo viability, 24 hours after transfusion.

4.6. Quality of Washed RBCs. Washed red blood cell concentrates are recommended for large-volume transfusions to potassium-sensitive patients, particularly neonates when fresh units are not available [304], for recipients who have a history of plasma-related transfusion reactions [305], and for the prevention of transfusion-related acute lung injury [306]. Recently, the use of washed RCCs has been proposed as a means to mitigate the accumulation of proinflammatory cells and molecules in stored RCCs to reduce the bioactivity of the product [307] and may be an effective means to address RBC degradation during storage.

RCCs can be washed in the blood bank using manual centrifugation and extraction methods, semiautomated cell processors, or just prior to transfusion using cell salvage devices [304, 308–310]. Manual washing and use of open cell processors hamper widespread use of washed RBCs as the resulting washed products have a limited storage duration. Due to concerns over the potential for bacterial contamination during washing of RCCs using an open processing system and decreased RBC viability when resuspended in saline rather than additive solution in the postwash period, a 24-hour expiry is applied to these products [311, 312]. This significantly constrains the window in which units can be prepared, transported, and transfused, resulting in high levels of component discards and inefficient inventory management practices. In contrast, semiautomated cell processors using closed system washing and modern additive solutions have the potential to improve the overall utilization of washed RCCs, as postwashing storage time of RCCs can be extended and the process can be more closely controlled.

The washing of RCCs has been shown to be an effective method to remove potassium, hemoglobin, and protein- or lipid-based immunomodulatory agents [310, 313–315] and may result in reduced adverse transfusion reactions [316–318]. In cardiac and neonatal patients [304, 307] and a number of animal models [319, 320], the use of washed RCCs can decrease the dose of labile plasma iron in the product and is accompanied by a blunting of posttransfusion inflammation. However, washing may also increase potassium concentration [310] while simultaneously removing a proportion of the original unit's erythrocytes, thereby resulting in a "low dose" product that may be less effective [286, 321–324]. It has been proposed that the method used to wash RCCs has an important impact on the quality characteristics of the final product [308, 309, 325, 326]. Provision of washed products is therefore usually reserved only for patients who have already developed significant adverse transfusion reactions to unwashed products.

4.7. Relationship between RBC In Vitro Parameters and Post-transfusion Outcomes. Quality control programs require that a number of in vitro parameters are monitored to ensure that component manufacturing is in a state of control and that RCCs meet specific physical characteristics. In most countries, blood manufacturers will ensure that RCCs meet general requirements for hematocrit, unit volume, total

hemoglobin per unit, sterility, residual leukocyte concentration, RBC recovery (typically for washed or cryopreserved units), and hemolysis. As discussed below, each of these parameters is intended to ensure that the physical characteristics of the product adhere to generally acceptable clinical specifications. However, the evidence for many of these specifications, particularly for specific patient groups requiring RCC, has not been clearly established.

Hematocrit or packed cell volume is a measurement of the ratio of the volume occupied by red blood cells to the total volume of the blood sample. Hematocrit is an important factor influencing the administration of RBC units as a high hematocrit will have a reduced flow and restricted movement through leukoreduction or microparticle filters. A low hematocrit will cause the administration of large product volumes resulting in potential circulatory overload in order to administer enough red blood cells to achieve the therapeutic effect of restoring oxygen carrying capacity of the recipient. Regulatory standards vary with each jurisdiction, but in general, most countries require the hematocrit to be ≤0.80 L/L in the majority of units tested.

The mass of hemoglobin in a unit of RCC or the concentration of hemoglobin in washed and deglycerolized RCC reflects the relative dosage of RBC transfused to patients. In order for the transfusion of a RBC unit to restore the oxygen carrying capacity of the recipient the RBC unit must contain adequate hemoglobin content. The total hemoglobin per unit metric is an indirect indication of the overall efficiency of the collection and manufacturing system. Assuming a fixed collection volume of whole blood with a physiologically normal hematocrit, the total hemoglobin per unit should fall between a set range when the manufacturing process is in control.

The residual leukocyte count is the total number of leukocytes in the RCC after leukoreduction processes such as leukocyte filtration, cell washing, cryopreservation, or pathogen reduction. Residual donor leukocytes are associated with potential adverse transfusion reactions in recipients. In an effort to avoid these adverse effects most blood systems utilize universal prestorage leukoreduction of all RCCs. The residual leukocyte concentration can be used as an indirect method of the overall efficiency of the leukoreduction process and filter performance.

Sterility is the state whereby a blood product is free from infectious microorganisms. Blood manufacturing is specifically concerned with ensuring the absence of any bacteria within the blood components. Maintaining a closed sterile system is essential to prevent bacterial contamination. During blood collection, the use of a sample diversion pouch and effective skin disinfection are used to minimize the probability of introducing bacteria into the whole blood unit. Many blood systems include random RCC sterility testing as part of their quality control program.

Unit volume is the total volume of the RCC unit following processing. The administration of high volumes of transfusion products can lead to circulatory overload. In order to avoid these adverse effects but still administer enough red blood cells to achieve the action of restoring the oxygen carrying capacity of the recipient, it is important to have consistent products with a high red blood cell mass to volume ratio (i.e., hematocrit). As physicians may prescribe a specific volume of a RBC product, the labeled volume should be an accurate representation of the volume of the product in the bag.

In the case of a RBC product, RBC recovery can be defined as the percentage of the total RBC available in a whole blood collection to the actual number appearing in an end-labeled product. RBC recovery is a relative measure of the overall efficiency of the cell washing or cryopreservation process. This measure reflects the mass of RBC that are lost during processing due to hemolysis and cells becoming trapped in tubing, leukoreduction filters, or transfer bags. Recovery is a useful measure for determining the overall efficiency of a manufacturing process.

Hemolysis is an end-point measure of the overall structural integrity of the RBC. RBC hemolysis can increase due to physical processing of the cells, temperature-dependent and storage time-dependent depletion of biochemical metabolites, and changes in extracellular storage solution characteristics (pH, osmolality, and tonicity) [327]. Hemolysis of stored red cells is a normal process and increases with storage time. Some degree of hemolysis is acceptable and expected in RCCs.

In addition to the standard quality control tests for quality, many blood systems may perform additional in vitro testing to evaluate the effect that their collection, processing, storage, and distribution systems have on the quality of the RCCs they issue to hospitals [250, 328, 329]. Gross morphological examination and scoring of RBCs, measurement of potassium leakage, analysis on an automated hematology analyzer, and measurements of osmotic fragility can be used to examine any prehemolytic physical changes to the RBCs. A saline stability test and extracellular vesicle enumeration and characterization using flow cytometry and dynamic light scattering can be used to assess the state of the RBC membrane. Functional assessment of glycolytic intermediates (ATP and 2,3-diphosphoglyceric acid), hemoglobin oxygen saturation (pO_2 and p50), and deformability (via ektacytometry or other methods) can give an indication of the O_2 delivering capacity of the RCC. In addition, advanced metabolomics and lipidomic tools can be used to assess subtle changes that occur to the RCC. As with many in vitro tests used in the assessment of blood components, the general relevance of these measures to the actual in vivo function of the RBCs is not well established and further understanding of the clinical relevance of these assessments is needed.

A requirement to have 75% of radiolabelled, autologous red blood cells survival for 24 h in circulation remains the current standard for the assessment of RCC quality by the FDA and many other national regulatory agencies [330]. As most damaged RBCs will be removed by circulation within 24 h, those cells that remain in circulation would be expected to be viable and functioning [331]. Efforts to develop biotin-labeling techniques and other methods to identify RBCs in circulation have been pursued as replacements to the use of radionucleii in in vivo survival testing [332]; indeed, most countries in Western Europe prohibit the administration of radiolabeled substances to healthy volunteers [333]. However,

the ability to remain in circulation may not be a true measure of whether a red cell can deliver oxygen or not. This has motivated groups to look to develop tools such as near-infrared spectroscopy [334] and plethysmography [335] to noninvasively monitor tissue oxygenation and microvascular blood flow in patients receiving RCC transfusions. While still in early development, these novel tools may provide a significant improvement in the ability to assess the in vivo efficacy of stored RCCs.

As the in vivo assessment of RCC quality will not be a routine quality control procedure that can be easily performed by blood manufacturers or blood transfusion services, developing in vitro tests that accurately predict in vivo survival and function is needed. As measurements of adenosine triphosphate levels in stored blood components correlate with in vivo survival, it has been suggested that levels of ATP should be at least 2.7 μmol/g Hb at the end of storage [331]. Early work has shown that changes in membrane deformability and microvesiculation correlate with in vivo survival of RCCs stored in different additive solutions [336]. While correlations can be easy to identify, understanding the pathophysiological basis for the correlation and the many factors which may affect the reliability and predictability of an in vitro biomarker of quality will be critical to the widespread adoption of the targets.

As new analytical methods are developed to evaluate the quality characteristics of stored red blood cells it is important to understand the context in which they will be used. From a blood component manufacturing perspective, having sensitive in vitro tools available to detect subtle changes in the collection, manufacturing, storage, and distribution is critical to being able to detect and respond quickly to process deviations. These measures may have no relationship or correlation with the efficacy or safety of the blood for transfusion and may only be specific to assuring manufacturing process control is maintained. Similarly, in vitro quality tests that detect changes in RCCs that may be critical to patient safety or to the efficacy of the product may be completely insensitive to specific manufacturing process changes. For these reasons, blood systems should not focus on developing expertise with one specific analytical method but need to have access to a large number of analytical methods if they are to truly understand product quality and what it means to their operation and to the patients that they serve.

5. Overall Conclusion

Existing determinants of blood product quality provide some information that is plausibly linked to predicting posttransfusion efficacy of their transfusion. For RCC and platelet concentrates, the evidence for most indications is stronger than that currently available for transfusable plasma. Nevertheless, controversies persist among transfusionists regarding what constitutes an appropriate transfusion and/or an appropriate dose of the blood product, in different clinical settings. Emerging tests offer potential improvements in strengthening the linkages between pretransfusion quality assessments and posttransfusion clinical efficacy and in determining which blood products are indeed of the highest quality. It must be remembered that blood products, unlike traditional pharmaceutical agents, are complex, multicomponent products. Improved characterization and quality assessment have an important role to play in furthering our understanding of how these products, and their modified forms, can best be used to benefit patients. At present there is insufficient evidence to endorse replacing current blood component quality tests with more complicated technologies, such as thrombin generation assays and viscoelastic methodologies for both platelets and plasma, or plethysmography for red cells; nevertheless, it is only through continuing to investigate emerging tests and cross-correlating them with existing measures that the field can improve. The relatively simple tests currently employed as quality markers, such as FVIII activity for plasma, pH, and platelet count for platelets, and hemoglobin-related parameters for red cells, remain of value and can be conducted in many settings with locally available instrumentation.

Disclosure

Because two of the authors (Jason P. Acker and William P. Sheffield) are members of the Canadian Blood Services Centre for Innovation, which receives funding from Health Canada, a department of the federal government of Canada, this article must contain the statement, "The views expressed herein do not necessarily represent the views of the federal government [of Canada]." Similarly, Denese C. Marks's funder requires inclusion of the statement, "Australian governments fund the Australian Red Cross Blood Service to provide blood, blood products and services to the Australian community."

Competing Interests

All authors declare they have no relevant conflict of interests.

Authors' Contributions

Jason P. Acker and Denese C. Marks contributed equally to this work.

Acknowledgments

The authors thank colleagues at Canadian Blood Services and the Australian Red Cross for insightful discussions related to this review. They assure any colleagues working in the area of blood quality whose work we did not cite that such oversights are not reflective of any perceived lack of quality in their publications but relate to practical limits on the length of this review.

References

[1] R. C. Arya, G. S. Wander, and P. Gupta, "Blood component therapy: which, when and how much," *Journal of Anaesthesiology Clinical Pharmacology*, vol. 27, no. 2, pp. 278–284, 2011.

[2] R. R. Vassallo and S. Murphy, "A critical comparison of platelet preparation methods," *Current Opinion in Hematology*, vol. 13, no. 5, pp. 323–330, 2006.

[3] M. A. Blajchman, "The clinical benefits of the leukoreduction of blood products," *Journal of Trauma—Injury, Infection and Critical Care*, vol. 60, no. 6, pp. S83–S88, 2006.

[4] M. D. Zielinski, D. H. Jenkins, J. D. Hughes, K. S. W. Badjie, and J. R. Stubbs, "Back to the future: the renaissance of whole-blood transfusions for massively hemorrhaging patients," *Surgery (United States)*, vol. 155, no. 5, pp. 883–886, 2014.

[5] S. F. O'Brien, Q.-L. Yi, W. Fan, V. Scalia, M. A. Fearon, and J.-P. Allain, "Current incidence and residual risk of HIV, HBV and HCV at Canadian Blood Services," *Vox Sanguinis*, vol. 103, no. 1, pp. 83–86, 2012.

[6] E. S. Cooper, A. W. Bracey, A. E. Horvath, J. N. Shanberge, T. L. Simon, and D. H. Yawn, "Practice parameter for the use of fresh-frozen plasma, cryoprecipitate, and platelets. Fresh-Frozen Plasma, Cryoprecipitate, and Platelets Administration Practice Guidelines Development Task Force of the College of American Pathologists," *The Journal of the American Medical Association*, vol. 271, no. 10, pp. 777–781, 1994.

[7] "Guidelines for red blood cell and plasma transfusion for adults and children," *Canadian Medical Association Journal*, vol. 156, no. 11, supplement, pp. S1–S24, 1997.

[8] "Expert Working Group Guidelines for red blood cell and plasma transfusion for adults and children," *Canadian Medical Association Journal*, vol. 156, no. 11, pp. S1–S24, 1997.

[9] British Committee for Standards in Haematology, "Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant," *British Journal of Haematology*, vol. 126, no. 1, pp. 11–28, 2004.

[10] M. E. Steiner, P. M. Ness, S. F. Assmann et al., "Effects of red-cell storage duration on patients undergoing cardiac surgery," *The New England Journal of Medicine*, vol. 372, no. 15, pp. 1419–1429, 2015.

[11] *AABB Standards for Blood Banks and Transfusion Services*, AABB Press, Bethesda, Md, USA, 30th edition, 2016.

[12] G. Liumbruno, F. Bennardello, A. Lattanzio, P. Piccoli, and G. Rossetti, "Recommendations for the transfusion of plasma and platelets," *Blood Transfusion*, vol. 7, no. 2, pp. 132–150, 2009.

[13] B. J. Hunt, "Bleeding and coagulopathies in critical care," *The New England Journal of Medicine*, vol. 370, no. 9, pp. 847–859, 2014.

[14] A. J. Gale, "Continuing education course #2: current understanding of hemostasis," *Toxicologic Pathology*, vol. 39, no. 1, pp. 273–280, 2011.

[15] L. Yang, S. Stanworth, S. Hopewell, C. Doree, and M. Murphy, "Is fresh-frozen plasma clinically effective? An update of a systematic review of randomized controlled trials (CME)," *Transfusion*, vol. 52, no. 8, pp. 1673–1686, 2012.

[16] S. J. Stanworth, S. J. Brunskill, C. J. Hyde, D. B. L. McClelland, and M. F. Murphy, "Is fresh frozen plasma clinically effective? a systematic review of randomized controlled trials," *British Journal of Haematology*, vol. 126, no. 1, pp. 139–152, 2004.

[17] M. H. Murad, J. R. Stubbs, M. J. Gandhi et al., "The effect of plasma transfusion on morbidity and mortality: a systematic review and meta-analysis," *Transfusion*, vol. 50, no. 6, pp. 1370–1383, 2010.

[18] G. A. Rock, K. H. Shumak, N. A. Buskard et al., "Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group," *The New England Journal of Medicine*, vol. 325, no. 6, pp. 393–397, 1991.

[19] G. Rock, D. Anderson, W. Clark et al., "Does cryosupernatant plasma improve outcome in thrombotic thrombocytopenic purpura? No answer yet," *British Journal of Haematology*, vol. 129, no. 1, pp. 79–86, 2005.

[20] S. J. Brunskill, A. Tusold, S. Benjamin, S. J. Stanworth, and M. F. Murphy, "A systematic review of randomized controlled trials for plasma exchange in the treatment of thrombotic thrombocytopenic purpura," *Transfusion Medicine*, vol. 17, no. 1, pp. 17–35, 2007.

[21] J. L. Callum and S. Rizoli, "Plasma transfusion for patients with severe hemorrhage: what is the evidence?" *Transfusion*, vol. 52, supplement 1, pp. 30S–37S, 2012.

[22] J. B. Holcomb, B. C. Tilley, S. Baraniuk et al., "Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial," *Journal of the American Medical Association*, vol. 313, no. 5, pp. 471–482, 2015.

[23] A. Kovács and A. Guttman, "Medicinal chemistry meets proteomics: fractionation of the human plasma proteome," *Current Medicinal Chemistry*, vol. 20, no. 4, pp. 483–490, 2013.

[24] R. Pieper, C. L. Gatlin, A. J. Makusky et al., "The human serum proteome: display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins," *Proteomics*, vol. 3, no. 7, pp. 1345–1364, 2003.

[25] E. J. Favaloro, S. Soltani, J. McDonald, E. Grezchnik, and L. Easton, "Cross-laboratory audit of normal reference ranges and assessment of ABO blood group, gender and age on detected levels of plasma coagulation factors," *Blood Coagulation and Fibrinolysis*, vol. 16, no. 8, pp. 597–605, 2005.

[26] M. Franchini and G. Lippi, "Fibrinogen replacement therapy: a critical review of the literature," *Blood Transfusion*, vol. 10, no. 1, pp. 23–27, 2012.

[27] R. Palla, F. Peyvandi, and A. D. Shapiro, "Rare bleeding disorders: diagnosis and treatment," *Blood*, vol. 125, no. 13, pp. 2052–2061, 2015.

[28] J. B. Lefkowitz, A. Weller, R. Nuss, P. J. Santiago-Borrero, D. L. Brown, and I. R. Ortiz, "A common mutation, Arg457→Gln, links prothrombin deficiencies in the Puerto Rican population," *Journal of Thrombosis and Haemostasis*, vol. 1, no. 11, pp. 2381–2388, 2003.

[29] P. M. Aggeler, "Physiological basis for transfusion therapy in hemorrhagic disorders: a critical review," *Transfusion*, vol. 1, pp. 71–86, 1961.

[30] D. J. Murray, J. Olson, R. Strauss, and J. H. Tinker, "Coagulation changes during packed red cell replacement of major blood loss," *Anesthesiology*, vol. 69, no. 6, pp. 839–845, 1988.

[31] S. T. Hiippala, G. J. Myllyla, and E. M. Vahtera, "Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates," *Anesthesia & Analgesia*, vol. 81, no. 2, pp. 360–365, 1995.

[32] W. N. Erber, "Massive blood transfusion in the elective surgical setting," *Transfusion and Apheresis Science*, vol. 27, no. 1, pp. 83–92, 2002.

[33] A. Tinmouth, "Assessing the rationale and effectiveness of frozen plasma transfusions: an evidence-based review," *Hematology/Oncology Clinics of North America*, vol. 30, no. 3, pp. 561–572, 2016.

[34] R. B. Counts, C. Haisch, T. L. Simon, N. G. Maxwell, D. M. Heimbach, and C. J. Carrico, "Hemostasis in massively transfused trauma patients," *Annals of Surgery*, vol. 190, no. 1, pp. 91–99, 1979.

[35] R. M. Kakaiya, E. E. Morse, and S. Panek, "Labile coagulation factors in thawed fresh frozen plasma prepared by two methods," *Vox Sanguinis*, vol. 46, no. 1, pp. 44–46, 1984.

[36] R. Cardigan, P. F. Van Der Meer, C. Pergande et al., "Coagulation factor content of plasma produced from whole blood stored for 24 hours at ambient temperature: results from an international multicenter BEST Collaborative study," *Transfusion*, vol. 51, supplement 1, pp. 50S–57S, 2011.

[37] W. P. Sheffield, V. Bhakta, C. Mastronardi, S. Ramirez-Arcos, D. Howe, and C. Jenkins, "Changes in coagulation factor activity and content of di(2-ethylhexyl)phthalate in frozen plasma units during refrigerated storage for up to five days after thawing," *Transfusion*, vol. 52, no. 3, pp. 493–502, 2012.

[38] N. A. Orlova, S. V. Kovnir, I. I. Vorobiev, A. G. Gabibov, and A. I. Vorobiev, "Blood clotting factor VIII: from evolution to therapy," *Acta Naturae*, vol. 5, no. 2, pp. 19–39, 2013.

[39] N. Matijevic, Y.-W. Wang, B. A. Cotton et al., "Better hemostatic profiles of never-frozen liquid plasma compared with thawed fresh frozen plasma," *Journal of Trauma and Acute Care Surgery*, vol. 74, no. 1, pp. 84–91, 2013.

[40] A. E. Pusateri, M. B. Given, M. A. Schreiber et al., "Dried plasma: state of the science and recent developments," *Transfusion*, vol. 56, supplement 2, pp. S128–S139, 2016.

[41] *Circular of Information for the Use of Human Blood and Blood Components*, AABB/American Red Cross/America's Blood Centers/The Armed Services Blood Program, Puget Sound , Wash, USA, 2013.

[42] A. F. Eder and M. A. Sebok, "Plasma components: FFP, FP24, and thawed plasma," *Immunohematology*, vol. 23, no. 4, pp. 150–157, 2007.

[43] *Blood Component Information Circular of Information*, Australian Red Cross Blood Transfusion Service, Melbourne, Australia, 2015.

[44] M. Franchini, E. J. Favaloro, and G. Lippi, "Mild hemophilia A," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 3, pp. 421–432, 2010.

[45] J. O'Donnell, E. G. D. Tuddenham, R. Manning, G. Kemball-Cook, D. Johnson, and M. Laffan, "High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction," *Thrombosis and Haemostasis*, vol. 77, no. 5, pp. 825–828, 1997.

[46] B. Nascimento, L. T. Goodnough, and J. H. Levy, "Cryoprecipitate therapy," *British Journal of Anaesthesia*, vol. 113, no. 6, pp. 922–934, 2014.

[47] D. F. O'Shaughnessy, C. Atterbury, P. B. Maggs et al., "Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant," *British Journal of Haematology*, vol. 126, no. 1, pp. 11–28, 2004.

[48] *Circular of Information*, Canadian Blood Services, Ottawa, Canada, 2016.

[49] A. H. Kamal, A. Tefferi, and R. K. Pruthi, "How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults," *Mayo Clinic Proceedings*, vol. 82, no. 7, pp. 864–873, 2007.

[50] J. B. Segal and W. H. Dzik, "Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review," *Transfusion*, vol. 45, no. 9, pp. 1413–1425, 2005.

[51] M. D. Lancé, "A general review of major global coagulation assays: thrombelastography, thrombin generation test and clot waveform analysis," *Thrombosis Journal*, vol. 13, article 1, 2015.

[52] G. A. Hans and M. W. Besser, "The place of viscoelastic testing in clinical practice," *British Journal of Haematology*, vol. 173, no. 1, pp. 37–48, 2016.

[53] C. Solomon, L. M. Asmis, and D. R. Spahn, "Is viscoelastic coagulation monitoring with ROTEM or TEG validated?" *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 76, no. 6, pp. 503–507, 2016.

[54] R. Cardigan, J. Sutherland, M. Garwood et al., "The effect of leucocyte depletion on the quality of fresh-frozen plasma," *British Journal of Haematology*, vol. 114, no. 1, pp. 233–240, 2001.

[55] M. Heiden, U. Salge, R. Henschler et al., "Plasma quality after whole-blood filtration depends on storage temperature and filter type," *Transfusion Medicine*, vol. 14, no. 4, pp. 297–304, 2004.

[56] K. S.-K. Chan and R. L. Sparrow, "Microparticle profile and procoagulant activity of fresh-frozen plasma is affected by whole blood leukoreduction rather than 24-hour room temperature hold," *Transfusion*, vol. 54, no. 8, pp. 1935–1944, 2014.

[57] P. F. van der Meer and D. de Korte, "The effect of holding times of whole blood and its components during processing on in vitro and in vivo quality," *Transfusion Medicine Reviews*, vol. 29, no. 1, pp. 24–34, 2015.

[58] R. N. I. Pietersz, D. de Korte, H. W. Reesink, W. J. A. Dekker, A. van den Ende, and J. A. Loos, "Storage of whole blood for up to 24 hours at ambient temperature prior to component preparation," *Vox Sanguinis*, vol. 56, no. 3, pp. 145–150, 1989.

[59] E. M. O'Neill, J. Rowley, M. Hansson-Wicher, S. McCarter, G. Ragno, and C. R. Valeri, "Effect of 24-hour whole-blood storage on plasma clotting factors," *Transfusion*, vol. 39, no. 5, pp. 488–491, 1999.

[60] C. Wilsher, M. Garwood, J. Sutherland, C. Turner, and R. Cardigan, "The effect of storing whole blood at 22∘C for up to 24 hours with and without rapid cooling on the quality of red cell concentrates and fresh-frozen plasma," *Transfusion*, vol. 48, no. 11, pp. 2338–2347, 2008.

[61] P. F. van der Meer and D. de Korte, "Active cooling of whole blood to room temperature improves blood component quality," *Transfusion*, vol. 51, no. 2, pp. 357–362, 2011.

[62] L. Thibault, A. Beauséjour, M. J. de Grandmont, R. Lemieux, and J.-F. Leblanc, "Characterization of blood components prepared from whole-blood donations after a 24-hour hold with the platelet-rich plasma method," *Transfusion*, vol. 46, no. 8, pp. 1292–1299, 2006.

[63] K. Serrano, K. Scammell, S. Weiss et al., "Plasma and cryoprecipitate manufactured from whole blood held overnight at room temperature meet quality standards," *Transfusion*, vol. 50, no. 2, pp. 344–353, 2010.

[64] E. Shinar, S. Etlin, O. Frenkel, and V. Yahalom, "The implementation of rapid cooling and overnight hold of whole blood at ambient temperature before processing into components in Israel," *Transfusion*, vol. 51, supplement 1, pp. 58S–64S, 2011.

[65] S. Wang, T. Wang, Y. Fan et al., "A comparison study of the blood component quality of whole blood held overnight at 4°C or room temperature," *Journal of Blood Transfusion*, vol. 2013, Article ID 523539, 7 pages, 2013.

[66] L. J. Dumont, J. A. Cancelas, L. A. Maes et al., "The bioequivalence of frozen plasma prepared from whole blood held overnight at room temperature compared to fresh-frozen plasma prepared within eight hours of collection," *Transfusion*, vol. 55, no. 3, pp. 476–484, 2015.

[67] D. Triulzi, J. Gottschall, E. Murphy et al., "A multicenter study of plasma use in the United States," *Transfusion*, vol. 55, no. 6, pp. 1313–1319, 2015.

[68] G. Carlebjork, M. Blomback, and P. Pihlstedt, "Freezing of plasma and recovery of factor VIII," *Transfusion*, vol. 26, no. 2, pp. 159–162, 1986.

[69] A.-M. Swärd-Nilsson, P.-O. Persson, U. Johnson, and S. Lethagen, "Factors influencing factor VIII activity in frozen plasma," *Vox Sanguinis*, vol. 90, no. 1, pp. 33–39, 2006.

[70] S. Runkel, H. Haubelt, W. Hitzler, and P. Hellstern, "The quality of plasma collected by automated apheresis and of recovered plasma from leukodepleted whole blood," *Transfusion*, vol. 45, no. 3, pp. 427–432, 2005.

[71] D. J. Triulzi, "AABB contributions to plasma safety," *Transfusion*, vol. 52, no. 1, pp. 5S–8S, 2012.

[72] A. Godier, C.-M. Samama, and S. Susen, "Plasma/platelets/red blood cell ratio in the management of the bleeding traumatized patient: does it matter?" *Current Opinion in Anaesthesiology*, vol. 25, no. 2, pp. 242–247, 2012.

[73] K. A. Downes, E. Wilson, R. Yomtovian, and R. Sarode, "Serial measurement of clotting factors in thawed plasma stored for 5 days," *Transfusion*, vol. 41, no. 4, p. 570, 2001.

[74] E. Scott, K. Puca, J. Heraly, J. Gottschall, and K. Friedman, "Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6∘C storage," *Transfusion*, vol. 49, no. 8, pp. 1584–1591, 2009.

[75] R. S. Sidhu, T. Le, B. Brimhall, and H. Thompson, "Study of coagulation factor activities in apheresed thawed fresh frozen plasma at 1–6°C for five days," *Journal of Clinical Apheresis*, vol. 21, no. 4, pp. 224–226, 2006.

[76] C. Von Heymann, M. K. Keller, C. Spies et al., "Activity of clotting factors in fresh-frozen plasma during storage at 4∘C over 6 days," *Transfusion*, vol. 49, no. 5, pp. 913–920, 2009.

[77] P. Cookson, S. Thomas, S. Marschner, R. Goodrich, and R. Cardigan, "In vitro quality of single-donor platelets treated with riboflavin and ultraviolet light and stored in platelet storage medium for up to 8 days," *Transfusion*, vol. 52, no. 5, pp. 983–994, 2012.

[78] R. Cardigan and L. Green, "Thawed and liquid plasma—what do we know?" *Vox Sanguinis*, vol. 109, no. 1, pp. 1–10, 2015.

[79] P. Hellstern, "Solvent/detergent-treated plasma: composition, efficacy, and safety," *Current Opinion in Hematology*, vol. 11, no. 5, pp. 346–350, 2004.

[80] P. F. Lindholm, K. Annen, and G. Ramsey, "Approaches to minimize infection risk in blood banking and transfusion practice," *Infectious Disorders—Drug Targets*, vol. 11, no. 1, pp. 45–56, 2011.

[81] B. Horowitz, R. Bonomo, A. M. Prince, S. N. Chin, B. Brotman, and R. W. Shulman, "Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma," *Blood*, vol. 79, no. 3, pp. 826–831, 1992.

[82] T.-E. Svae, W. Frenzel, A. Heger, and J. Römisch, "Quality differences between solvent/detergent plasmas and fresh-frozen plasma," *Transfusion Medicine*, vol. 17, no. 4, pp. 318–321, 2007.

[83] A. S. Lawrie, L. Green, M. T. Canciani et al., "The effect of prion reduction in solvent/detergent-treated plasma on haemostatic variables," *Vox Sanguinis*, vol. 99, no. 3, pp. 232–238, 2010.

[84] J.-C. Osselaer, C. Debry, M. Goffaux et al., "Coagulation function in fresh-frozen plasma prepared with two photochemical treatment methods: methylene blue and amotosalen," *Transfusion*, vol. 48, no. 1, pp. 108–117, 2008.

[85] L. Backholer, M. Wiltshire, S. Proffitt, P. Cookson, and R. Cardigan, "Paired comparison of methylene blue- and amotosalen-treated plasma and cryoprecipitate," *Vox Sanguinis*, vol. 110, no. 4, pp. 352–361, 2016.

[86] R. Cardigan, K. Philpot, P. Cookson, and R. Luddington, "Thrombin generation and clot formation in methylene blue-treated plasma and cryoprecipitate," *Transfusion*, vol. 49, no. 4, pp. 696–703, 2009.

[87] T. Hubbard, L. Backholer, M. Wiltshire, R. Cardigan, and R. A. S. Ariëns, "Effects of riboflavin and amotosalen photoactivation systems for pathogen inactivation of fresh-frozen plasma on fibrin clot structure," *Transfusion*, vol. 56, no. 1, pp. 41–48, 2016.

[88] V. S. Hornsey, O. Drummond, A. Morrison, L. McMillan, I. R. MacGregor, and C. V. Prowse, "Pathogen reduction of fresh plasma using riboflavin and ultraviolet light: effects on plasma coagulation proteins," *Transfusion*, vol. 49, no. 10, pp. 2167–2172, 2009.

[89] K. Izutsu, "Stabilization of therapeutic proteins in aqueous solutions and freeze-dried solids: an overview," *Methods in Molecular Biology*, vol. 1129, pp. 435–441, 2014.

[90] A. Sailliol, C. Martinaud, A. P. Cap et al., "The evolving role of lyophilized plasma in remote damage control resuscitation in the French Armed Forces Health Service," *Transfusion*, vol. 53, supplement 1, pp. 65S–71S, 2013.

[91] J. Bux, D. Dickhörner, and E. Scheel, "Quality of freeze-dried (lyophilized) quarantined single-donor plasma," *Transfusion*, vol. 53, no. 12, pp. 3203–3209, 2013.

[92] C. Martinaud, C. Civadier, S. Ausset, C. Verret, A.-V. Deshayes, and A. Sailliol, "In vitro hemostatic properties of french lyophilized plasma," *Anesthesiology*, vol. 117, no. 2, pp. 339–346, 2012.

[93] T. Burnouf, "Modern plasma fractionation," *Transfusion Medicine Reviews*, vol. 21, no. 2, pp. 101–117, 2007.

[94] M. Radosevich and T. Burnouf, "Intravenous immunoglobulin G: trends in production methods, quality control and quality assurance," *Vox Sanguinis*, vol. 98, no. 1, pp. 12–28, 2010.

[95] J. Curnow, L. Pasalic, and E. J. Favaloro, "Treatment of von willebrand disease," *Seminars in Thrombosis and Hemostasis*, vol. 42, no. 2, pp. 133–146, 2016.

[96] A. Casini, P. de Moerloose, and M. Neerman-Arbez, "Clinical features and management of congenital fibrinogen deficiencies," *Seminars in Thrombosis and Hemostasis*, vol. 42, no. 4, pp. 366–374, 2016.

[97] J. N. Goldstein, M. A. Refaai, T. J. Milling Jr. et al., "Four-factor prothrombin complex concentrate versus plasma for rapid vitamin K antagonist reversal in patients needing urgent surgical or invasive interventions: a phase 3b, open-label, non-inferiority, randomised trial," *The Lancet*, vol. 385, no. 9982, pp. 2077–2087, 2015.

[98] C. Tersteeg, A. Schiviz, S. F. De Meyer et al., "Potential for recombinant ADAMTS13 as an effective therapy for acquired thrombotic thrombocytopenic purpura," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 35, no. 11, pp. 2336–2342, 2015.

[99] M. P. Zeller, K. S. Al-Habsi, M. Golder, G. M. Walsh, and W. P. Sheffield, "Plasma and plasma protein product transfusion: a canadian blood services centre for innovation symposium," *Transfusion Medicine Reviews*, vol. 29, no. 3, pp. 181–194, 2015.

[100] B. Whitaker, S. Rajbhandary, S. Kleinman, A. Harris, and N. Kamani, "Trends in United States blood collection and transfusion: results from the 2013 AABB Blood Collection, Utilization,

and Patient Blood Management Survey," *Transfusion*, vol. 56, no. 9, pp. 2173–2183, 2016.

[101] A. Tinmouth, T. Thompson, D. M. Arnold et al., "Utilization of frozen plasma in Ontario: a provincewide audit reveals a high rate of inappropriate transfusions," *Transfusion*, vol. 53, no. 10, pp. 2222–2229, 2013.

[102] A. W. Shih, E. Kolesar, S. Ning, N. Manning, D. M. Arnold, and M. A. Crowther, "Evaluation of the appropriateness of frozen plasma usage after introduction of prothrombin complex concentrates: A retrospective study," *Vox Sanguinis*, vol. 108, no. 3, pp. 274–280, 2015.

[103] C.-H. Hui, I. Williams, and K. Davis, "Clinical audit of the use of fresh-frozen plasma and platelets in a tertiary teaching hospital and the impact of a new transfusion request form," *Internal Medicine Journal*, vol. 35, no. 5, pp. 283–288, 2005.

[104] S. Pybus, A. MacCormac, A. Houghton, V. Martlew, and J. Thachil, "Inappropriateness of fresh frozen plasma for abnormal coagulation tests," *Journal of the Royal College of Physicians of Edinburgh*, vol. 42, no. 4, pp. 294–300, 2013.

[105] S. J. Stanworth, L. J. Estcourt, G. Powter et al., "A no-prophylaxis platelet-transfusion strategy for hematologic cancers," *The New England Journal of Medicine*, vol. 368, no. 19, pp. 1771–1780, 2013.

[106] R. M. Kaufman, B. Djulbegovic, T. Gernsheimer et al., "Platelet transfusion: a clinical practice guideline from the AABB," *Annals of Internal Medicine*, vol. 162, no. 3, pp. 205–213, 2015.

[107] M. J. Cohen and S. A. Christie, "New understandings of post injury coagulation and resuscitation," *International Journal of Surgery*, vol. 33, pp. 242–245, 2016.

[108] R. N. I. Pietersz, H. W. Reesink, S. Panzer et al., "Bacterial contamination in platelet concentrates," *Vox Sanguinis*, vol. 106, no. 3, pp. 256–283, 2014.

[109] P. F. van der Meer, "PAS or plasma for storage of platelets? A concise review," *Transfusion Medicine*, vol. 26, no. 5, pp. 339–342, 2016.

[110] J. N. Thon, P. Schubert, and D. V. Devine, "Platelet Storage Lesion: a new understanding from a proteomic perspective," *Transfusion Medicine Reviews*, vol. 22, no. 4, pp. 268–279, 2008.

[111] B. G. Solheim, O. Flesland, J. Seghatchian, and F. Brosstad, "Clinical implications of red blood cell and platelet storage lesions: an overview," *Transfusion and Apheresis Science*, vol. 31, no. 3, pp. 185–189, 2004.

[112] C. Saunders, G. Rowe, K. Wilkins, and P. Collins, "Impact of glucose and acetate on the characteristics of the platelet storage lesion in platelets suspended in additive solutions with minimal plasma," *Vox Sanguinis*, vol. 105, no. 1, pp. 1–10, 2013.

[113] K. M. Reddoch, H. F. Pidcoke, R. K. Montgomery et al., "Hemostatic function of apheresis platelets stored at 4∘C and 22∘C," *Shock*, vol. 41, no. 1, pp. 54–61, 2014.

[114] L. Johnson, S. Tan, B. Wood, A. Davis, and D. C. Marks, "Refrigeration and cryopreservation of platelets differentially affect platelet metabolism and function: a comparison with conventional platelet storage conditions," *Transfusion*, vol. 56, no. 7, pp. 1807–1818, 2016.

[115] L. Johnson, C. P. Coorey, and D. C. Marks, "The hemostatic activity of cryopreserved platelets is mediated by phosphatidylserine-expressing platelets and platelet microparticles," *Transfusion*, vol. 54, no. 8, pp. 1917–1926, 2014.

[116] F. Bertolini and S. Murphy, "A multicenter inspection of the swirling phenomenon in platelet concentrates prepared in routine practice," *Transfusion*, vol. 36, no. 2, pp. 128–132, 1996.

[117] T. VandenBroeke, L. J. Dumont, S. Hunter et al., "Platelet storage solution effects on the accuracy of laboratory tests for platelet function: A Multi-laboratory study," *Vox Sanguinis*, vol. 86, no. 3, pp. 183–188, 2004.

[118] B. K. Kim and M. G. Baldini, "The platelet response to hypotonic shock. Its value as an indicator of platelet viability after storage," *Transfusion*, vol. 14, no. 2, pp. 130–138, 1974.

[119] S. Holme, G. Moroff, and S. Murphy, "A multi-laboratory evaluation of in vitro platelet assays: the tests for extent of shape change and response to hypotonic shock. Biomedical Excellence for Safer Transfusion Working Party of the International Society of Blood Transfusion," *Transfusion*, vol. 38, no. 1, pp. 31–40, 1998.

[120] P. Järemo, "Some correlations between light transmission changes and some commonly used in vitro assays for the assessment of platelet concentrates," *European Journal of Haematology*, vol. 58, no. 3, pp. 181–185, 1997.

[121] E. M. Huang and T. C. Detwiler, "Characteristics of the synergistic actions of platelet agonists," *Blood*, vol. 57, no. 4, pp. 685–691, 1981.

[122] L. Johnson, K. M. Winter, T. Hartkopf-Theis, S. Reid, M. Kwok, and D. C. Marks, "Evaluation of the automated collection and extended storage of apheresis platelets in additive solution," *Transfusion*, vol. 52, no. 3, pp. 503–509, 2012.

[123] V. S. Hornsey, K. McColl, O. Drummond et al., "Extended storage of platelets in SSP+ platelet additive solution," *Vox Sanguinis*, vol. 91, no. 1, pp. 41–46, 2006.

[124] J. W. N. Akkerman, "Regulation of carbohydrate metabolism in platelets. A review," *Thrombosis and Haemostasis*, vol. 39, no. 3, pp. 712–724, 1978.

[125] J. G. Zhang, C. J. Carter, B. Culibrk et al., "Buffy-coat platelet variables and metabolism during storage in additive solutions or plasma," *Transfusion*, vol. 48, no. 5, pp. 847–856, 2008.

[126] G. Moroff, J. Kurtz, S. Seetharaman et al., "Comparative in vitro evaluation of apheresis platelets stored with 100% plasma or 65% platelet additive solution III/35% plasma and including periods without agitation under simulated shipping conditions," *Transfusion*, vol. 52, no. 4, pp. 834–843, 2012.

[127] L. J. Dumont and T. VandenBroeke, "Seven-day storage of apheresis platelets: report of an in vitro study," *Transfusion*, vol. 43, no. 2, pp. 143–150, 2003.

[128] D. W. C. Dekkers, I. M. De Cuyper, P. F. van der Meer, A. J. Verhoeven, and D. De Korte, "Influence of pH on stored human platelets," *Transfusion*, vol. 47, no. 10, pp. 1889–1895, 2007.

[129] P. Metcalfe, L. M. Williamson, C. P. M. Reutellngsperger, I. Swann, W. H. Ouwehand, and A. H. Goodall, "Activation during preparation of therapeutic platelets affects deterioration during storage: a comparative flow cytometric study of different production methods," *British Journal of Haematology*, vol. 98, no. 1, pp. 86–95, 1997.

[130] A.-M. Albanyan, P. Harrison, and M. F. Murphy, "Markers of platelet activation and apoptosis during storage of apheresis- and buffy coat-derived platelet concentrates for 7 days," *Transfusion*, vol. 49, no. 1, pp. 108–117, 2009.

[131] F. Cognasse, F. Boussoulade, P. Chavarin et al., "Release of potential immunomodulatory factors during platelet storage," *Transfusion*, vol. 46, no. 7, pp. 1184–1189, 2006.

[132] S. M. Picker, "In-vitro assessment of platelet function," *Transfusion and Apheresis Science*, vol. 44, no. 3, pp. 305–319, 2011.

[133] G. C. Leitner, J. List, M. Horvath, B. Eichelberger, S. Panzer, and P. Jilma-Stohlawetz, "Additive solutions differentially affect metabolic and functional parameters of platelet concentrates," *Vox Sanguinis*, vol. 110, no. 1, pp. 20–26, 2016.

[134] M. Böck, S. Rahrig, D. Kunz, G. Lutze, and M. U. Heim, "Platelet concentrates derived from buffy coat and apheresis: biochemical and functional differences," *Transfusion Medicine*, vol. 12, no. 5, pp. 317–324, 2002.

[135] S. Murphy and F. H. Gardner, "Platelet storage at 22 degrees C; metabolic, morphologic, and functional studies," *Journal of Clinical Investigation*, vol. 50, no. 2, pp. 370–377, 1971.

[136] *Guidance for Industry*, US Department of Health and Human Services, Washington, DC, USA, 2009.

[137] L. J. Dumont, D. F. Dumont, Z. M. Unger et al., "A randomized controlled trial comparing autologous radiolabeled in vivo platelet (PLT) recoveries and survivals of 7-day-stored PLT-rich plasma and buffy coat PLTs from the same subjects," *Transfusion*, vol. 51, no. 6, pp. 1241–1248, 2011.

[138] L. J. Dumont, J. A. Cancelas, D. F. Dumont et al., "A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide-frozen autologous platelets in healthy volunteers," *Transfusion*, vol. 53, no. 1, pp. 128–137, 2013.

[139] B. Diedrich, P. Sandgren, B. Jansson, H. Gulliksson, L. Svensson, and A. Shanwell, "In vitro and in vivo effects of potassium and magnesium on storage up to 7 days of apheresis platelet concentrates in platelet additive solution," *Vox Sanguinis*, vol. 94, no. 2, pp. 96–102, 2008.

[140] A. Bikker, E. Bouman, S. Sebastian et al., "Functional recovery of stored platelets after transfusion," *Transfusion*, vol. 56, no. 5, pp. 1030–1037, 2016.

[141] R. P. Goodrich, J. Li, H. Pieters, R. Crookes, J. Roodt, and A. D. P. Heyns, "Correlation of *in vitro* platelet quality measurements with *in vivo* platelet viability in human subjects," *Vox Sanguinis*, vol. 90, no. 4, pp. 279–285, 2006.

[142] S. J. Slichter, J. Corson, M. K. Jones et al., "Exploratory studies of extended storage of apheresis platelets in a platelet additive solution (PAS)," *Blood*, vol. 123, no. 2, pp. 271–280, 2014.

[143] J. C. Zimring, S. Slichter, K. Odem-Davis et al., "Metabolites in stored platelets associated with platelet recoveries and survivals," *Transfusion*, vol. 56, no. 8, pp. 1974–1983, 2016.

[144] C. R. Valeri, G. Ragno, and S. Khuri, "Freezing human platelets with 6 percent dimethyl sulfoxide with removal of the supernatant solution before freezing and storage at −80 degrees C without postthaw processing," *Transfusion*, vol. 45, no. 12, pp. 1890–1898, 2005.

[145] L. Johnson, S. Reid, S. Tan, D. Vidovic, and D. C. Marks, "PAS-G supports platelet reconstitution after cryopreservation in the absence of plasma," *Transfusion*, vol. 53, no. 10, pp. 2268–2277, 2013.

[146] C. R. Valeri, R. Srey, J. P. Lane, and G. Ragno, "Effect of WBC reduction and storage temperature on PLTs frozen with 6 percent DMSO for as long as 3 years," *Transfusion*, vol. 43, no. 8, pp. 1162–1167, 2003.

[147] F. Noorman, R. Strelitski, and J. Badloe, "Frozen platelets can be stored for 4 years at—80°C without affecting in vitro recovery, morphology, receptor expression, or coagulation profile," *Transfusion*, vol. 54, no. S2, pp. 15A–279A, 2014.

[148] P. A. Daly, C. A. Schiffer, J. Aisner, and P. H. Wiernik, "Successful transfusion of platelets cryopreserved for more than 3 years," *Blood*, vol. 54, no. 5, pp. 1023–1027, 1979.

[149] C. C. M. Lelkens, J. G. Koning, B. de Kort, I. B. G. Floot, and F. Noorman, "Experiences with frozen blood products in the Netherlands military," *Transfusion and Apheresis Science*, vol. 34, no. 3, pp. 289–298, 2006.

[150] J. Badloe and F. Noorman, "The Netherlands experience with frozen −80°C red cells, plasma and platelets in combat casualty care," *Transfusion*, vol. 51, no. S3, pp. 1A–297A, 2011.

[151] R. I. Handin and C. R. Valeri, "Improved viability of previously frozen platelets," *Blood*, vol. 40, no. 4, pp. 509–513, 1972.

[152] C. R. Valeri, H. Feingold, and L. D. Marchionni, "A simple method for freezing human platelets using 6% dimethylsulfoxide and storage at −80∘C," *Blood*, vol. 43, no. 1, pp. 131–136, 1974.

[153] C. R. Valeri, "Hemostatic effectiveness of liquid-preserved and previously frozen human platelets," *The New England Journal of Medicine*, vol. 290, no. 7, pp. 353–358, 1974.

[154] S. Yılmaz, R. A. Çetinkaya, İ. Eker et al., "Freezing of apheresis platelet concentrates in 6% dimethyl sulfoxide: the first preliminary study in Turkey," *Turkish Journal of Hematology*, vol. 33, no. 1, pp. 28–33, 2016.

[155] M. C. Reade, D. C. Marks, L. Johnson, D. O. Irving, and A. D. Holley, "Frozen platelets for rural Australia: the CLIP trial," *Anaesthesia and Intensive Care*, vol. 41, no. 6, pp. 804–805, 2013.

[156] J. P. Crowley, A. Rene, and C. R. Valeri, "Changes in platelet shape and structure after freeze preservation," *Blood*, vol. 44, no. 4, pp. 599–603, 1974.

[157] J. I. Spector, E. M. Skrabut, and C. R. Valeri, "Oxygen consumption, platelet aggregation and release reactions in platelets freeze-preserved with dimethylsulfoxide," *Transfusion*, vol. 17, no. 2, pp. 99–109, 1977.

[158] V. S. Hornsey, L. McMillan, A. Morrison, O. Drummond, I. R. MacGregor, and C. V. Prowse, "Freezing of buffy coat-derived, leukoreduced platelet concentrates in 6 percent dimethyl sulfoxide," *Transfusion*, vol. 48, no. 12, pp. 2508–2514, 2008.

[159] C. G. Zaroulis, J. I. Spector, C. P. Emerson, and C. R. Valeri, "Therapeutic transfusions of previously frozen washed human platelets," *Transfusion*, vol. 19, no. 4, pp. 371–378, 1979.

[160] C. A. Schiffer, J. Aisner, and P. H. Wiernik, "Clinical experience with transfusion of cryopreserved platelets," *British Journal of Haematology*, vol. 34, no. 3, pp. 377–385, 1976.

[161] C. A. Schiffer, J. Aisner, J. P. Dutcher, P. A. Daly, and P. H. Wiernik, "A clinical program of platelet cryopreservation," *Progress in Clinical and Biological Research*, vol. 88, pp. 165–180, 1982.

[162] B. Gerber, L. Alberio, S. Rochat et al., "Safety and efficacy of cryopreserved autologous platelet concentrates in HLA-alloimmunized patients with hematologic malignancies," *Transfusion*, vol. 56, no. 10, pp. 2426–2437, 2016.

[163] S. F. Khuri, N. Healey, H. MacGregor et al., "Comparison of the effects of transfusions of cryopreserved and liquid-preserved platelets on hemostasis and blood loss after cardiopulmonary bypass," *Journal of Thoracic and Cardiovascular Surgery*, vol. 117, no. 1, pp. 172–184, 1999.

[164] L. Johnson, S. Raynel, J. Seghatchian, and D. C. Marks, "Platelet microparticles in cryopreserved platelets: potential mediators of haemostasis," *Transfusion and Apheresis Science*, vol. 53, no. 2, pp. 146–152, 2016.

[165] M. R. Barnard, H. MacGregor, G. Ragno et al., "Fresh, liquid-preserved, and cryopreserved platelets: adhesive surface receptors and membrane procoagulant activity," *Transfusion*, vol. 39, no. 8, pp. 880–888, 1999.

[166] K. Nogami, "The utility of thromboelastography in inherited and acquired bleeding disorders," *British Journal of Haematology*, vol. 174, no. 4, pp. 503–514, 2016.

[167] P. Cookson, A. Lawrie, L. Green et al., "Thrombin generation and coagulation factor content of thawed plasma and platelet concentrates," *Vox Sanguinis*, vol. 108, no. 2, pp. 160–168, 2015.

[168] I. J. Bontekoe, P. F. van der Meer, and D. de Korte, "Determination of thromboelastographic responsiveness in stored single-donor platelet concentrates," *Transfusion*, vol. 54, no. 6, pp. 1610–1618, 2014.

[169] J. Cid, G. Escolar, A. Galan et al., "In vitro evaluation of the hemostatic effectiveness of cryopreserved platelets," *Transfusion*, vol. 56, no. 3, pp. 580–586, 2016.

[170] R. Al Dieri, B. de Laat, and H. C. Hemker, "Thrombin generation: what have we learned?" *Blood Reviews*, vol. 26, no. 5, pp. 197–203, 2012.

[171] T. Z. Tegegn, S. H. De Paoli, M. Orecna et al., "Characterization of procoagulant extracellular vesicles and platelet membrane disintegration in DMSO-cryopreserved platelets," *Journal of Extracellular Vesicles*, vol. 5, Article ID 30422, 2016.

[172] I. Eker, S. Yilmaz, R. A. Cetinkaya et al., "Generation of platelet microparticles after cryopreservation of apheresis platelet concentrates contribute to the hemostatic activity," *Turkish Journal of Haematology*, 2016.

[173] R. Cardigan, J. Sutherland, M. Garwood et al., "In vitro function of buffy coat-derived platelet concentrates stored for 9 days in CompoSol, PASII or 100% plasma in three different storage bags," *Vox Sanguinis*, vol. 94, no. 2, pp. 103–112, 2008.

[174] L. Johnson, M. C. Reade, R. A. Hyland, S. Tan, and D. C. Marks, "In vitro comparison of cryopreserved and liquid platelets: potential clinical implications," *Transfusion*, vol. 55, no. 4, pp. 838–847, 2015.

[175] L. M. Currie, B. Lichtiger, S. A. Livesey, W. Tansey, D. J. Yang, and J. Connor, "Enhanced circulatory parameters of human platelets cryopreserved with second-messenger effectors: an in vivo study of 16 volunteer platelet donors," *British Journal of Haematology*, vol. 105, no. 3, pp. 826–831, 1999.

[176] J. F. W. Keuren, E. J. P. Magdeleyns, J. W. P. Govers-Riemslag, T. Lindhout, and J. Curvers, "Effects of storage-induced platelet microparticles on the initiation and propagation phase of blood coagulation," *British Journal of Haematology*, vol. 134, no. 3, pp. 307–313, 2006.

[177] S. Raynel, M. P. Padula, D. C. Marks, and L. Johnson, "Cryopreservation alters the membrane and cytoskeletal protein profile of platelet microparticles," *Transfusion*, vol. 55, no. 10, pp. 2422–2432, 2015.

[178] S. D. Bohling, M. B. Pagano, M. R. Stitzel, C. Ferrell, W. Yeung, and W. L. Chandler, "Comparison of clot-based vs chromogenic factor Xa procoagulant phospholipid activity assays," *American Journal of Clinical Pathology*, vol. 137, no. 2, pp. 185–192, 2012.

[179] L. Ayers, M. Kohler, P. Harrison et al., "Measurement of circulating cell-derived microparticles by flow cytometry: sources of variability within the assay," *Thrombosis Research*, vol. 127, no. 4, pp. 370–377, 2011.

[180] J.-M. Freyssinet and F. Toti, "Formation of procoagulant microparticles and properties," *Thrombosis Research*, vol. 125, no. 1, pp. S46–S48, 2010.

[181] S. E. Headland, H. R. Jones, A. S. V. D'Sa, M. Perretti, and L. V. Norling, "Cutting-edge analysis of extracellular microparticles using ImageStream^X imaging flow cytometry," *Scientific Reports*, vol. 4, article 5237, 2014.

[182] S. Murphy and F. H. Gardner, "Effect of storage temperature on maintenance of platelet viability—deleterious effect of refrigerated storage," *The New England Journal of Medicine*, vol. 280, no. 20, pp. 1094–1098, 1969.

[183] A. Charlton, J. Wallis, J. Robertson, D. Watson, A. Iqbal, and H. Tinegate, "Where did platelets go in 2012? A survey of platelet transfusion practice in the North of England," *Transfusion Medicine*, vol. 24, no. 4, pp. 213–218, 2014.

[184] B. Wood, M. P. Padula, D. C. Marks, and L. Johnson, "Refrigerated storage of platelets initiates changes in platelet surface marker expression and localization of intracellular proteins," *Transfusion*, vol. 56, no. 10, pp. 2548–2559, 2016.

[185] K. M. Hoffmeister, T. W. Felbinger, H. Falet et al., "The clearance mechanism of chilled blood platelets," *Cell*, vol. 112, no. 1, pp. 87–97, 2003.

[186] D. E. Van Der Wal, V. X. Du, K. S. L. Lo, J. T. Rasmussen, S. Verhoef, and J. W. N. Akkerman, "Platelet apoptosis by cold-induced glycoprotein Ibα clustering," *Journal of Thrombosis and Haemostasis*, vol. 8, no. 11, pp. 2554–2562, 2010.

[187] K. M. Hoffmeister and H. Falet, "Platelet clearance by the hepatic Ashwell-Morrell receptor: mechanisms and biological significance," *Thrombosis Research*, vol. 141, supplement 2, pp. S68–S72, 2016.

[188] V. Rumjantseva, P. K. Grewal, H. H. Wandall et al., "Dual roles for hepatic lectin receptors in the clearance of chilled platelets," *Nature Medicine*, vol. 15, no. 11, pp. 1273–1280, 2009.

[189] G. Baimukanova, B. Miyazawa, D. R. Potter et al., "The effects of 22°C and 4°C storage of platelets on vascular endothelial integrity and function," *Transfusion*, vol. 56, supplement 1, pp. S52–S64, 2016.

[190] J. A. Bynum, M. Adam Meledeo, T. M. Getz et al., "Bioenergetic profiling of platelet mitochondria during storage: 4◦C storage extends platelet mitochondrial function and viability," *Transfusion*, vol. 56, no. 1, pp. S76–S84, 2016.

[191] P. M. Nair, H. F. Pidcoke, A. P. Cap, and A. K. Ramasubramanian, "Effect of cold storage on shear-induced platelet aggregation and clot strength," *Journal of Trauma and Acute Care Surgery*, vol. 77, no. 3, supplement 2, pp. S88–S93, 2014.

[192] P. Sandgren, M. Hansson, H. Gulliksson, and A. Shanwell, "Storage of buffy-coat-derived platelets in additive solutions at 4◦C and 22◦C: flow cytometry analysis of platelet glycoprotein expression," *Vox Sanguinis*, vol. 93, no. 1, pp. 27–36, 2007.

[193] A. P. Cap, "Platelet storage: a license to chill!," *Transfusion*, vol. 56, no. 1, pp. 13–16, 2016.

[194] *Canadian Blood Services Annual Report 2014-2015: How we Connect*, Canadian Blood Services, Ottawa, Canada, 2015.

[195] *Rapport Annuel 2012-2013 d'Hema-Quebec*, Hema-Quebec, Quebec, Canada, 2012.

[196] World Health Organization, *WHO Global Blood Safety and Availability Fact Sheet No. 279*, World Health Organization, Geneva, Switzerland, 2009.

[197] M. Raghavan and P. E. Marik, "Anemia, allogenic blood transfusion, and immunomodulation in the critically ill," *Chest*, vol. 127, no. 1, pp. 295–307, 2005.

[198] E. Bennett-Guerrero, Y. Zhao, S. M. O'Brien et al., "Variation in use of blood transfusion in coronary artery bypass graft surgery," *The Journal of the American Medical Association*, vol. 304, no. 14, pp. 1568–1575, 2010.

[199] N. Mohandas and J. A. Chasis, "Red blood cell deformability, membrane material properties and shape: regulation by transmembrane, skeletal and cytosolic proteins and lipids," *Seminars in Hematology*, vol. 30, no. 3, pp. 171–192, 1993.

[200] L. H. Derick, S.-C. Liu, A. H. Chishti, and J. Palek, "Protein immunolocalization in the spread erythrocyte membrane skeleton," *European Journal of Cell Biology*, vol. 57, no. 2, pp. 317–320, 1992.

[201] S. Peter Klinken, "Red blood cells," *International Journal of Biochemistry and Cell Biology*, vol. 34, no. 12, pp. 1513–1518, 2002.

[202] D. Bratosin, J. Estaquier, J. C. Ameisen, and J. Montreuil, "Molecular and cellular mechanisms of erythrocyte programmed cell death: impact on blood transfusion," *Vox sanguinis*, vol. 83, pp. 307–310, 2002.

[203] C. Donadee, N. J. H. Raat, T. Kanias et al., "Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion," *Circulation*, vol. 124, no. 4, pp. 465–476, 2011.

[204] M. T. Gladwin, T. Kanias, and D. B. Kim-Shapiro, "Hemolysis and cell-free hemoglobin drive an intrinsic mechanism for human disease," *The Journal of Clinical Investigation*, vol. 122, no. 4, pp. 1205–1208, 2012.

[205] P. C. Minneci, K. J. Deans, H. Zhi et al., "Hemolysis-associated endothelial dysfunction mediated by accelerated NO inactivation by decompartmentalized oxyhemoglobin," *The Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3409–3417, 2005.

[206] S. Sassa, "Why heme needs to be degraded to iron, biliverdin IXα, and carbon monoxide?" *Antioxidants and Redox Signaling*, vol. 6, no. 5, pp. 819–824, 2004.

[207] R. E. Fleming and P. Ponka, "Iron overload in human disease," *New England Journal of Medicine*, vol. 366, no. 4, pp. 348–359, 2012.

[208] E. A. Hod and S. L. Spitalnik, "Stored red blood cell transfusions: iron, inflammation, immunity, and infection," *Transfusion Clinique et Biologique*, vol. 19, no. 3, pp. 84–89, 2012.

[209] T. Kanias and J. P. Acker, "Biopreservation of red blood cells—the struggle with hemoglobin oxidation," *The FEBS Journal*, vol. 277, no. 2, pp. 343–356, 2010.

[210] E. A. Hod, N. Zhang, S. A. Sokol et al., "Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation," *Blood*, vol. 115, no. 21, pp. 4284–4292, 2010.

[211] A. Tinmouth and I. Chin-Yee, "The clinical consequences of the red cell storage lesion," *Transfusion Medicine Reviews*, vol. 15, no. 2, pp. 91–107, 2001.

[212] K. L. Scott, J. Lecak, and J. P. Acker, "Biopreservation of red blood cells: past, present, and future," *Transfusion Medicine Reviews*, vol. 19, no. 2, pp. 127–142, 2005.

[213] D. Orlov and K. Karkouti, "The pathophysiology and consequences of red blood cell storage," *Anaesthesia*, vol. 70, p. 29-e12, 2015.

[214] I. Chin-Yee, N. Arya, and M. S. d'almeida, "The red cell storage lesion and its implication for transfusion," *Transfusion Science*, vol. 18, no. 3, pp. 447–458, 1997.

[215] L. C. Wolfe, "The membrane and the lesions of storage in preserved red cells," *Transfusion*, vol. 25, no. 3, pp. 185–203, 1985.

[216] J. R. Hess, "An update on solutions for red cell storage," *Vox Sanguinis*, vol. 91, no. 1, pp. 13–19, 2006.

[217] H. Bessos and J. Seghatchian, "Red cell storage lesion: the potential impact of storage-induced CD47 decline on immunomodulation and the survival of leucofiltered red cells," *Transfusion and Apheresis Science*, vol. 32, no. 2, pp. 227–232, 2005.

[218] D. Bratosin, J. Mazurier, J. P. Tissier et al., "Cellular and molecular mechanisms of senescent erythrocyte phagocytosis by macrophages. A review," *Biochimie*, vol. 80, no. 2, pp. 173–195, 1998.

[219] C. F. Hogman and H. T. Meryman, "Storage parameters affecting red blood cell survival and function after transfusion," *Transfusion Medicine Reviews*, vol. 13, no. 4, pp. 275–296, 1999.

[220] I. Chin-Yee, N. Arya, and M. S. d'Almeida, "The red cell storage lesion and its implication for transfusion," *Transfusion Science*, vol. 18, no. 3, pp. 447–458, 1997.

[221] A. Stewart, S. Urbaniak, M. Turner, and H. Bessos, "Red cell storage lesion: the potential impact of storage-induced CD47 decline on immunomodulation and the survival of leucofiltered red cells," *Transfusion*, vol. 45, no. 9, pp. 1496–1503, 2005.

[222] P. A. Kurup, P. Arun, N. S. Gayathri, C. R. Dhanya, and A. R. Indu, "Modified formulation of CPDA for storage of whole blood, and of SAGM for storage of red blood cells, to maintain the concentration of 2,3-diphosphoglycerate," *Vox Sanguinis*, vol. 85, no. 4, pp. 253–261, 2003.

[223] J. R. Hess and T. G. Greenwalt, "Storage of red blood cells: new approaches," *Transfusion Medicine Reviews*, vol. 16, no. 4, pp. 283–295, 2002.

[224] T. Hovav, S. Yedgar, N. Manny, and G. Barshtein, "Alteration of red cell aggregability and shape during blood storage," *Transfusion*, vol. 39, no. 3, pp. 277–281, 1999.

[225] T. L. Berezina, S. B. Zaets, C. Morgan et al., "Influence of storage on red blood cell rheological properties," *Journal of Surgical Research*, vol. 102, no. 1, pp. 6–12, 2002.

[226] R. Almizraq, J. D. R. Tchir, J. L. Holovati, and J. P. Acker, "Storage of red blood cells affects membrane composition, microvesiculation, and in vitro quality," *Transfusion*, vol. 53, no. 10, pp. 2258–2267, 2013.

[227] R. T. Card, "Red cell membrane changes during storage," *Transfusion Medicine Reviews*, vol. 2, no. 1, pp. 40–47, 1988.

[228] G. Deplaine, I. Safeukui, F. Jeddi et al., "The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro," *Blood*, vol. 117, no. 8, pp. e88–e95, 2011.

[229] S. M. Frank, B. Abazyan, M. Ono et al., "Decreased erythrocyte deformability after transfusion and the effects of erythrocyte storage duration," *Anesthesia and Analgesia*, vol. 116, no. 5, pp. 975–981, 2013.

[230] S. J. Brunskill, K. L. Wilkinson, C. Doree, M. Trivella, and S. Stanworth, "Transfusion of fresher versus older red blood cells for all conditions," *Cochrane Database of Systematic Reviews*, no. 5, Article ID CD010801, 2015.

[231] L. M. G. Van De Watering, "Age of blood: does older blood yield poorer outcomes?" *Current Opinion in Hematology*, vol. 20, no. 6, pp. 526–532, 2013.

[232] W. A. Flegel, C. Natanson, and H. G. Klein, "Does prolonged storage of red blood cells cause harm?" *British Journal of Haematology*, vol. 165, no. 1, pp. 3–16, 2014.

[233] C. Lelubre and J.-L. Vincent, "Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review," *Critical Care*, vol. 17, no. 2, article R66, 2013.

[234] T. Burnouf, M.-L. Chou, H. Goubran, F. Cognasse, O. Garraud, and J. Seghatchian, "An overview of the role of microparticles/microvesicles in blood components: are they clinically beneficial or harmful?" *Transfusion and Apheresis Science*, vol. 53, no. 2, pp. 137–145, 2015.

[235] J. Simak and M. P. Gelderman, "Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers," *Transfusion Medicine Reviews*, vol. 20, no. 1, pp. 1–26, 2006.

[236] F. K. Keating, S. Butenas, M. K. Fung, and D. J. Schneider, "Platelet-white blood cell (WBC) interaction, WBC apoptosis, and procoagulant activity in stored red blood cells," *Transfusion*, vol. 51, no. 5, pp. 1086–1095, 2011.

[237] R. E. Waugh, M. Narla, C. W. Jackson, T. J. Mueller, T. Suzuki, and G. L. Dale, "Rheological properties of senescent erythrocytes: loss of surface area and volume with red blood cell age," *Blood*, vol. 79, no. 5, pp. 1351–1358, 1992.

[238] A. Dhabangi, B. Ainomugisha, C. Cserti-Gazdewich et al., "Effect of transfusion of red blood cells with longer vs shorter storage duration on elevated blood lactate levels in children with severe anemia: the total randomized clinical trial," *Journal of the American Medical Association*, vol. 314, no. 23, pp. 2514–2523, 2015.

[239] D. A. Fergusson, P. Hébert, D. L. Hogan et al., "Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial," *The Journal of the American Medical Association*, vol. 308, no. 14, pp. 1443–1451, 2012.

[240] C. F. Högman and H. T. Meryman, "Red blood cells intended for transfusion: quality criteria revisited," *Transfusion*, vol. 46, no. 1, pp. 137–142, 2006.

[241] A. Chabanel, M. Masse, and S. Begue, "National French observatory of the quality of blood components for transfusion," *Transfusion Clinique et Biologique*, vol. 15, no. 3, pp. 85–90, 2008.

[242] K. Radwanski, F. Cognasse, O. Garraud, J.-M. Payrat, and K. Min, "Comparison of apheresis and 24h RT held red cell concentrates by measurement of storage lesion parameters and neutrophil activating factors during 42-day storage," *Transfusion and Apheresis Science*, vol. 48, no. 2, p. 169, 2013.

[243] C. F. Hogman, L. Eriksson, K. Hedlund, and J. Wallvik, "The bottom and top system: a new technique for blood component preparation and storage," *Vox Sanguinis*, vol. 55, no. 4, pp. 211–217, 1988.

[244] F. Bennardello, C. Fidone, V. Spadola et al., "The prevention of adverse reactions to transfusions in patients with haemoglobinopathies: a proposed algorithm," *Blood Transfusion*, vol. 11, no. 3, pp. 377–384, 2013.

[245] G. M. D'Amici, C. Mirasole, A. D'Alessandro, T. Yoshida, L. J. Dumont, and L. Zolla, "Red blood cell storage in SAGM and AS3: a comparison through the membrane two-dimensional electrophoresis proteome," *Blood Transfusion*, vol. 10, supplement 2, pp. s46–s54, 2012.

[246] C. F. Högman, "Liquid-stored red blood cells for transfusion: a status report," *Vox Sanguinis*, vol. 76, no. 2, pp. 67–77, 1999.

[247] L. M. G. van de Watering, "Effects of red blood cell storage in heavily transfused patients," *Current Opinion in Anaesthesiology*, vol. 26, no. 2, pp. 204–207, 2013.

[248] B. Bicalho, A. S. Pereira, and J. P. Acker, "Buffy coat (top/bottom)- and whole-blood filtration (top/top)-produced red cell concentrates differ in size of extracellular vesicles," *Vox Sanguinis*, vol. 109, no. 3, pp. 214–220, 2015.

[249] A. L. Hansen, J. D. R. Kurach, T. R. Turner et al., "The effect of processing method on the in vitro characteristics of red blood cell products," *Vox Sanguinis*, vol. 108, no. 4, pp. 350–358, 2015.

[250] J. P. Acker, A. L. Hansen, J. D. R. Kurach, T. R. Turner, I. Croteau, and C. Jenkins, "A quality monitoring program for red blood cell components: in vitro quality indicators before and after implementation of semiautomated processing," *Transfusion*, vol. 54, no. 10, pp. 2534–2543, 2014.

[251] S. Bakkour, J. P. Acker, D. M. Chafets et al., "Manufacturing method affects mitochondrial DNA release and extracellular vesicle composition in stored red blood cells," *Vox Sanguinis*, vol. 111, no. 1, pp. 22–32, 2016.

[252] S. M. Picker, S. M. Radojska, and B. S. Gathof, "In vitro quality of red blood cells (RBCs) collected by multicomponent apheresis compared to manually collected RBCs during 49 days of storage," *Transfusion*, vol. 47, no. 4, pp. 687–696, 2007.

[253] S. Holme, M. Dean Elfath, and P. Whitley, "Evaluation of in vivo and in vitro quality of apheresis-collected RBC stored for 42 days," *Vox Sanguinis*, vol. 75, no. 3, pp. 212–217, 1998.

[254] N. M. Heddle, D. M. Arnold, J. P. Acker et al., "Red blood cell processing methods and in-hospital mortality: a transfusion registry cohort study," *The Lancet Haematology*, vol. 3, no. 5, pp. e246–e254, 2016.

[255] R. A. Middelburg, D. Van Stein, B. Zupanska et al., "Female donors and transfusion-related acute lung injury: a case-referent study from the International TRALI Unisex Research Group," *Transfusion*, vol. 50, no. 11, pp. 2447–2454, 2010.

[256] A. Jordan, D. Chen, Q. -. Yi, T. Kanias, M. T. Gladwin, and J. P. Acker, "Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data," *Vox Sanguinis*, vol. 111, no. 1, pp. 8–15, 2016.

[257] J. S. Raval, J. H. Waters, A. Seltsam et al., "Menopausal status affects the susceptibility of stored RBCs to mechanical stress," *Vox Sanguinis*, vol. 100, no. 4, pp. 418–421, 2011.

[258] V. L. Tzounakas, A. G. Kriebardis, I. S. Papassideri, and M. H. Antonelou, "Donor-variation effect on red blood cell storage lesion: a close relationship emerges," *PROTEOMICS—Clinical Applications*, vol. 10, no. 8, pp. 791–804, 2016.

[259] M. Detraglia, F. B. Cook, D. M. Stasiw, and L. C. Cerny, "Erythrocyte fragility in aging," *Biomembranes*, vol. 345, no. 2, pp. 213–219, 1974.

[260] V. L. Tzounakas, H. T. Georgatzakou, A. G. Kriebardis et al., "Donor variation effect on red blood cell storage lesion: a multivariable, yet consistent, story," *Transfusion*, vol. 56, no. 6, pp. 1274–1286, 2016.

[261] T. Kanias, D. Sinchar, D. Osei-Hwedieh et al., "Testosterone-dependent sex differences in red blood cell hemolysis in storage, stress, and disease," *Transfusion*, vol. 56, no. 10, pp. 2571–2583, 2016.

[262] R. Sparrow and K. A. Payne, "Donor factors, rather than physical variables of red blood cell components determine the level of hemolysis at 42 days of storage," *Transfusion*, vol. 55, no. S3, pp. 55A–56A, 2015.

[263] T. Kanias, M. C. Lanteri, S. M. Keating et al., "Genetic, ethnic and gender determinants of red blood storage and stress hemolysis," *Transfusion*, vol. 55, no. S3, pp. 38A–39A, 2015.

[264] T. J. Van't Erve, B. A. Wagner, S. M. Martin et al., "The heritability of hemolysis in stored human red blood cells," *Transfusion*, vol. 55, no. 6, pp. 1178–1185, 2015.

[265] J. S. Raval, J. H. Waters, A. Seltsam et al., "The use of the mechanical fragility test in evaluating sublethal RBC injury during storage," *Vox Sanguinis*, vol. 99, no. 4, pp. 325–331, 2010.

[266] M. Ciavatti, A. Jouvenceaux, B. Chataing et al., "Changes in the erythrocyte membrane during blood preservation. Influence of progesterone," *Revues Francais Transfusion Immunohematologie*, vol. 19, no. 4, pp. 539–554, 1976.

[267] J. M. Rifkind, K. Araki, J. G. Mohanty, and T. Suda, "Age dependent changes in erythrocyte membrane function," *Progress in clinical and biological research*, vol. 195, pp. 159–172, 1985.

[268] N. J. Rencricca, J. Solomon, W. J. Fimian Jr., D. Howard, V. Rizzoli, and F. Stohlman Jr., "The effect of testosterone on erythropoiesis," *Scandinavian Journal of Haematology*, vol. 6, no. 6, pp. 431–436, 1969.

[269] A. Jordan, Q.-L. Yi, and J. P. Acker, "Age matters: how donor characteristics influence red cell product quality," *Transfusion*, vol. 55, no. S3, pp. 77A–78A, 2015.

[270] R. A. Middelburg, E. Briët, and J. G. Van der Bom, "Mortality after transfusions, relation to donor sex," *Vox Sanguinis*, vol. 101, no. 3, pp. 221–229, 2011.

[271] M. Chassé, A. Tinmouth, S. W. English et al., "Association of blood donor age and sex with recipient survival after red blood cell transfusion," *JAMA Internal Medicine*, vol. 176, no. 9, pp. 1307–1314, 2016.

[272] H. Bjursten, A. Dardashti, J. Björk, P. Wierup, L. Algotsson, and P. Ederoth, "Transfusion of sex-mismatched and non-leukocyte-depleted red blood cells in cardiac surgery increases mortality," *Journal of Thoracic and Cardiovascular Surgery*, 2015.

[273] R. L. Barty, R. J. Cook, Y. Liu et al., "Exploratory analysis of the association between donor sex and in-hospital mortality in transfusion recipients," *Transfusion*, vol. 55, no. S3, pp. 23A–24A, 2015.

[274] M. Desmarets, L. Bardiaux, E. Benzenine et al., "Effect of storage time and donor sex of transfused red blood cells on 1-year survival in patients undergoing cardiac surgery: an observational study," *Transfusion*, vol. 56, no. 5, pp. 1213–1222, 2016.

[275] S. K. Vasan, F. Chiesa, K. Rostgaard et al., "Lack of association between blood donor age and survival of transfused patients," *Blood*, vol. 127, no. 5, pp. 658–661, 2016.

[276] J. Treleaven, A. Gennery, J. Marsh et al., "Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force," *British Journal of Haematology*, vol. 152, no. 1, pp. 35–51, 2011.

[277] G. Moroff and N. L. C. Luban, "The irradiation of blood and blood components to prevent graft-versus-host disease: technical issues and guidelines," *Transfusion Medicine Reviews*, vol. 11, no. 1, pp. 15–26, 1997.

[278] H. M. Zbikowska and A. Antosik, "Irradiation dose-dependent oxidative changes in red blood cells for transfusion," *International Journal of Radiation Biology*, vol. 88, no. 9, pp. 654–660, 2012.

[279] R. Katharia, R. Chaudhary, and P. Agarwal, "Prestorage gamma irradiation induces oxidative injury to red cells," *Transfusion and Apheresis Science*, vol. 48, no. 1, pp. 39–43, 2013.

[280] G. Moroff and N. L. C. Luban, "The influence of gamma irradiation on red cell and platelet properties," *Transfusion Science*, vol. 15, no. 2, pp. 141–148, 1994.

[281] K. Raghavendran, J. Nemzek, L. M. Napolitano, and P. R. Knight, "Aspiration-induced lung injury," *Critical Care Medicine*, vol. 39, no. 4, pp. 818–826, 2011.

[282] H. Relevy, A. Koshkaryev, N. Manny, S. Yedgar, and G. Barshtein, "Blood banking-induced alteration of red blood cell flow properties," *Transfusion*, vol. 48, no. 1, pp. 136–146, 2008.

[283] R. M. Patel, J. D. Roback, K. Uppal, T. Yu, D. P. Jones, and C. D. Josephson, "Metabolomics profile comparisons of irradiated and nonirradiated stored donor red blood cells," *Transfusion*, vol. 55, no. 3, pp. 544–552, 2015.

[284] D. Xu, M. Peng, Z. Zhang, G. Dong, Y. Zhang, and H. Yu, "Study of damage to red blood cells exposed to different doses of γ-ray irradiation," *Blood Transfusion*, vol. 10, no. 3, pp. 321–330, 2012.

[285] H. El Kenz, F. Corazza, P. Van Der Linden, S. Chabab, and C. Vandenvelde, "Potassium content of irradiated packed red blood cells in different storage media: is there a need for additive solution-dependent recommendations for infant transfusion?" *Transfusion and Apheresis Science*, vol. 49, no. 2, pp. 249–253, 2013.

[286] S. K. Harm, J. S. Raval, J. Cramer, J. H. Waters, and M. H. Yazer, "Haemolysis and sublethal injury of RBCs after routine blood bank manipulations," *Transfusion Medicine*, vol. 22, no. 3, pp. 181–185, 2012.

[287] A. C. Lee, L. L. Reduque, N. L. C. Luban, P. M. Ness, B. Anton, and E. S. Heitmiller, "Transfusion-associated hyperkalemic cardiac arrest in pediatric patients receiving massive transfusion," *Transfusion*, vol. 54, no. 1, pp. 244–254, 2014.

[288] A. Vraets, Y. Lin, and J. L. Callum, "Transfusion-associated hyperkalemia," *Transfusion Medicine Reviews*, vol. 25, no. 3, pp. 184–196, 2011.

[289] K. Serrano, D. Chen, A. L. Hansen et al., "The effect of timing of gamma-irradiation on hemolysis and potassium release in leukoreduced red cell concentrates stored in SAGM," *Vox Sanguinis*, vol. 106, no. 4, pp. 379–381, 2014.

[290] D. de Korte, H. Croxon, J. Petrick et al., "Timing of gamma irradiation and sex of blood donor influences in vitro characteristics of red cell concentrates," *Transfusion*, vol. 55, no. S3, p. 38A, 2015.

[291] P. Mazur, "Basic problems in cryobiology," in *Advances in Cryogenic Engineering*, K. D. Timmerhaus, Ed., pp. 28–37, Plenum Press, New York, NY, USA, 1964.

[292] J. Lecak, K. Scott, C. Young, J. Hannon, and J. P. Acker, "Evaluation of red blood cells stored at −80°C in excess of 10 years," *Transfusion*, vol. 44, no. 9, pp. 1306–1313, 2004.

[293] C. R. Valeri, G. Ragno, L. E. Pivacek et al., "An experiment with glycerol-frozen red blood cells stored at -80°C for up to 37 years," *Vox Sanguinis*, vol. 79, no. 3, pp. 168–174, 2000.

[294] M. A. Schreiber, B. H. McCully, J. B. Holcomb et al., "Transfusion of cryopreserved packed red blood cells is safe and effective after trauma: a prospective randomized trial," *Annals of Surgery*, vol. 262, no. 3, pp. 426–432, 2015.

[295] D. A. Hampton, C. Wiles, L. J. Fabricant et al., "Cryopreserved red blood cells are superior to standard liquid red blood cells," *Journal of Trauma and Acute Care Surgery*, vol. 77, no. 1, pp. 20–27, 2014.

[296] J. E. Lovelock, "The mechanism of the protective action of glycerol against haemolysis by freezing and thawing," *Biochimica et Biophysica Acta*, vol. 11, pp. 28–36, 1953.

[297] A. U. Smith, "Prevention of hæmolysis during freezing and thawing of red blood-cells," *The Lancet*, vol. 256, no. 6644, pp. 910–911, 1950.

[298] A. W. Rowe, E. Eyster, and A. Kellner, "Liquid nitrogen preservation of red blood cells for transfusion: a low glycerol—rapid freeze procedure," *Cryobiology*, vol. 5, no. 2, pp. 119–128, 1968.

[299] H. W. Krijnen, J. J. De Wit, A. C. J. Kuivenhoven, J. A. Loos, and H. K. Prins, "Glycerol treated human red cells frozen with liquid nitrogen," *Vox Sanguinis*, vol. 9, pp. 559–572, 1964.

[300] J. H. Pert, P. K. Schork, and R. Moore, "Low-temperature preservation of human erythrocytes: biochemical and clinical aspects," *Bibliotheca haematologica*, vol. 19, pp. 47–53, 1964.

[301] H. T. Meryman and M. Hornblower, "A method for freezing and washing red blood cells using a high glycerol concentration," *Transfusion*, vol. 12, no. 3, pp. 145–156, 1972.

[302] J. L. Tullis, M. M. Ketchel, H. M. Pyle et al., "Studies on the in vivo survival of glycerolized and frozen human red blood cells," *The Journal of the American Medical Association*, vol. 168, no. 4, pp. 399–404, 1958.

[303] *Standards for Blood Banks and Transfusion Services*, vol. 127, American Association of Blood Banks, Bethesda, Md, USA, 22nd edition, 2003.

[304] M. F. O'Leary, P. Szklarski, T. M. Klein, and P. P. Young, "Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population," *Transfusion*, vol. 51, no. 5, pp. 955–960, 2011.

[305] A. A. R. Tobian, W. J. Savage, D. J. Tisch, S. Thoman, K. E. King, and P. M. Ness, "Prevention of allergic transfusion reactions to platelets and red blood cells through plasma reduction," *Transfusion*, vol. 51, no. 8, pp. 1676–1683, 2011.

[306] C. C. Silliman, E. E. Moore, J. L. Johnson, R. J. Gonzalez, and W. L. Biffl, "Transfusion of the injured patient: proceed with caution," *Shock*, vol. 21, no. 4, pp. 291–299, 2004.

[307] J. M. Cholette, K. F. Henrichs, G. M. Alfieris et al., "Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: results of a prospective, randomized, controlled clinical trial," *Pediatric Critical Care Medicine*, vol. 13, no. 3, pp. 290–299, 2012.

[308] T. Smith, W. Riley, and D. Fitzgerald, "In vitro comparison of two different methods of cell washing," *Perfusion*, vol. 28, no. 1, pp. 34–37, 2013.

[309] M. Gruber, A. Breu, M. Frauendorf, T. Seyfried, and E. Hansen, "Washing of banked blood by three different blood salvage devices," *Transfusion*, vol. 53, no. 5, pp. 1001–1009, 2013.

[310] A. Hansen, Q.-L. Yi, and J. P. Acker, "Quality of red blood cells washed using the ACP 215 cell processor: assessment of optimal pre- and postwash storage times and conditions," *Transfusion*, vol. 53, no. 8, pp. 1772–1779, 2013.

[311] *Standards for Blood Banks and Transfusion Services*, AABB, Bethesda, Md, USA, 2012.

[312] *CAN/CSA-Z902-10 Blood and Blood Components*, Canadian Standards Association, Mississauga, Canada, 2010.

[313] A. L. Hansen, T. R. Turner, J. D. R. Kurach, and J. P. Acker, "Quality of red blood cells washed using a second wash sequence on an automated cell processor," *Transfusion*, vol. 55, no. 10, pp. 2415–2421, 2015.

[314] C. B. Tóth, J. Kramer, J. Pinter, M. Thék, and J. E. Szabó, "IgA content of washed red blood cell concentrates," *Vox Sanguinis*, vol. 74, no. 1, pp. 13–14, 1998.

[315] L. R. Bryant, L. Holland, and S. Corkern, "Optimal leukocyte removal from refrigerated blood with the IBM 2991 blood cell processor," *Transfusion*, vol. 18, no. 4, pp. 469–471, 1978.

[316] B. Wenz, "Clinical and laboratory precautions that reduce the adverse reactions, alloimmunization, infectivity, and possibly immunomodulation associated with homologous transfusions," *Transfusion Medicine Reviews*, vol. 4, no. 4, supplement 1, pp. 3–7, 1990.

[317] K. L. Lannan, J. Sahler, S. L. Spinelli, R. P. Phipps, and N. Blumberg, "Transfusion immunomodulation—the case for leukoreduced and (perhaps) washed transfusions," *Blood Cells, Molecules, and Diseases*, vol. 50, no. 1, pp. 61–68, 2013.

[318] M. A. Refaai and N. Blumberg, "Transfusion immunomodulation from a clinical perspective: an update," *Expert Review of Hematology*, vol. 6, no. 6, pp. 653–663, 2013.

[319] I. Corteś-Puch, D. Wang, J. Sun et al., "Washing older blood units before transfusion reduces plasma iron and improves outcomes in experimental canine pneumonia," *Blood*, vol. 123, no. 9, pp. 1403–1411, 2014.

[320] R. M. Belizaire, A. T. Makley, E. M. Campion et al., "Resuscitation with washed aged packed red blood cell units decreases the proinflammatory response in mice after hemorrhage," *Journal of Trauma and Acute Care Surgery*, vol. 73, no. 2, supplement 1, pp. S128–S133, 2012.

[321] V. Weisbach, W. Riego, E. Strasser et al., "The in vitro quality of washed, prestorage leucocyte-depleted red blood cell concentrates," *Vox Sanguinis*, vol. 87, no. 1, pp. 19–26, 2004.

[322] R. de Vroege, W. R. Wildevuur, J. A. G. Muradin, D. Graves, and W. van Oeveren, "Washing of stored red blood cells by an autotransfusion device before transfusion," *Vox Sanguinis*, vol. 92, no. 2, pp. 130–135, 2007.

[323] B. Westphal-Varghese, M. Erren, M. Westphal et al., "Processing of stored packed red blood cells using autotransfusion devices decreases potassium and microaggregates: a prospective, randomized, single-blinded in vitro study," *Transfusion Medicine*, vol. 17, no. 2, pp. 89–95, 2007.

[324] R. Mancini, L. Marinelli, N. Mirante et al., "Evaluation of haemoglobin, haematocrit, haemolysis, residual protein content and leucocytes in 345 red blood cell concentrates used for the treatment of patients with β-thalassaemia," *Blood Transfusion*, vol. 10, no. 1, pp. 39–44, 2012.

[325] T. J. Contreras and C. R. Valeri, "A comparison of methods to wash liquid-stored red blood cells and red blood cells frozen with high or low concentrations of glycerol," *Transfusion*, vol. 16, no. 6, pp. 539–565, 1976.

[326] A. L. Hansen, T. R. Turner, Q.-L. Yi, and J. P. Acker, "Quality of red blood cells washed using an automated cell processor with and without irradiation," *Transfusion*, vol. 54, no. 6, pp. 1585–1594, 2014.

[327] S. O. Sowemimo-Coker, "Red blood cell hemolysis during processing," *Transfusion Medicine Reviews*, vol. 16, no. 1, pp. 46–60, 2002.

[328] D. de Korte and A. J. Verhoeven, "Quality determinants of erythrocyte destined for transfusion," *Cellular and molecular biology (Noisy-le-Grand, France)*, vol. 50, no. 2, pp. 187–195, 2004.

[329] J. R. Hess, "Measures of stored red blood cell quality," *Vox Sanguinis*, vol. 107, no. 1, pp. 1–9, 2014.

[330] L. J. Dumont and J. P. Aubuchon, "Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials," *Transfusion*, vol. 48, no. 6, pp. 1053–1060, 2008.

[331] W. A. Heaton, "Evaluation of posttransfusion recovery and survival of transfused red cells," *Transfusion medicine reviews*, vol. 6, no. 3, pp. 153–169, 1992.

[332] K. J. Wardrop, R. L. Tucker, and E. P. Anderson, "Use of an in vitro biotinylation technique for determination of posttransfusion viability of stored canine packed red blood cells," *American Journal of Veterinary Research*, vol. 59, no. 4, pp. 397–400, 1998.

[333] D. M. Mock, J. A. Widness, P. Veng-Pedersen et al., "Measurement of posttransfusion red cell survival with the biotin label," *Transfusion Medicine Reviews*, vol. 28, no. 3, pp. 114–125, 2014.

[334] J. Creteur, A. P. Neves, and J.-L. Vincent, "Near-infrared spectroscopy technique to evaluate the effects of red blood cell transfusion on tissue oxygenation," *Critical Care*, vol. 13, supplement 5, article S11, 2009.

[335] M. G. Risbano, T. Kanias, D. Triulzi et al., "Effects of aged stored autologous red blood cells on human endothelial function," *American Journal of Respiratory and Critical Care Medicine*, vol. 192, no. 10, pp. 1223–1233, 2015.

[336] S. O. Sowemimo-Coker, J. Acker, M. Narla et al., "Development of a statistical model for predicting in vivo viability of red blood cells: importance of red cell membrane damages," *Transfusion*, vol. 55, no. S3, pp. 56A–57A, 2015.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 6140239, 11 pages
http://dx.doi.org/10.1155/2016/6140239

Review Article

Could Microparticles Be the Universal Quality Indicator for Platelet Viability and Function?

Elisabeth Maurer-Spurej[1,2,3] and Kate Chipperfield[3,4]

[1] *LightIntegra Technology Inc., 650-999 West Broadway, Vancouver, BC, Canada V5Z 1K5*
[2] *Canadian Blood Services, Centre for Blood Research, 2350 Health Sciences Mall, Vancouver, BC, Canada*
[3] *Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada*
[4] *Hematopathology, Department of Pathology and Laboratory Medicine, British Columbia Children's Hospital, 4480 Oak Street, Room 2K49 Vancouver, BC, Canada V6H 3V4*

Correspondence should be addressed to Elisabeth Maurer-Spurej; emaurer@mail.ubc.ca

Received 19 August 2016; Revised 27 October 2016; Accepted 6 November 2016

Academic Editor: Denese Marks

High quality means good fitness for the intended use. Research activity regarding quality measures for platelet transfusions has focused on platelet storage and platelet storage lesion. Thus, platelet quality is judged from the manufacturer's point of view and regulated to ensure consistency and stability of the manufacturing process. Assuming that fresh product is always superior to aged product, maintaining in vitro characteristics should preserve high quality. However, despite the highest in vitro quality standards, platelets often fail in vivo. This suggests we may need different quality measures to predict platelet performance after transfusion. Adding to this complexity, platelets are used clinically for very different purposes: platelets need to circulate when given as prophylaxis to cancer patients and to stop bleeding when given to surgery or trauma patients. In addition, the emerging application of platelet-rich plasma injections exploits the immunological functions of platelets. Requirements for quality of platelets intended to prevent bleeding, stop bleeding, or promote wound healing are potentially very different. Can a single measurable characteristic describe platelet quality for all uses? Here we present microparticle measurement in platelet samples, and its potential to become the universal quality characteristic for platelet production, storage, viability, function, and compatibility.

1. Introduction

High quality performance is achieved when the best tool or process is employed for the intended use. Currently, with the end-goal of high quality platelet products for transfusion, platelet concentrate production, manipulation, and storage are tailored to maintaining platelet viability. Anticoagulation, consistency, and stability of the manufacturing process, limited exposure to stress, and optimal storage conditions are tightly controlled parameters to preserve platelet viability in vitro and prevent degradation, also known as platelet storage lesion. It is assumed that most donors donate viable platelets and that viability is lost due to the storage lesion; donor variability is not considered a major contributing factor [1]. It then follows that patients needing viable platelets that remain in circulation for some time would benefit most from the freshest product. Traditionally, in vitro platelet quality measures have been based on these assumptions [2]. Parameters like CD62 expression, response to ADP, or hypotonic shock are measured because there is a physiological rationale behind changes in these measures that occur with both activation and aging of platelets. Platelet release of microparticles has also been shown to follow platelet activation and increase with aging of platelet products [3]. Thus, platelet quality is assessed from the manufacturer's point of view and regulated to ensure consistency and stability of the manufacturing process [4].

Because the emphasis is to detect degradation from the beginning to the end of the current 5-day shelf life [5, 6], the resolution of current quality measures has been tuned to be high for small changes on the "resting/viable" end of the quality spectrum but becomes low on the "activated/functional"

end of the quality spectrum. Additional changes are seen upon extended shelf life which become more important as bacterially tested or pathogen inactivated platelets may be stored for 7 days. Some sensitive markers start to fail if the storage lesion effects are too big. However, correlation between different in vitro tests improves with the inclusion of data outside the highly variable normal range [7]. It is therefore not surprising that these measures are often poor predictors of clinical outcome [8] and that studies do not consistently find that in vitro parameters correlate well with in vivo outcome [8–11]. Irrespective of product age, we observed that 17% (1 in 6) of platelet transfusions did not produce the expected posttransfusion platelet count increments in hematology-oncology patients [12]. One possible cause of this unexpectedly high rate of poor clinical outcome is that platelet viability is highly influenced by donor characteristics. Indeed, approximately 33% of normal donors donate preactivated platelets as indicated by high microparticle levels [13]. Cancer patients typically need platelets not because they are actively bleeding, but because they are at risk of bleeding due to low platelet counts secondary to disease and/or therapy. In these patients, transfused platelets are required to stay in circulation, ready to respond to nontraumatic microvascular bleeding. Due to their reduced viability, preactivated platelets are not recommended for storage or for prophylaxis in cancer patients [14]. In contrast, preactivated platelets are thought to be beneficial when immediate haemostatic function is required to stop acute bleeding, particularly important for surgical or trauma patients [15].

Platelets are used clinically for very different purposes and platelet quality must be compatible with the respective purpose of transfusion. Consequently, the ideal quality parameter to predict platelet performance after transfusion must be able to differentiate between resting/viable and preactivated/highly functional platelets with similar resolution across the entire viability-functionality spectrum.

Activated platelets shed microparticles [16], and platelets are the major source of circulating microparticles [17, 18]. In this review, we explore the possibility that microparticle content as a measure of platelet fragmentation and heterogeneity may fulfill the requirement for a universal quality indicator for platelet production, storage, viability, function, and compatibility [19–21]. We assess the heterogeneity of platelet concentrates from the three different perspectives of production, prophylactic transfusion, and therapeutic transfusion.

2. Platelet Quality from the Perspective of Production

2.1. Platelet Quality Measures. In vitro measures have been based on the assumption that fresh platelets are better than old platelets. Thus, discoid platelets with low expression of CD62 [22] and other activation markers and low release of intracellular or metabolic substances are deemed high quality, whereas the opposite is deemed poor quality [23]. The panel of in vitro tests also includes functional measures such as the response of platelets to ADP or hypotonic shock [24, 25].

2.2. Homogeneous Compared to Heterogeneous Platelets. If platelets are more viable—that is, fit to survive storage, transportation, gamma irradiation, and other processes that might happen prior to transfusion—then they are by definition less functional. This has been known since the 1970s when investigators sought to define the right storage temperature for platelet concentrates. Several research teams reported that preservation of platelets for storage and optimal radiolabel recovery and survival caused a reversible dysfunction of platelets' hemostatic capability. In short, room temperature stored platelets were more viable but refrigerated platelets showed better function assessed by increased haemostasis in aspirin-treated volunteers or thrombocytopenic patients [26, 27]. Keeping platelets viable is an important role of anticoagulants. Concentrates rich in viable platelets are homogeneous in their composition, containing primarily discoid platelets and few or no microparticles or microaggregates (Figure 1(a)) [13].

In contrast, aged, chilled, or otherwise activated platelets are expected to be heterogeneous, containing platelets with high polydispersity due to a large spread of different morphologies, high surface expression of activation markers, many microparticles, and the presence of microaggregates [21, 28, 29] (Figure 1(b)). Heterogeneous platelets are known to be more functional but less viable. This is shown in animal experiments where heterogeneous platelets do not survive long in circulation, particularly in animals with inflammatory conditions [30]. Considering that aged platelets are often heterogeneous, these platelets are likely primed for cell death [31].

2.3. Microparticles as Quality Indicators. The heterogeneity of platelet concentrates increases with storage [10, 13, 32, 33] and with pathogen-reduction processing [5, 13] and varies greatly between normal donors [13, 34–36]. The largest contributor to platelet heterogeneity is the microparticle content (Figure 1(b)). Microparticles, also known as extracellular vesicles, are abundant in certain platelet concentrates where microparticles contain extracellular mitochondria [37, 38]. Microparticles are implicated as a transport and delivery system of mediators participating in hemostasis, thrombosis, vascular repair, and inflammation, acting both locally and systemically under physiologic as well as pathophysiologic conditions [17, 39–41]. Microparticles express membrane-associated proteins and are able to transfer receptors, growth factors, and microRNA between cells [32, 39, 40, 42]. If microparticles contain mitochondria they might be associated with adverse inflammatory reactions in recipients [38]. Many of the details of the origin and composition of microparticles are under investigation and, due to the limitations of some testing systems, results may be controversial [43].

Two important questions have long been proposed for investigation: first, whether microparticles in blood products have a potentially pathogenic effect, and second, how blood product processing and storage affect microparticle release [32]. More recently, regulatory agencies are recognizing the importance of microparticles as quality indicators due to their potential physiological and pathophysiologic roles. The US

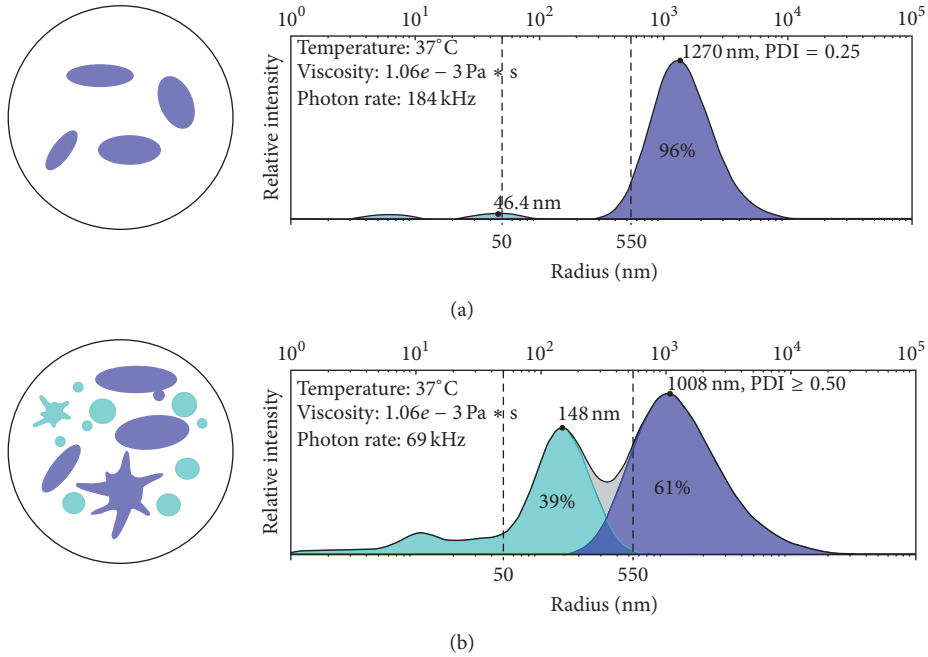


FIGURE 1: Example of dynamic light scattering test results showing the contribution of exosome-sized particles (radii below 50 nm), microparticles (radii 50–550 nm), platelets, and microaggregates (radii above 550 nm). (a) Homogeneous platelets (few or no microparticles, platelets with predominantly discoid shape with low polydispersity, and narrow blue peak). (b) Heterogeneous platelets (many microparticles, platelets with high polydispersity, and broad blue peak).

Food and Drug Administration acknowledged the importance of microparticles in transfusion medicine because microparticles are present in both plasma and cellular blood products [32]. Finally, Paul-Ehrlich Institute in Germany licensed ThromboLUX (Table 1) microparticle testing for use in transport validation.

2.4. Assessment of Microparticles in Blood. Several well-established research technologies have been used and described in the literature for the measurement of microparticles in blood and other body fluids (Table 1), including dynamic light scattering (DLS) as used in ThromboLUX (LightIntegra Technology Inc.) [13, 44], flow cytometry (FC) [37], and ELISA [45, 46]. The optical system, small sample volume, and specific software used in ThromboLUX address the challenges other DLS instruments face with testing platelet-rich plasma or platelet concentrates [20, 43]. The qNano Gold (Izon Science) uses size exclusion chromatography and resistive pulse sensing [47], and a combination of dynamic light scattering and particle tracking is used by NanoSight (Malvern) [48]. Excellent recent reviews describe all but the latest dynamic light scattering testing methods and how they can be used in various biological media including blood [49, 50].

2.5. Limitations of Microparticle Tests. Here we review studies on the limitations of currently available microparticle tests (Table 2). It is generally recognized that accurate determination of microparticle concentration with flow cytometric methods is problematic [51–53]. Flow cytometric methods have limitations in cases where microvesicles form aggregates or complexes with each other or with cells. In comparison, dynamic light scattering assays are not designed as whole blood assays and therefore require centrifugation to obtain PRP. Large aggregates present in whole blood would be removed during centrifugation but aggregates that formed in concentrates over time or with certain product manipulations would result in a broadening of the platelet population and increase of the polydispersity index for platelets. If a microparticle assay does not require ultracentrifugation, artifacts from sample preparation are unlikely, and the assay can be conducted in the presence of platelets and other particles. Microparticles and chylomicrons are differentiated neither by scattering-based flow cytometry or dynamic light scattering in platelet-rich plasma, nor by nanoparticle tracking analysis (NTA) or tunable resistive pulse sensing (TRPS) in platelet-poor plasma [54], which will affect accurate cell-derived microparticle quantification in lipid-rich samples.

Use of flow cytometry could lead to an underestimation of microparticle content if smaller microparticles are not counted [50, 55, 56]. Flow cytometry is capable of analyzing platelet microparticles <1 micron in size in plasma sources and may be more accurate than ELISA, which may fail to immobilize platelet microparticles >100 nm in diameter [55]. Smaller microparticles are not detected by standard flow cytometry because they are excluded when the operator cuts out electronic noise by setting thresholds [57], but high-sensitivity flow cytometry (hs-FCM) can now discriminate previously undetectable small microparticles in plasma samples [56]. In contrast to flow cytometry, underestimation of microparticle content is not relevant to dynamic light scattering-based assays, which provide

Table 1: Comparison of microparticle testing technologies.

Technology	Principle	Manufacturer	Invasive[1]	Standards, dilutions required	Trained specialist required	MP separation required	Prep. time [min]	Time/test [min]	Daily maintenance
ThromboLUX	DLS	LightIntegra	No	No	No	No	5	8	No
Flow cytometer	Static light scattering, fluorescence	Abbott, Becton Dickinson amongst others	No	Yes	Yes	Yes	30+	5–10	Yes
Micro flow cytometer	Static light scattering, fluorescence	Apogee flow systems	No	Yes	Yes	Yes	30+	5–10*	Yes
qNano Gold	Size exclusion chromatography	Izon Science	Yes	Yes	Yes	Yes	20†	10–15	No
NanoSight	DLS particle tracking	Malvern	Yes	Yes	Yes	Yes	15	6–75	No
ELISA	Double antibody sandwich technique	JIMRO Co. Ltd., Diagnostica Stago	No	Yes	Yes	Yes	30+	5–10	No

[1]The test is considered invasive if the required sample volume is larger than what can be aseptically obtained from a tubing segment.

*Consumables only usable for 6–8 hours. †Isolation of microparticles by differential centrifugation or size exclusion chromatography required, dynamic light scattering (DLS), microparticles (MP), and enzyme-linked immunosorbent assay (ELISA).

Table 2: Overview of published clinical microparticle studies.

Performance topic	Reference	Type of microparticle assay	Total number of subjects in study	Concentration [MP/L]	Summary statement
Accurate enumeration of microparticles (especially in the presence of platelets or other particles)	Balvers et al. 2015 [51]	FC	20 (10 trauma patients; 10 healthy)	7.5×10^3	Flow cytometry does not count microparticles if bound in complexes; reported concentration is about 10^6 lower than reported elsewhere; sample was prepared at low temperature
	Jayachandran et al. 2011 [52]	FC	118 (58 assayed for plasma microparticles)	N/A	Flow cytometry does not detect aggregates
	van Ierssel et al. 2012 [53]	FC	13 in vitro lipid (5 coronary heart disease; 8 healthy); 5 in vivo lipid, healthy	2.5×10^8 (EMP only)	Flow cytometry data are affected by high circulating levels of lipids
Size of microparticles (below the detection limit of many technologies)	Leong et al. 2011 [55]	FC	6 (acute myocardial infarction; healthy)	3×10^9	Platelet microparticle size is below stated detection limits of most flow cytometers. However, study confirmed that flow cytometry is capable of analyzing microparticles from plasma; approximately 2-fold for acute myocardial infarction (AMI) patient
	Robert et al. 2012 [56]	FC	40 (30 coronary disease; 10 healthy)	2.0×10^9 (1.1×10^{10} with high sensitivity FCM)	Standard flow cytometry does not detect small microparticles. High-sensitivity flow cytometry allows measurement of previously undetectable microparticles; approximately 10-fold for coronary patients
Probe/marker selection	Hou et al. 2011 [77]	FC	20 healthy donors	1×10^9 (fresh) 1.5×10^{10} (day 9)	Annexin V does not bind to membranes at low phosphatidyl-serine levels and is Ca^{2+} dependent; lactadherin is proposed as an alternative
	Iversen et al. 2013 [58]	FC	49 (20 healthy; 29 systemic lupus erythematosus)	9×10^9	Annexin V binding is Ca^{2+} dependent, resulting in potential clotting of plasma; approximately 2-fold for patients with systemic lupus erythematosus (SLE)
	Lanuti et al. 2012 [78]	FC	34 (20 diabetes; 14 healthy)	1.1×10^8 (EMP only)	Endothelial microparticles and circulating endothelial cells share markers such as CD144 and CD146 leading to overestimation; approximately 2-fold for patients with type 2 diabetes (Iversen et al. published endothelial microparticle concentration to be a factor 10 lower than platelet microparticles)
	Bohling et al. 2012 [45]	ELISA, clot-based and chromogenic and flow cytometry	75 (24 healthy, 28 trauma, 23 nontrauma (patients taking warfarin, heparin, or lupus anticoagulants))	4×10^{10}	The performance characteristics of a clot-based versus chromogenic procoagulant phospholipid assay were compared and low correlation found; neither assay was considered optimal
Standardization of methods	Marchetti et al. 2014 [61]	ELISA, clot-based and thrombin generation	145 (72 control, 73 essential thrombocythemia)		The performance characteristics of clot-based procoagulant phospholipid assay and thrombin generation assay were compared

Table 2: Continued.

Performance topic	Reference	Type of microparticle assay	Total number of subjects in study	Concentration [MP/L]	Summary statement
Method selection	Strasser et al. 2013 [62]	FC, prothrombinase ELISA, clot-based ELISA	31 healthy donors	1.2×10^9	The performance characteristics of a clot-based procoagulant phospholipid assay, prothrombinase assay, and flow cytometry were compared
	Labrie et al. 2013 [20]	DLS	24 apheresis platelet concentrates from normal volunteers	1.5×10^{12}	ThromboLUX microparticle assay was compared to flow cytometry and correlated highly
	Xu et al. 2011 [44]	DLS	160 (81 platelet-rich plasma, 79 apheresis platelet concentrates)	2×10^{11}	ThromboLUX microparticle assay was compared to flow cytometry [51]; values were calculated from reported relative content but concentrations are not published

Flow cytometry (FC) and dynamic light scattering (DLS).

qualitative and quantitative information for microparticles/extracellular vesicles in the radius range of 1 nm to 550 nm. However, DLS-based assays do not differentiate between cellular fragments shed from platelets, red blood cells, white blood cells, or endothelial cells. The NanoSight can differentiate the cellular origin of microparticles based on its fluorescence capability. However, the limitations of this technique as described in the operator's manual—chamber leaks, bubbles, and the risk of contamination from cleaning and reusing the chamber—suggest that this method may not be conducive to routine use.

Selecting the right probe or marker for microparticle detection is another important issue. Annexin V is widely used in flow cytometry to select entire microparticle populations based on binding to exposed phosphatidylserine. Calcium- dependent binding can result in plasma clotting, but a modification using heparin was successfully tested [58]. When targeting Annexin V for microparticle detection, loss of sensitivity occurs when phosphatidylserine levels are low. Whether all microparticles bind Annexin V and whether a high concentration of Annexin V-binding microparticles relates to poor viability (poor posttransfusion recovery) are still unanswered questions.

Standardization is an unresolved issue in microparticle detection. It is now well accepted that accurate bead standards with appropriate refractive indices to gate microparticles by flow cytometry still need to be developed [47, 59]. Currently, comparing data from different studies is difficult due to the wide variety of methods for microparticle determination used by different laboratories [60]. Dedicated instruments configured to perform microparticle screening have the advantage of reduced assay-to-assay and system-to-system variability. Method-to-method standardization is also being investigated in other systems. For example, the clot-based procoagulant phospholipid assay correlates significantly with a thrombin generation assay [61] and the study authors suggest that the thrombin generation assay may be the more sensitive measure for procoagulant activity of microparticles carrying active tissue factor.

Methods for microparticle detection show good correlations of results, although comparability of counts by flow cytometry and microparticle activity may be limited due to different assay principles [62]. Counts are based on detecting the intensity of scattered or fluorescent light as microparticles move through the laser beam of a flow cytometer while microparticle activity tests rely on chemical reactions of microparticle components. Relative platelet microparticle counts measured by flow cytometry were shown to strongly correlate with the microparticle content measured by one dynamic light scattering assay in both platelet-rich plasma and apheresis platelet concentrates [20, 44]. However, when the reported relative microparticle content is converted to concentrations, the numbers obtained by dynamic light scattering are 100–1000 times higher than those reported by others (Table 2). This is possibly due to the use of native or fixed samples without differential centrifugation to remove platelets prior to testing. The much lower microparticle concentrations detected by flow cytometry could be related to (1) beads being inadequate as size standards [47], (2) loss of microparticles below the electronic threshold [44], and (3) limitations such as swarm detection [57, 59].

Currently there is no consensus on the best measures for accuracy of microparticle concentration values, size detection, probe selection, standardization, or appropriateness for testing of specific samples, and research is ongoing. Methods such as ThromboLUX cannot be used to characterize various microparticle subpopulations; however, they do allow routine microparticle screening of platelet concentrates at various points of the product life cycle [12, 20, 44].

3. Platelet Quality Measures for Prophylactic Transfusion

3.1. Platelet Viability. We observed that ThromboLUX-measured microparticle content in fresh, normal-donor platelet-rich plasma was inversely associated with radiolabeled platelet recovery in autologous transfusions (unpublished results). The mechanism of how microparticles could reduce platelet recovery after reinfusion is not known. Three scenarios have been suggested: (1) microparticles might have a direct effect on the recipient's immune system, (2) the factors that generate microparticles also mark the platelets for removal from circulation, and/or (3) microparticle generation indicates platelet activation and preactivated platelets are consumed by daily vascular maintenance. In a recent publication our collaborators on this unpublished work found lipid oxidation products–which are linked to platelet activation and heterogeneity–to be associated with poor posttransfusion performance [63]. If homogeneous autologous platelet transfusions give better recovery it might be expected that patients receiving allogeneic transfusions for prophylaxis would also benefit when platelets are homogeneous. Here it is suggested that homogeneous, viable platelets give better recovery measured as count increments.

3.2. Platelet Refractoriness and Platelet Compatibility. Platelet refractoriness, a situation in which the patient does not show the expected response to the platelet transfusion [64], is a complication seen in up to 27% of platelet recipients [65]. Platelet refractoriness is defined as two consecutive platelet transfusions resulting in insufficient corrected (platelet) count increments (CCI). The threshold below which a CCI is deemed insufficient depends on the time point of measurement: a CCI less than 5,000–7,500 platelets/μL measured in the recipient's blood sample drawn 1 hour after transfusion characterizes poor recovery; a CCI less than 5,000 platelets/μL in a sample drawn 24 hours after transfusion characterizes poor survival. Patients with immune refractoriness show low posttransfusion CCI at both 1 hour and 24 hours after transfusion which may or may not be addressed with HLA/HPA matched platelet concentrates [65, 66]. However, often, even when there is no documented alloimmunisation, the 1-hour platelet increment is satisfactory followed by a significant decrease in platelet count at 24-hour posttransfusion. Poor platelet quality was suggested as one reason why transfused cancer patients may show especially poor platelet survival at 24 hours [12]. In addition to the impact on patient care, inpatient hospital costs for a platelet-refractory patient

(approximately US$ 104,000) are more than double compared to nonrefractory patients, with hospital stays 21 days longer [67].

Microparticles are prothrombotic inflammatory markers. Patients who become refractory to platelet transfusion often have concurrent fever or systemic inflammation that might be detectable as elevated microparticles [68, 69]. Homogeneous platelets may therefore be the best choice for cancer patients at risk for platelet refractoriness while heterogeneous platelets may be incompatible with patients challenged by preexisting inflammation. It follows that platelet transfusions from donors with high microparticle content may only be compatible with patients without preexisting inflammation. It is conceivable that transfusing heterogeneous platelets to patients whose immune systems are more activated can push them to the tipping point to become platelet-refractory. Avoiding transfusion of heterogeneous platelets for prophylactic use might prevent refractoriness. Interestingly, a very similar two-hit concept has been suggested for red blood cell transfusions based on dog studies: dogs with bacterial infection (first hit) receiving older red blood cells containing higher concentrations of microparticles (second hit) had a much higher risk of mortality [70, 71].

4. Platelet Quality for Therapeutic Use

4.1. Platelet Hemostatic Function. Heterogeneous platelet concentrates contain preactivated platelets, which are fit to react quickly once they enter circulation [15, 21, 28]. Thus, heterogeneous platelets are highly functional and have been shown to stop bleeding faster than homogeneous, viable platelets [28].

5. Platelet Quality for Platelet-Rich Plasma Injections

Platelet-rich plasma (PRP) injections are currently not managed by blood operators because they are autologous products: patients are phlebotomized of a small volume of whole blood which is then processed and reinjected to treat a variety of conditions including chronic tendon injuries, osteoarthritis, and bone regeneration. There are no clear quality standards for PRP injections and the current existing variability has been described previously [72]. The mechanism by which PRP injections exert their healing properties is still not known but the abundance of growth factors present in platelets [73] and the bactericidal and other immune functions platelets possess [74] are thought to play a major role. In the context of this review it is conceivable that microparticles also play a role in these autologous treatments. Samples from patients who already suffer from systemic inflammation might show microparticles as indicators of systemic inflammation and thus be predictors of a reduced likelihood of treatment success. On the other hand, it has been suggested that microparticles are carriers of growth factors and might significantly contribute to the healing properties of PRP [75]. This is an area that requires further study.

6. Future Direction

Implementation of microparticle measurements for quality control of platelet concentrates could address the impact of pathogen inactivation, platelet additive solutions, and 7-day storage. Inventory management based on this measure could lead to optimization of patient care and reduce cost at the same time.

Platelet viability would best be described in terms of post-transfusion platelet recovery at 24 hours, which is inversely associated with microparticle content. Clinical studies are needed to confirm our currently unpublished pilot data as well as the animal experiments that seem to support the hypothesis that patients with existing inflammation (first hit) receiving a transfusion of heterogeneous platelets (second hit) have a high risk of becoming refractory.

7. Conclusion

The technology for testing of microparticle content as a marker of the heterogeneity of platelet concentrates is a developing field. The selection of the most appropriate method of measurement for each situation remains to be determined. However, the compatibility of homogeneous versus heterogeneous platelet concentrates for clinical use is becoming clear. For prophylaxis, giving homogeneous, viable platelets to cancer patients should be advantageous because these are expected to circulate and not be immediately removed from circulation. For use as a therapeutic agent to stop bleeding, concentrates rich in heterogeneous platelets might react better, as was shown with chilled and preactivated platelets. Thus, by measuring the composition of a platelet concentrate, the performance of the concentrate during storage and its resilience to additional stress could be determined and inform its optimal use.

Implementation of routine screening of platelet concentrates requires a quick and easy, noninvasive test that measures platelet characteristics meaningful to all aspects of platelet quality. We have proposed microparticle content to be that characteristic parameter. The FDA recommends determination of platelet microparticle content but until recently there was no quick and easy test method to achieve this. Some technological challenges of dynamic light scattering have been resolved with ThromboLUX, which does not characterize microparticle subpopulations and as such is not an in-depth research tool but allows routine microparticle screening of platelet-rich plasma or platelet concentrates.

Potential new research could address the reason for the heterogeneity of platelet donors, ways to influence the subsequent heterogeneity of the donated product, which could either decrease the heterogeneity for prophylactic use, for example, by nanofiltration [76], or increase the heterogeneity for therapeutic use, for example, by chilling. Finally, the clinical and health economic impact of platelet quality determination and subsequent inventory management warrants investigation.

Competing Interests

Elisabeth Maurer-Spurej declares a conflict of interest as the principal inventor of ThromboLUX and founder of LightIntegra Technology Inc. Kate Chipperfield was an independent principal investigator of several ThromboLUX evaluation studies with institutional approvals and has not personally gained from the work that was supported by Canadian Blood Services and LightIntegra Technology Inc.

Acknowledgments

Stephen Arthur provided professional writing and editing of the article with funding from LightIntegra Technology Inc.

References

[1] K. Mittal and R. Kaur, "Platelet storage lesion: an update," *Asian Journal of Transfusion Science*, vol. 9, no. 1, pp. 1–3, 2015.

[2] S. Holme, "Storage and quality assessment of platelets," *Vox Sanguinis*, vol. 74, no. 2, pp. 207–216, 1998.

[3] A. Pienimaeki-Roemer, K. Kuhlmann, A. Böttcher et al., "Lipidomic and proteomic characterization of platelet extracellular vesicle subfractions from senescent platelets," *Transfusion*, vol. 55, no. 3, pp. 507–521, 2015.

[4] A. Farrugia and E. Vamvakas, "Toward a patient-based paradigm for blood transfusion," *Journal of Blood Medicine*, vol. 5, pp. 5–13, 2014.

[5] L. Johnson, P. Schubert, S. Tan, D. V. Devine, and D. C. Marks, "Extended storage and glucose exhaustion are associated with apoptotic changes in platelets stored in additive solution," *Transfusion*, vol. 56, no. 2, pp. 360–368, 2016.

[6] L. Johnson, R. Hyland, S. Tan et al., "In vitro quality of platelets with low plasma carryover treated with ultraviolet C light for pathogen inactivation," *Transfusion Medicine and Hemotherapy*, vol. 43, no. 3, pp. 190–197, 2016.

[7] W. A. Heaton, "In-vivo and in-vitro evaluation of stored platelet products," in *Platelet Transfusion Therapy*, J. D. Sweeny and M. Lazano, Eds., AABB Press, Bethesda, Md, USA, 2013.

[8] S. Murphy, "Utility of in vitro tests in predicting the in vivo viability of stored PLTs," *Transfusion*, vol. 44, no. 4, pp. 618–619, 2004.

[9] R. P. Goodrich, J. Z. Li, H. Pieters, R. Crookes, J. Roodt, and A. D. P. Heyns, "Correlation of in vitro platelet quality measurements with in vivo platelet viability in human subjects," *Vox Sanguinis*, vol. 90, no. 4, pp. 279–285, 2006.

[10] T. O. Apelseth and T. Hervig, "In vitro evaluation of platelet concentrates during storage: platelet counts and markers of platelet destruction," *Transfusion and Apheresis Science*, vol. 37, no. 3, pp. 261–268, 2007.

[11] T. O. Apelseth, Ø. Bruserud, T. Wentzel-Larsen, and T. Hervig, "Therapeutic efficacy of platelet transfusion in patients with acute leukemia: an evaluation of methods," *Transfusion*, vol. 50, no. 4, pp. 766–775, 2010.

[12] E. Maurer-Spurej, A. Labrie, C. Pittendreigh et al., "Platelet quality measured with dynamic light scattering correlates with transfusion outcome in hematologic malignancies," *Transfusion*, vol. 49, no. 11, pp. 2276–2284, 2009.

[13] E. Maurer-Spurej, R. Larsen, A. Labrie, A. Heaton, and K. Chipperfield, "Microparticle content of platelet concentrates is predicted by donor microparticles and is altered by production methods and stress," *Transfusion and Apheresis Science*, vol. 55, no. 1, pp. 35–43, 2016.

[14] C. A. Schiffer, K. C. Anderson, C. L. Bennett et al., "Platelet transfusion for patients with cancer: clinical practice guidelines of the American Society of Clinical Oncology," *Journal of Clinical Oncology*, vol. 19, no. 5, pp. 1519–1538, 2001.

[15] T. M. Getz, R. K. Montgomery, J. A. Bynum, J. K. Aden, H. F. Pidcoke, and A. P. Cap, "Storage of platelets at 4∘C in platelet additive solutions prevents aggregate formation and preserves platelet functional responses," *Transfusion*, vol. 56, no. 6, pp. 1320–1328, 2016.

[16] J. Ripoche, "Blood platelets and inflammation: their relationship with liver and digestive diseases," *Clinics and Research in Hepatology and Gastroenterology*, vol. 35, no. 5, pp. 353–357, 2011.

[17] R. Flaumenhaft, "Formation and fate of platelet microparticles," *Blood Cells, Molecules, and Diseases*, vol. 36, no. 2, pp. 182–187, 2006.

[18] J. F. W. Keuren, E. J. P. Magdeleyns, J. W. P. Govers-Riemslag, T. Lindhout, and J. Curvers, "Effects of storage-induced platelet microparticles on the initiation and propagation phase of blood coagulation," *British Journal of Haematology*, vol. 134, no. 3, pp. 307–313, 2006.

[19] L. Johnson, M. C. Reade, R. A. Hyland, S. Tan, and D. C. Marks, "In vitro comparison of cryopreserved and liquid platelets: potential clinical implications," *Transfusion*, vol. 55, no. 4, pp. 838–847, 2015.

[20] A. Labrie, A. Marshall, H. Bedi, and E. Maurer-Spurej, "Characterization of platelet concentrates using dynamic light scattering," *Transfusion Medicine and Hemotherapy*, vol. 40, no. 2, pp. 93–100, 2013.

[21] L. Johnson, S. Tan, B. Wood, A. Davis, and D. C. Marks, "Refrigeration and cryopreservation of platelets differentially affect platelet metabolism and function: a comparison with conventional platelet storage conditions," *Transfusion*, vol. 56, no. 7, pp. 1807–1818, 2016.

[22] E. Levin, K. Serrano, D. V. Devine et al., "Standardization of CD62P measurement: results of an international comparative study," *Vox Sanguinis*, vol. 105, pp. 38–46, 2013.

[23] E. Maurer-Spurej and K. Chipperfield, "Past and future approaches to assess the quality of platelets for transfusion," *Transfusion Medicine Reviews*, vol. 21, no. 4, pp. 295–306, 2007.

[24] S. Holme, "In vitro assays used in the evaluation of the quality of stored platelets: correlation with in vivo assays," *Transfusion and Apheresis Science*, vol. 39, no. 2, pp. 161–165, 2008.

[25] O. M. Akay, E. Gündüz, H. Başyiğit, and Z. Gulbas, "Platelet function testing during 5-day storage of single and random donor plateletpheresis," *Transfusion and Apheresis Science*, vol. 36, no. 3, pp. 285–289, 2007.

[26] C. R. Valeri, "Circulation and hemostatic effectiveness of platelets stored at 4 C or 22 C: studies in aspirin-treated normal Volunteers," *Transfusion*, vol. 16, no. 1, pp. 20–23, 1976.

[27] G. A. Becker, M. Tuccelli, T. Kunicki, M. K. Chalos, and R. H. Aster, "Studies of platelet concentrates stored at 22 C and 4 C," *Transfusion*, vol. 13, no. 2, pp. 61–68, 1973.

[28] K. M. Reddoch, H. F. Pidcoke, R. K. Montgomery et al., "Hemostatic function of apheresis platelets stored at 4°C and 22°C," *Shock*, vol. 41, supplement 1, pp. 54–61, 2014.

[29] J. Neumüller, C. Meisslitzer-Ruppitsch, A. Ellinger et al., "Monitoring of platelet activation in platelet concentrates using

transmission electron microscopy," *Transfusion Medicine and Hemotherapy*, vol. 40, no. 2, pp. 101–107, 2013.

[30] Y. Lu, Q. Li, Y.-Y. Liu et al., "Inhibitory effect of caffeic acid on ADP-induced thrombus formation and platelet activation involves mitogen-activated protein kinases," *Scientific Reports*, vol. 5, Article ID 13824, 2015.

[31] K. D. Mason, M. R. Carpinelli, J. I. Fletcher et al., "Programmed anuclear cell death delimits platelet life span," *Cell*, vol. 128, no. 6, pp. 1173–1186, 2007.

[32] J. Simak and M. P. Gelderman, "Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers," *Transfusion Medicine Reviews*, vol. 20, no. 1, pp. 1–26, 2006.

[33] E. Maurer-Spurej, G. Pfeiler, N. Maurer, H. Lindner, O. Glatter, and D. V. Devine, "Room temperature activates human blood platelets," *Laboratory Investigation*, vol. 81, no. 4, pp. 581–592, 2001.

[34] M. Phang, L. Lincz, M. Seldon, and M. L. Garg, "Acute supplementation with eicosapentaenoic acid reduces platelet microparticle activity in healthy subjects," *Journal of Nutritional Biochemistry*, vol. 23, no. 9, pp. 1128–1133, 2012.

[35] S.-Y. Wu, J. Mayneris-Perxachs, J. A. Lovegrove, S. Todd, and P. Yaqoob, "Fish-oil supplementation alters numbers of circulating endothelial progenitor cells and microparticles independently of eNOS genotype," *American Journal of Clinical Nutrition*, vol. 100, no. 5, pp. 1232–1243, 2014.

[36] M. Sossdorf, G. P. Otto, R. A. Claus, H. H. W. Gabriel, and W. Lösche, "Cell-derived microparticles promote coagulation after moderate exercise," *Medicine & Science in Sports & Exercise*, vol. 43, no. 7, pp. 1169–1176, 2011.

[37] E. Boilard, A.-C. Duchez, and A. Brisson, "The diversity of platelet microparticles," *Current Opinion in Hematology*, vol. 22, no. 5, pp. 437–444, 2015.

[38] "Boudreau LH, Duchez A-C, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation," *Blood*, vol. 125, no. 5, p. 890, 2015.

[39] F. Cognasse, H. Hamzeh-Cognasse, S. Laradi et al., "The role of microparticles in inflammation and transfusion: a concise review," *Transfusion and Apheresis Science*, vol. 53, no. 2, pp. 159–167, 2016.

[40] T. Burnouf, M.-L. Chou, H. Goubran, F. Cognasse, O. Garraud, and J. Seghatchian, "An overview of the role of microparticles/microvesicles in blood components: are they clinically beneficial or harmful?" *Transfusion and Apheresis Science*, vol. 53, no. 2, pp. 137–145, 2015.

[41] B. Laffont, A. Corduan, M. Rousseau et al., "Platelet microparticles reprogram macrophage gene expression and function," *Thrombosis and Haemostasis*, vol. 115, no. 2, pp. 311–323, 2016.

[42] H. A. Goubran, T. Burnouf, J. Stakiw, and J. Seghatchian, "Platelet microparticle: a sensitive physiological "fine tuning" balancing factor in health and disease," *Transfusion and Apheresis Science*, vol. 52, no. 1, pp. 12–18, 2015.

[43] A. Black, A. Pienimaeki-Roemer, O. Kenyon, E. Orsó, and G. Schmitz, "Platelet-derived extracellular vesicles in plateletpheresis concentrates as a quality control approach," *Transfusion*, vol. 55, no. 9, pp. 2184–2196, 2015.

[44] Y. Xu, N. Nakane, and E. Maurer-Spurej, "Novel test for microparticles in platelet-rich plasma and platelet concentrates using dynamic light scattering," *Transfusion*, vol. 51, no. 2, pp. 363–370, 2011.

[45] S. D. Bohling, M. B. Pagano, M. R. Stitzel, C. Ferrell, W. Yeung, and W. L. Chandler, "Comparison of clot-based vs chromogenic factor Xa procoagulant phospholipid activity assays," *American Journal of Clinical Pathology*, vol. 137, no. 2, pp. 185–192, 2012.

[46] S. Nomura, A. Shouzu, K. Taomoto et al., "Assessment of an ELISA kit for platelet-derived microparticles by joint research at many institutes in Japan," *Journal of Atherosclerosis and Thrombosis*, vol. 16, no. 6, pp. 878–887, 2009.

[47] E. van der Pol, F. Coumans, Z. Varga, M. Krumrey, and R. Nieuwland, "Innovation in detection of microparticles and exosomes," *Journal of Thrombosis and Haemostasis*, vol. 11, no. 1, pp. 36–45, 2013.

[48] C. Gercel-Taylor, S. Atay, R. H. Tullis, M. Kesimer, and D. D. Taylor, "Nanoparticle analysis of circulating cell-derived vesicles in ovarian cancer patients," *Analytical Biochemistry*, vol. 428, no. 1, pp. 44–53, 2012.

[49] T. Burnouf, H. A. Goubran, M.-L. Chou, D. Devos, and M. Radosevic, "Platelet microparticles: detection and assessment of their paradoxical functional roles in disease and regenerative medicine," *Blood Reviews*, vol. 28, no. 4, pp. 155–166, 2014.

[50] B. P. Foster, T. Balassa, T. D. Benen et al., "Extracellular vesicles in blood, milk and body fluids of the female and male urogenital tract and with special regard to reproduction," *Critical Reviews in Clinical Laboratory Sciences*, pp. 1–17, 2016.

[51] K. Balvers, N. Curry, D. J. B. Kleinveld et al., "Endogenous microparticles drive the proinflammatory host immune response in severely injured trauma patients," *Shock*, vol. 43, no. 4, pp. 317–321, 2015.

[52] M. Jayachandran, R. D. Litwiller, B. D. Lahr et al., "Alterations in platelet function and cell-derived microvesicles in recently menopausal women: relationship to metabolic syndrome and atherogenic risk," *Journal of Cardiovascular Translational Research*, vol. 4, no. 6, pp. 811–822, 2011.

[53] S. H. van Ierssel, V. Y. Hoymans, E. M. van Craenenbroeck et al., "Endothelial microparticles (EMP) for the assessment of endothelial function: an in vitro and in vivo study on possible interference of plasma lipids," *PLoS ONE*, vol. 7, no. 2, Article ID e31496, 2012.

[54] M. Mork, S. Pedersen, J. Botha, S. M. Lund, and S. R. Kristensen, "Preanalytical, analytical, and biological variation of blood plasma submicron particle levels measured with nanoparticle tracking analysis and tunable resistive pulse sensing," *Scandinavian Journal of Clinical And Laboratory Investigation*, vol. 76, no. 5, pp. 349–360, 2016.

[55] H. S. Leong, T. J. Podor, B. Manocha, and J. D. Lewis, "Validation of flow cytometric detection of platelet microparticles and liposomes by atomic force microscopy," *Journal of Thrombosis and Haemostasis*, vol. 9, no. 12, pp. 2466–2476, 2011.

[56] S. Robert, R. Lacroix, P. Poncelet et al., "High-sensitivity flow cytometry provides access to standardized measurement of small-size microparticles-brief report," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 4, pp. 1054–1058, 2012.

[57] E. Van der Pol, M. J. C. Van Gemert, A. Sturk, R. Nieuwland, and T. G. Van Leeuwen, "Single vs. swarm detection of microparticles and exosomes by flow cytometry," *Journal of Thrombosis and Haemostasis*, vol. 10, no. 5, pp. 919–930, 2012.

[58] L. V. Iversen, O. Østergaard, C. T. Nielsen, S. Jacobsen, and N. H. H. Heegaard, "A heparin-based method for flow cytometric analysis of microparticles directly from platelet-poor plasma in calcium containing buffer," *Journal of Immunological Methods*, vol. 388, no. 1-2, pp. 49–59, 2013.

[59] W. L. Chandler, "Measurement of microvesicle levels in human blood using flow cytometry," *Cytometry Part B: Clinical Cytometry*, vol. 90, no. 4, pp. 326–336, 2016.

[60] J. Alijotas-Reig, C. Palacio-Garcia, E. Llurba, and M. Vilardell-Tarres, "Cell-derived microparticles and vascular pregnancy complications: a systematic and comprehensive review," *Fertility and Sterility*, vol. 99, no. 2, pp. 441–449, 2013.

[61] M. Marchetti, C. J. Tartari, L. Russo et al., "Phospholipid-dependent procoagulant activity is highly expressed by circulating microparticles in patients with essential thrombocythemia," *American Journal of Hematology*, vol. 89, no. 1, pp. 68–73, 2014.

[62] E. F. Strasser, S. Happ, D. R. Weiss, A. Pfeiffer, R. Zimmermann, and R. Eckstein, "Microparticle detection in platelet products by three different methods," *Transfusion*, vol. 53, no. 1, pp. 156–166, 2013.

[63] J. C. Zimring, S. Slichter, K. Odem-Davis et al., "Metabolites in stored platelets associated with platelet recoveries and survivals," *Transfusion*, vol. 56, no. 8, pp. 1974–1983, 2016.

[64] P. Rebulla, "A mini-review on platelet refractoriness," *Haematologica*, vol. 90, no. 2, pp. 247–253, 2005.

[65] S. J. Slichter, K. Davis, H. Enright et al., "Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients," *Blood*, vol. 105, no. 10, pp. 4106–4114, 2005.

[66] J. R. Hess, F. L. Trachtenberg, S. F. Assmann et al., "Clinical and laboratory correlates of platelet alloimmunization and refractoriness in the PLADO trial," *Vox Sanguinis*, vol. 111, no. 3, pp. 281–291, 2016.

[67] K. R. Meehan, C. O. Matias, S. S. Rathore et al., "Platelet transfusions: utilization and associated costs in a tertiary care hospital," *American Journal of Hematology*, vol. 64, no. 4, pp. 251–256, 2000.

[68] E. Boilard, P. A. Nigrovic, K. Larabee et al., "Platelets amplify inflammation in arthritis via collagen-dependent microparticle production," *Science*, vol. 327, no. 5965, pp. 580–583, 2010.

[69] P. Diehl, F. Nagy, V. Sossong et al., "Increased levels of circulating microparticles in patients with severe aortic valve stenosis," *Thrombosis and Haemostasis*, vol. 99, no. 4, pp. 711–719, 2008.

[70] I. Cortés-Puch, K. E. Remy, S. B. Solomon et al., "In a canine pneumonia model of exchange transfusion, altering the age but not the volume of older red blood cells markedly alters outcome," *Transfusion*, vol. 55, no. 11, pp. 2564–2575, 2015.

[71] W. A. Flegel, C. Natanson, and H. G. Klein, "Does prolonged storage of red blood cells cause harm?" *British Journal of Haematology*, vol. 165, no. 1, pp. 3–16, 2014.

[72] C. S. Cohn, E. Lockhart, and J. J. McCullough, "The use of autologous platelet-rich plasma in the orthopedic setting," *Transfusion*, vol. 55, no. 7, pp. 1812–1820, 2015.

[73] J. Textor, "Platelet-Rich Plasma (PRP) as a therapeutic agent: platelet biology, growth factors and a review of the literature," in *Platelet-Rich Plasma: Regenerative Medicine: Sports Medicine, Orthopedic, and Recovery of Musculoskeletal Injuries*, Lecture Notes in Bioengineering, pp. 61–94, Springer, Berlin, Germany, 2014.

[74] M. R. Yeaman and A. S. Bayer, "Chapter 37-antimicrobial host defense," in *Platelets*, pp. 767–801, Elsevier, Amsterdam, The Netherlands, 2013.

[75] E.-S. Kim, J.-J. Kim, and E.-J. Park, "Angiogenic factor-enriched platelet-rich plasma enhances in vivo bone formation around alloplastic graft material," *Journal of Advanced Prosthodontics*, vol. 2, no. 1, pp. 7–13, 2010.

[76] M.-L. Chou, L.-T. Lin, D. Devos, and T. Burnouf, "Nanofiltration to remove microparticles and decrease the thrombogenicity of plasma: in vitro feasibility assessment," *Transfusion*, vol. 55, no. 10, pp. 2433–2444, 2015.

[77] J. Hou, Y. Fu, J. Zhou et al., "Lactadherin functions as a probe for phosphatidylserine exposure and as an anticoagulant in the study of stored platelets," *Vox Sanguinis*, vol. 100, no. 2, pp. 187–195, 2011.

[78] P. Lanuti, F. Santilli, M. Marchisio et al., "A novel flow cytometric approach to distinguish circulating endothelial cells from endothelial microparticles: relevance for the evaluation of endothelial dysfunction," *Journal of Immunological Methods*, vol. 380, no. 1-2, pp. 16–22, 2012.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 6260792, 7 pages
http://dx.doi.org/10.1155/2016/6260792

Research Article

Stability of Thawed Apheresis Fresh-Frozen Plasma Stored for up to 120 Hours at 1°C to 6°C

William P. Sheffield,[1] Varsha Bhakta,[1] Qi-Long Yi,[2] and Craig Jenkins[3]

[1]*Centre for Innovation, Canadian Blood Services, Hamilton, ON, Canada*
[2]*Department of Epidemiology and Surveillance, Canadian Blood Services, Ottawa, ON, Canada*
[3]*Centre for Innovation, Canadian Blood Services, Ottawa, ON, Canada*

Correspondence should be addressed to William P. Sheffield; sheffiel@mcmaster.ca

Received 16 August 2016; Revised 21 October 2016; Accepted 30 October 2016

Academic Editor: Rajendra K. Chaudhary

Regulations concerning the storage of transfusable plasma differ internationally. In Canada, plasma obtained from whole blood donations and frozen within 24 hours of phlebotomy (frozen plasma, FP) may be thawed and transfused within 120 hours of refrigerated storage. However, plasma frozen within 8 hours of phlebotomy following apheresis donation (FFPA) must be transfused within 24 hours of thawing and refrigeration. Our objectives were to measure coagulation factors (F) V, VII, and VIII, fibrinogen activities, and the prothrombin time (PT) in thawed refrigerated FFPA at 0, 24, and 120 hours of storage and to compare these values to those in thawed refrigerated FP. Fibrinogen activity remained unchanged over time, while mean factor levels in 28 FFPA units declined by 17% (FV), 19.7% (FVII), and 54.6% (FVIII) over 120 hours, while PT values rose to 7.6%. Factor activities were significantly higher in FFPA than FP after 120 hours of refrigerated storage. Residual FVIII activities in thawed FFPA met predefined noninferiority criteria compared to thawed FP after 120 hours. These results support a change in Canadian regulations to permit transfusion of thawed FFPA made in a closed system and refrigerated for up to 120 hours, one that could reduce wastage of transfusable plasma.

1. Introduction

The ability to freeze transfusable plasma provides both advantages and disadvantages to blood operators and transfusionists. Freezing plasma preserves coagulation factor and other plasma protein activities and makes possible a long shelf life of the frozen product, ranging from one to three years [1]. It also complicates the rapid provision of plasma therapy to patients in urgent need of this intervention because of the time required to thaw frozen plasma. Transfusable plasma is indicated for the prevention or treatment of bleeding due either to genetic deficiencies of coagulation factors for which no purified concentrate is available or to acquired coagulopathy in the setting of disseminated intravascular coagulation, cardiac surgery, warfarin reversal, or massive transfusion [2–8]. It is also indicated as a replacement fluid in plasma exchange for thrombotic thrombocytopenic purpura (TTP), a high volume procedure [9, 10]. European regulations require that plasma be infused as soon as possible after thawing, but in North America, a longer shelf life for thawed, refrigerated plasma is permitted [1].

In the United States and in Canada, the designation "fresh-frozen plasma" (FFP) is applied to plasma that is frozen within 8 hours of phlebotomy. In Canada, transfusable plasma may also be obtained from whole blood donations maintained at 20 to 24°C provided that it is frozen within 24 hours of phlebotomy, yielding frozen plasma (FP). In the United States, two FP-type products may be produced for transfusion: PF24, which is plasma frozen within 24 hours of phlebotomy from whole blood that has been refrigerated within 8 hours of phlebotomy, and PF24RT24, which differs from PF24 only in that the whole blood from which it is derived may be maintained at room temperature for up to 24 hours. FDA guidelines permit FFP, PF24, and PF24RT24 to be thawed and refrigerated for up to 24 hours prior to transfusion; they may all also be relabelled as "Thawed

Plasma" and refrigerated for a total of 120 hours (5 days) prior to transfusion, provided that they have been produced in a functionally closed system and are not used in interstate commerce [11]. In large part, the three products are used interchangeably by transfusion medicine practitioners [12, 13]. In Canada, FP may be thawed and refrigerated up to 120 hours prior to transfusion, but thawed FFP must currently be transfused after at most 24 hours of refrigerated storage.

Prior to 2011, Canadian regulators enforced the same 24-hour outdate for thawed refrigerated FP or FFP. We obtained data that allowed for these regulations to be changed by conducting a stability study of refrigerated thawed FP [14]. We demonstrated noninferiority of Canadian FP to PF24 (called FP24 at that time) with respect to coagulation factor VIII (FVIII) activity values in thawed FP refrigerated for 120 hours in an American study by Scott et al. [15], one now cited in FDA guidance documents and a Circular of Information prepared by the AABB, the American Red Cross (ARC), America's Blood Centers (ABC), and the Armed Services Blood Program (ASBP) [11]. We further demonstrated similarity of fibrinogen, FV, and FVII activities and of prothrombin time values to those reported by Scott et al. [14, 15].

We previously elected not to include FFP in our stability studies of thawed plasma. In Canada, excepting the province of Quebec, FFP is currently only available if produced by apheresis (FFPA) [8]. With a view to a potential extension in shelf life of thawed FFPA, in the present study we tested the hypothesis that FVIII activity in thawed refrigerated FFPA was noninferior to FVIII activity in thawed refrigerated FP. We framed the hypothesis in this manner for statistical purposes, and because Canadian regulators accepted this kind of design in our previous study [15] that led to shelf life extension for FP. FVIII is the coagulation factor whose quality control in transfusable plasma is regulated in Canada. Biologically one would expect thermolabile FVIII activity to be better conserved in FFPA due to shorter exposure time of plasma to elevated temperatures. We report data in support of this hypothesis and further show acceptable conservation of fibrinogen, FV, and FVII activities and prothrombin time in thawed refrigerated FFPA.

2. Materials and Methods

2.1. Design. This study was designed to test the hypothesis that FVIII activity in FFPA would be noninferior to that in FP after both products had been thawed and maintained at 1°–6°C for 120 hours. Because such a shelf life is only considered safe in a transfusable product made in a functionally closed system, we specifically investigated Concurrent Plasma, which is FFPA produced in ~200 mL volumes using a Trima Accel device (Terumo BCT, Lakewood, CO, USA) at the same time as an apheresis platelet product, using Anticoagulant Citrate Dextrose-Formula A (ACD-A) (ACD-FFPA). Sample size was estimated on the basis of the observed FVIII values at 120 hours in our FP stability study [14] and the conservative assumption that the mean ACD-FFPA FVIII values and standard deviations would be identical, which yielded a noninferiority margin of >0.423 IU/mL for $n = 28$. Noninferiority was defined a priori as being confirmed if the lower bound of the 95% confidence interval of the data set was greater than the predetermined noninferiority margin.

Accordingly, 28 ACD-FFPA units produced following standard Canadian Blood Services operating procedures were removed from inventory as an extension of quality control and designated for use in this study. This group comprised 14 non-O type (A, B, or AB) and 14 type O units. Units were tested prior to their one-year expiry date, after having being frozen for between 5 and 11.5 months. For comparisons to FP, we used data from our previous study [14], specifically selecting the results of the 27 units processed using MacoPharma collection sets because these remain in use by the Canadian blood operator and because of the similarity in the size of that data set to the current $n = 28$ study.

2.2. Unit Sampling. All ACD-FFPA units were shipped frozen on dry ice from two production sites to the testing site. Units were then stored at −80°C until they were thawed by immersion in a 37°C DH8 (Helmer, Noblesville, IN, USA) quick thaw water bath. Units were then transferred to a Biological Safety Cabinet and mixed gently by brief end-to-end rocking, and aliquots were removed via a sterile sampling coupler using a syringe and a needle. The process was repeated 24 and 120 hours after thaw. At each time point, aliquots were removed and immediately tested, with a backup sample being frozen at −80°C. Units were then returned to the laboratory, where they were stored in a Helmer IB125 refrigerator maintained at 1°–6°C.

2.3. Unit Testing. Units were tested in groups of 9 or 10 over a three-week period at the timed intervals described above. All samples were tested on an STA Compact Max automated coagulation analyzer following manufacturer's instructions (Diagnostica Stago, Asnieres, France).

2.4. Graphical and Statistical Analysis. Graphical representations of data were produced using GraphPad Prism 6.04 (GraphPad Software, San Diego, CA, USA). Statistical analysis was facilitated using GraphPad InStat or Statistical Package for the Social Sciences (SPSS; IBM, Armonk, NY, USA). Additional details are provided in the text and/or figure legends.

3. Results

3.1. Noninferiority of Thawed ACD-FFPA after 120 Hours of Refrigerated Storage to FP. After 120 hours of refrigerated storage, the FVIII activity of ACD-FFPA was 0.786 ± 0.23 IU/mL (mean ± SD, $n = 28$). The lower bound of the 95% confidence interval of the data set was calculated to be 0.7124 IU/mL; given that this was greater than the predetermined noninferiority margin of 0.423, noninferiority of stored ACD-FFPA was confirmed with respect to FVIII activity.

3.2. Effects of Extended Storage on Coagulation-Related Test Parameters. As shown in Figure 1 and Table 1, all coagulation factor activities that were tested in ACD-FFPA declined with

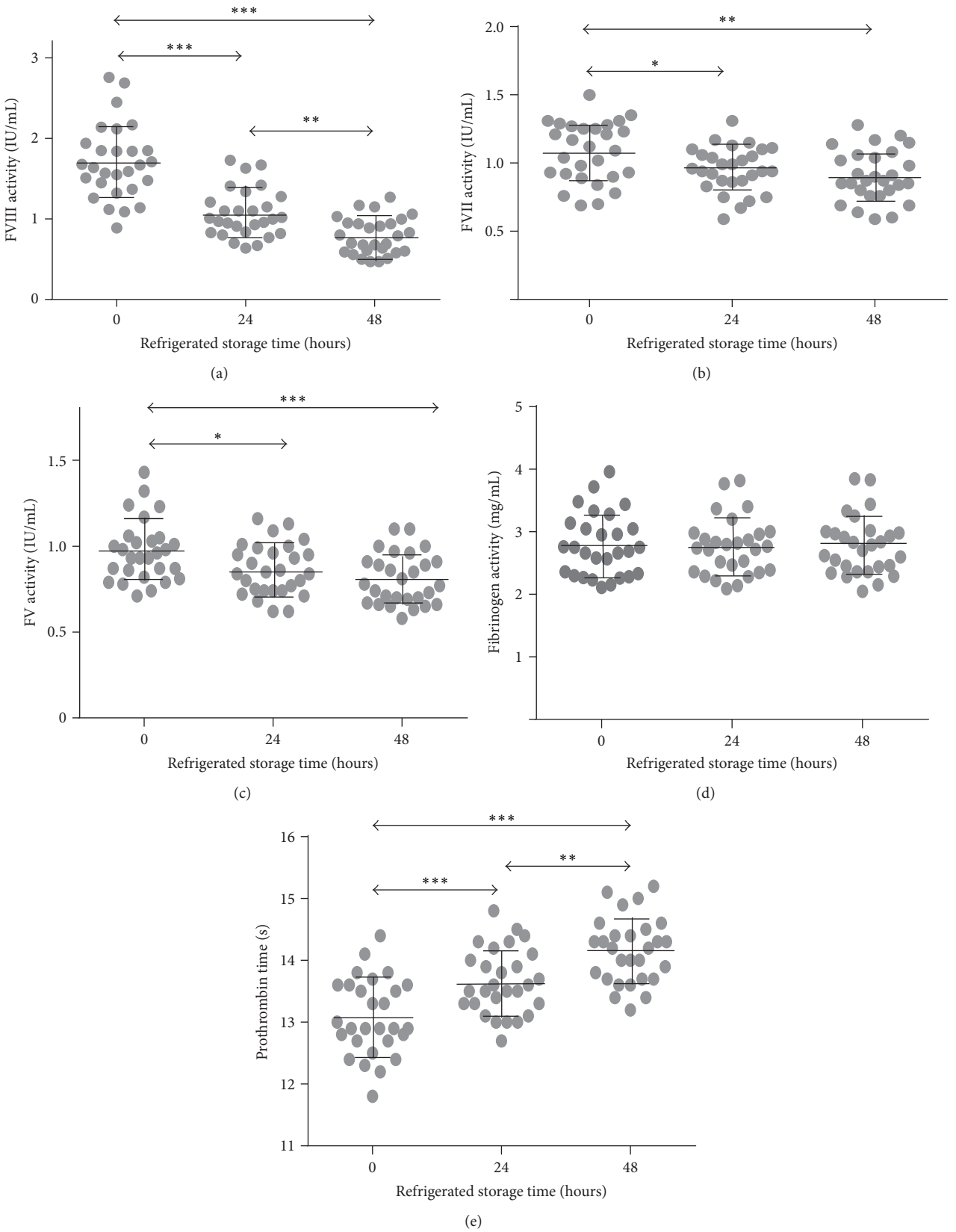


Figure 1: Coagulation parameters in ACD-FFPA. ACD-FFPA units were tested for the activities or times shown on the y-axes after thawing (0 hours) and after 24 or 120 hours of refrigerated storage. Grey points represent individual values, while horizontal lines depict the mean and error bars one SD above or below the mean. Lines with arrowheads identify statistical differences among groups by two-way ANOVA with Tukey's post hoc tests: $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$.

Table 1: Stability of coagulation parameters in ACD-FFPA.

TEST	Time of refrigerated storage (hours)		
	0	24	120
FV (IU/mL)	0.971 ± 0.18	0.864 ± 0.15 (89)*	0.806 ± 0.15 (83)***
FVII (IU/mL)	1.08 ± 0.22	0.955 ± 0.19 (88)*	0.904 ± 0.16 (84)***
FVIII (IU/mL)	1.73 ± 0.46	1.07 ± 0.30 (62)***	0.786 ± 0.23 (45)***
Fibrinogen (grams/L)	2.79 ± 0.50	2.76 ± 0.45 (99)	2.77 ± 0.47 (99)
PT (seconds)	13.1 ± 0.62	13.7 ± 0.53 (105)***	14.1 ± 0.53 (108)***

Values are reported as the mean ± one standard deviation; $^{*}p < 0.05$ and $^{***}p < 0.001$ versus 0 hours value for each parameter. Parenthetical values are the activity or clotting time at time t as a percentage of that at time $t = 0$.

refrigerated storage time, with the exception of fibrinogen activity, which remained unchanged. The largest decline was observed in FVIII activity, which declined by 38.2% in the first 24 hours and by 54.6% over 120 hours. Part of the magnitude of this decline could be ascribed to the high FVIII activity of ACD-FFPA at thaw (1.73 ± 0.46 IU/mL, compared to our previously determined FP FVIII activity values of 0.901 ± 0.32) [14]. All units in this study had ≥0.70 IU/mL FVIII at thaw, a proportion declining to 96.4% after 24 hours of refrigerated storage and 53.6% after 120 hours.

Reductions in FV and FVII activity followed a similar trajectory, declining on average 11.0 and 11.6%, respectively, in the first 24 hours, and 17.0 and 19.7% after 120 hours. PT values increased by 4.6% in the first 24 hours and by 7.6% after 120 hours.

3.3. Comparison to FP Stored under the Same Conditions. All parameters tested with ACD-FFPA were compared to previous values for FP following 120 hours of refrigerated storage [14]. As shown in Figure 2 and Table 2, four of the five parameters indicated a greater capacity to support coagulation of ACD-FFPA than FP. FVIII, FVII, and FV activities were significantly higher in ACD-FFPA following extended refrigerated storage. Similarly, mean PT values were also significantly lower, indicative of more rapid clotting of stored ACD-FFPA than stored FP. Only mean fibrinogen activities in stored ACD-FFPA were lower than in stored FP, by approximately 15%.

4. Discussion

The primary finding of this study was that ACD-FFPA thawed and maintained at 1°–6°C for 120 hours was noninferior with respect to FVIII activity to FP treated in the same way. Regulators in Canada, the United Kingdom, and Europe, but not the United States, require quality control of transfusable plasma for this labile coagulation factor; in Canada and the United Kingdom, 75% of units tested must contain at least 0.7 IU/mL FVIII activity at thaw [1]. All units tested in this study surpassed this threshold at thaw. As there are no regulations regarding minimum FVIII levels that must be maintained for thawed plasma prior to transfusion, we compared those in ACD-FFPA to those in FP at the end of the storage period. A historical control group published in 2012 was used for the FP data set [14]. Not only were FVIII activity levels significantly higher than those in FP, this finding also held true for another factor considered labile (FV), for a vitamin K-dependent factor (FVII), and for a more global hemostasis test, the PT. Only in fibrinogen activity did stored FP exhibit an apparent minor advantage of 15% greater levels than stored FFPA. It is not clear why fibrinogen activity would be greater in FP than FFPA, but our data set for such values in the current study of ACD-FFPA (with a range of 2.05 to 3.85 g/L) is fully consistent with a study of over 1000 healthy blood donors, 98% of whom were found to exhibit fibrinogen activity levels of 1.8 to 4.2 g/L [16].

Higher levels of FVIII, a labile coagulation factor, are expected in FFP over FP due to the shorter [15] time period between phlebotomy and freezing of the product [17]. Kakaiya et al. reported that plasma from CPDA-1-anticoagulated whole blood donations contained 1.02 ± 0.25 IU FVIII/mL if frozen within 8 hours of phlebotomy, compared to units prepared from refrigerated whole blood donations and frozen within 18–20 hours of phlebotomy, which contained significantly less FVIII activity, 0.55 ± 0.20 IU FVIII/mL; slower processing was associated with reduced FVII but not FV or FXI activities [18]. Similarly Scott et al. reported 0.81 ± 0.19 IU/mL for FFP and 0.66 ± 0.17 IU/mL ($p < 0.05$) for FP24 for FVIII activities at thaw, without significant differences among other clotting factors [15]. We previously demonstrated, at a time when both FFP and FP were made from Canadian whole blood donations, significantly greater FVIII activity in FFP than FP in type O donations (0.89 ± 0.23 IU/mL versus 0.72 ± 0.23 IU/mL) [19].

Our study design allowed us to eliminate trivial explanations for the differences in FVIII activity between ACD-FFPA and FP, such as the known association between ABO blood type and FVIII levels because we compared balanced groups of 14 type O and 14 type A ACD-FFPA units to 14 type O and 13 type non-O FP units [15]. Donors with type O blood have approximately 25% lower levels of FVIII activity, a phenomenon thought to reflect protection by ABO antigens of clearance determinants on von Willebrand Factor, which carries FVIII in the circulation [20].

Although we did not test FFPA or FP for ADAMTS13 in this study or its predecessor, others have demonstrated that this plasma component, of probable importance in plasma exchange in TTP, is present in similar amounts in FFP, FP-type plasma, and cryoprecipitate-poor plasma and is stable in each case to refrigerated storage for 5 days [21]. In addition, we also found ADAMTS13 to be stable in cryosupernatant plasma thawed and refrigerated for 5 days [22].

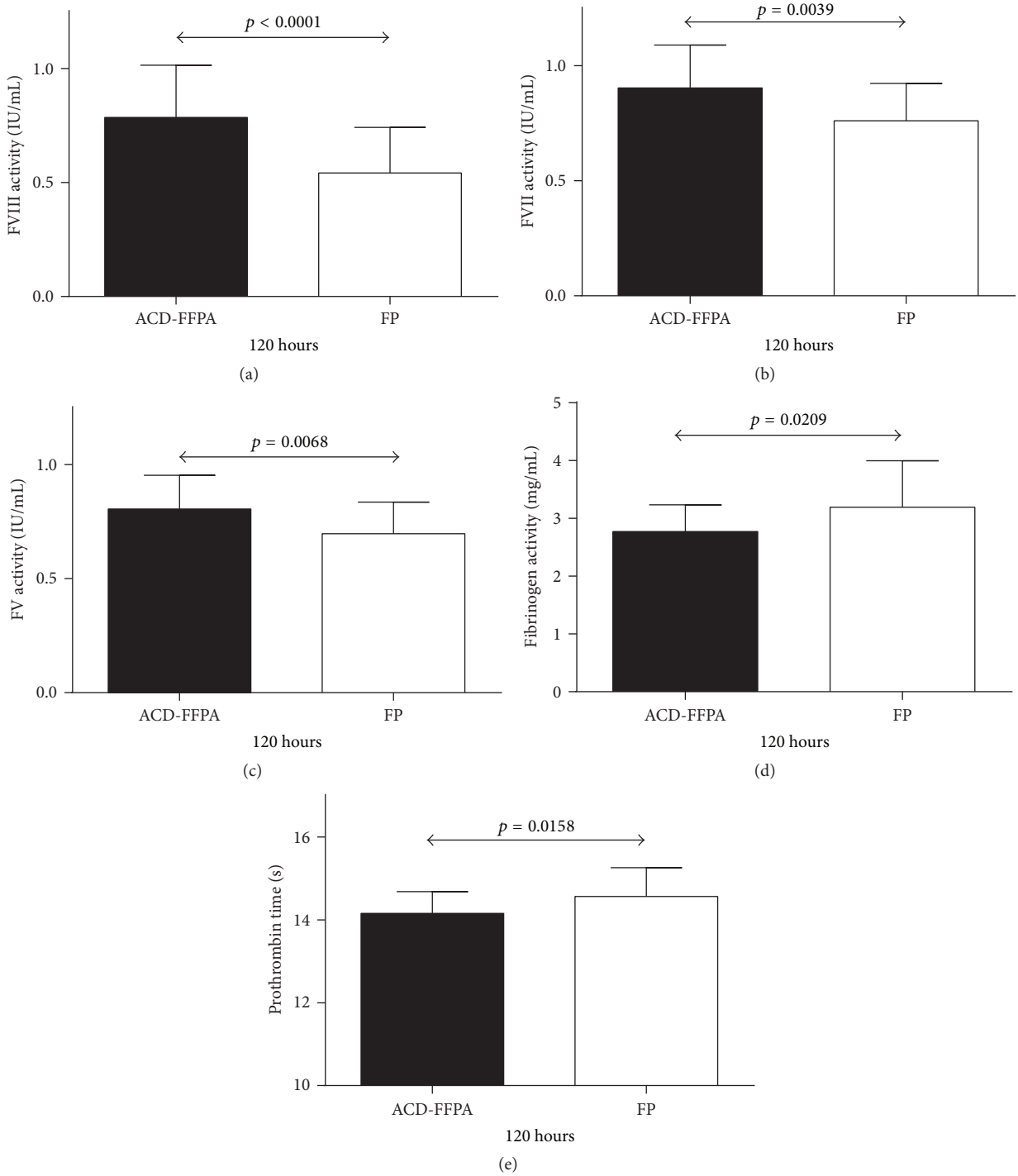


Figure 2: Comparison of coagulation parameters in ACD-FFPA and FP. ACD-FFPA units (solid bars) or FP units (open bars) were tested for the activities or times shown on the y-axes after 120 hours of refrigerated storage. Lines with arrowheads identify statistical differences between the two groups, with p values above the lines. Data sets (b–e) passing tests of normality and of similarity of standard deviation were tested using the unpaired t-test, while Welch's t-test was applied to the data in (a).

Only a single previous report in the biomedical literature concerning the quality of ACD-FFPA prepared concurrently to platelets in a functionally closed environment can be found. Sidhu et al. prepared a group of 20 ACD-FFPA units, comprising five units from each ABO blood group [23]. Because these investigators did not sample the units at thaw, but instead at 24, 72, and 120 hours after thaw, a comparison of recoveries between studies is not possible. However, FVIII activities of 0.767 ± 0.048 (mean ± SE) after 120 hours of refrigerated storage were observed, values very similar

Table 2: Comparison of coagulation parameters in ACD-FFPA and FP[a] at 120 h.

TEST	ACD-FFPA[b]	FP[c]	p value
FV (IU/mL)	0.806 ± 0.15	0.697 ± 0.14	0.0068
FVII (IU/mL)	0.904 ± 0.19	0.761 ± 0.16	0.0039
FVIII (IU/mL)	0.786 ± 0.23	0.542 ± 0.20	<0.0001
Fibrinogen (grams/L)	2.77 ± 0.47	3.193 ± 0.81	0.0209
PT (seconds)	14.1 ± 0.53	14.6 ± 0.69	0.0158

[a]Values are reported as the mean ± one standard deviation. Statistical comparisons were made using an unpaired t-test in all cases, except for FVIII, for which the unpaired t-test, Welch corrected, was employed. [b]From current study. [c]From [11].

to those observed in our current study, although it is not clear if the differences in ABO distribution in their sample population had any effect. We elected to use 50% type O and 50% nontype O in our group of analyzed units to more closely mimic our donor population in Canada (46% type O), whereas Sidhu et al. used a 25% type O and a 75% nontype O mixture of units [20]. Sidhu et al. also found no change in fibrinogen or FVII levels between Days 1 and 5 of refrigerated storage and a statistically significant loss of FV activity, mirroring our results [23]. Similarly, Neisser-Svae et al. found residual FVIII activities in thawed FFP of 0.75 ± 0.13 IU/mL (mean ± SD) after 5 days of refrigerated storage [24], and von Heymann et al. reported median FVIII values of 0.75 with an interquartile (25–75%) range of 0.68 to 0.88 IU/mL for thawed FFP, again showing strong similarity to our results with ACD-FFPA [25].

In our previous stability studies of thawed FP and thawed cryosupernatant plasma, at the conclusion of the studies, we tested the residual products in the bag for bacterial contamination [14, 22]. A total of 90 units were negative for growth in the BacT-ALERT system employed in Canada for mandatory screening of platelet products. Since bacterial contamination of plasma is a very rare event, these findings suggested that our procedures were sufficiently robust to avoid contamination of plasma units by airborne microbes despite multiple sampling events. For this reason BacT-ALERT testing was not performed in the current study.

One reason that we did not study FFPA stability when we previously addressed the issue of thawed FP stability was that some FFPA in the Canadian blood operator's inventory is generated in a functionally open system, necessitating transfusion within four hours of thawing, and some (ACD-FFPA) is not. However, in the interval since that initial study, the trend towards the maintenance of trauma packs containing prethawed FP units by Canadian trauma centres for use in massive transfusion has intensified [26]. Hospitals cannot currently follow plasma-sparing practices, such as releasing thawed trauma pack FP for transfusion to nontrauma patients on Day 4 of refrigerated storage with FFPA due to its current short regulated shelf life. Concerns over potential confusion between FFPA from functionally open and closed systems have also dissipated, as Concurrent Plasma, ACD-FFPA, is now uniquely identifiable in the Canadian Blood Services inventory due to not only its anticoagulant, but also more importantly due to its being the only FFP distributed to Canadian hospitals in ~200 mL volumes. The results of this study provide support to changing the permitted refrigerated shelf life of ACD-FFPA to 120 hours, aligning Canadian practice to that in the United States, where transfusion of thawed relabeled FFP or PF24 stored refrigerated for up to 120 hours has been permitted since 1998, with no apparent negative consequences for patients [12, 13].

Disclosure

Since the Canadian Blood Services Centre for Innovation receives funding from Health Canada, a department of the Federal Government of Canada, this article must contain the statement, "The views expressed herein do not necessarily represent the views of the federal government [of Canada]."

Competing Interests

All authors declare they have no relevant conflict of interests.

References

[1] R. Cardigan and L. Green, "Thawed and liquid plasma—what do we know?" *Vox Sanguinis*, vol. 109, no. 1, pp. 1–10, 2015.

[2] B. J. Barrett, "Practice parameter for the use of fresh-frozen plasma, cryoprecipitate, and platelets," *Journal of the American Medical Association*, vol. 271, no. 10, pp. 777–781, 1994.

[3] E. Crosby, D. Ferguson, H. A. Hume et al., "Guidelines for red blood cell and plasma transfusion for adults and children," *Canadian Medical Association Journal*, vol. 156, no. 11, pp. S1–S24, 1997.

[4] "BCSH guidelines for the use of fresh frozen plasma (updated)," *British Journal of Haematology*, vol. 126, pp. 11–28, 2004.

[5] *CAN/CSA-Z902-10: Blood and Blood Components*, Canadian Standards Association, 2010.

[6] Council of Europe, *Council of Europe: Guide to the Preparation, Use and Quality Assurance of Blood Components*, Directorate for the Quality of Medicines & Health Care of the Council of Europe, Strasbourg, France, 18th edition, 2015.

[7] AABB, *Standards for Blood Banks and Transfusion Services*, AABB Press, Bethesda, Md, USA, 30th edition, 2016.

[8] *Circular of Information*, Canadian Blood Services, Ottawa, Canada, 2016.

[9] G. Rock, D. Anderson, W. Clark et al., "Does cryosupernatant plasma improve outcome in thrombotic thrombocytopenic purpura? No answer yet," *British Journal of Haematology*, vol. 129, no. 1, pp. 79–86, 2005.

[10] G. A. Rock, K. H. Shumak, N. A. Buskard et al., "Comparison of plasma exchange with plasma infusion in the treatment of

thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group," *The New England Journal of Medicine*, vol. 325, no. 6, pp. 393–397, 1991.

[11] *Circular of Information for the Use of Human Blood and Blood Components*, AABB/American Red Cross/America's Blood Centers/The Armed Services Blood Program, Puget Sound, Washington, DC, USA, 2013.

[12] A. F. Eder and M. A. Sebok, "Plasma components: FFP, FP24, and thawed Plasma," *Immunohematology*, vol. 23, no. 4, pp. 150–157, 2007.

[13] D. Triulzi, J. Gottschall, E. Murphy et al., "A multicenter study of plasma use in the United States," *Transfusion*, vol. 55, no. 6, pp. 1313–1319, 2015.

[14] W. P. Sheffield, V. Bhakta, C. Mastronardi, S. Ramirez-Arcos, D. Howe, and C. Jenkins, "Changes in coagulation factor activity and content of di(2-ethylhexyl) phthalate in frozen plasma units during refrigerated storage for up to five days after thawing," *Transfusion*, vol. 52, no. 3, pp. 493–502, 2012.

[15] E. Scott, K. Puca, J. Heraly, J. Gottschall, and K. Friedman, "Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6°C storage," *Transfusion*, vol. 49, no. 8, pp. 1584–1591, 2009.

[16] O. Weisert and M. Jeremic, "Plasma fibrinogen levels in 1,016 regular blood donors. I. The influence of age and sex on mean values and percentiles," *Vox Sanguinis*, vol. 27, no. 2, pp. 176–185, 1974.

[17] P. F. van der Meer and D. de Korte, "The effect of holding times of whole blood and its components during processing on in vitro and in vivo quality," *Transfusion Medicine Reviews*, vol. 29, no. 1, pp. 24–34, 2015.

[18] R. M. Kakaiya, E. E. Morse, and S. Panek, "Labile coagulation factors in thawed fresh frozen plasma prepared by two methods," *Vox Sanguinis*, vol. 46, no. 1, pp. 44–46, 1984.

[19] W. P. Sheffield, V. Bhakta, C. Jenkins, and D. V. Devine, "Conversion to the buffy coat method and quality of frozen plasma derived from whole blood donations in Canada," *Transfusion*, vol. 50, no. 5, pp. 1043–1049, 2010.

[20] L. Gallinaro, M. G. Cattini, M. Sztukowska et al., "A shorter von willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von willebrand factor," *Blood*, vol. 111, no. 7, pp. 3540–3545, 2008.

[21] E. A. Scott, K. E. Puca, B. C. Pietz, B. K. Duchateau, and K. D. Friedman, "Comparison and stability of ADAMTS13 activity in therapeutic plasma products," *Transfusion*, vol. 47, no. 1, pp. 120–125, 2007.

[22] V. Bhakta, C. Jenkins, S. Ramirez-Arcos, and W. P. Sheffield, "Stability of relevant plasma protein activities in cryosupernatant plasma units during refrigerated storage for up to 5 days postthaw," *Transfusion*, vol. 54, no. 2, pp. 418–425, 2014.

[23] R. S. Sidhu, T. Le, B. Brimhall, and H. Thompson, "Study of coagulation factor activities in apheresed thawed fresh frozen plasma at 1–6°C for five days," *Journal of Clinical Apheresis*, vol. 21, no. 4, pp. 224–226, 2006.

[24] A. Neisser-Svae, L. Trawnicek, A. Heger, T. Mehta, and D. Triulzi, "Five-day stability of thawed plasma: solvent/detergent-treated plasma comparable with fresh-frozen plasma and plasma frozen within 24 hours," *Transfusion*, vol. 56, no. 2, pp. 404–409, 2016.

[25] C. von Heymann, M. K. Keller, C. Spies et al., "Activity of clotting factors in fresh-frozen plasma during storage at 4 C over 6 days," *Transfusion*, vol. 49, no. 5, pp. 913–920, 2009.

[26] J. L. Callum and S. Rizoli, "Plasma transfusion for patients with severe hemorrhage: what is the evidence?" *Transfusion*, vol. 52, supplement 1, pp. 30S–37S, 2012.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 3059848, 6 pages
http://dx.doi.org/10.1155/2016/3059848

Research Article

Mitigating the Risk of Transfusion-Transmitted Dengue in Australia

Kelly Rooks,[1] Clive R. Seed,[2] Jesse J. Fryk,[1] Catherine A. Hyland,[1] Robert J. Harley,[3] Jerry A. Holmberg,[4] Denese C. Marks,[5] Robert L. P. Flower,[1] and Helen M. Faddy[1,6]

[1]*Research and Development, Australian Red Cross Blood Service, Brisbane, QLD, Australia*
[2]*Medical Services, Australian Red Cross Blood Service, Perth, WA, Australia*
[3]*Medical Services, Australian Red Cross Blood Service, Brisbane, QLD, Australia*
[4]*Grifols Diagnostic Solutions, Inc., Emeryville, CA, USA*
[5]*Research and Development, Australian Red Cross Blood Service, Sydney, NSW, Australia*
[6]*School of Medicine, The University of Queensland, Brisbane, QLD, Australia*

Correspondence should be addressed to Helen M. Faddy; hfaddy@redcrossblood.org.au

Received 18 August 2016; Accepted 16 October 2016

Academic Editor: Erwin Strasser

Dengue viruses (DENV 1–4) are a risk to transfusion safety, with several transfusion-transmitted (TT) cases reported globally. DENV 1–4 are endemic in over 100 countries, with seasonal outbreaks occurring in northeastern Australia. To mitigate TT-DENV risk in Australia, fresh blood components are not manufactured from donors returning from any area (domestic/overseas) with known dengue transmission. Alternatively, TT-DENV risk may be mitigated using an appropriate blood donor screening assay. We aimed to determine the rate of dengue infection in donors during dengue outbreaks in Australia. Plasma samples were collected from blood donors during local dengue outbreaks. All samples were tested for the presence of DENV RNA and selected samples were tested for DENV antigen (nonstructural protein 1, NS1) with two assays. No donors residing in high risk areas had detectable levels of DENV RNA or NS1 and no cases of DENV viremia were detected in blood donors residing in areas of Australia experiencing DENV outbreaks. Definitive conclusions could not be drawn from this study; however, the lack of detection of DENV RNA or antigen in donations suggests that the current risk of TT-DENV is low and maintaining the fresh component restriction for "at-risk" donors is appropriate.

1. Introduction

Dengue is one of the most important arboviral pathogens worldwide, with an estimated 390 million infections per year [1]. Of these estimated dengue infections, only 96 million manifest clinically, with the majority of infections therefore asymptomatic [2]. Dengue is emerging or reemerging across the globe, with transmission occurring in over 100 countries each year [3].

There are four serotypes of dengue virus (DENV): DENV-1, DENV-2, DENV-3 and DENV-4. DENV are mosquito-borne, with the primary vector being *Aedes aegypti*. This urban-adapted mosquito is distributed throughout tropical and subtropical climates, giving rise to endemic and epidemic DENV transmission in both developing and developed nations [4]. A secondary vector capable of transmitting DENV, *Aedes albopictus*, has increased in geographic range in recent years, which may contribute to the increasing number of dengue infections [4].

Almost 75% of the DENV global disease burden is in the Southeast Asian and Western Pacific Regions [5]. In Australia, seasonal outbreaks occur in the northeast of the country [1, 6]. One of the largest DENV epidemics in Australia's history occurred in 2008/2009, with distinct outbreaks in Cairns, Innisfail, and Townsville [7]. Collectively, this epidemic resulted in over 1,000 confirmed infections. Another sizeable DENV outbreak occurred in northeastern Australia in the summer of 2012/2013, resulting in 534 confirmed cases [8].

Given the high rate of asymptomatic DENV infection, this virus poses a risk to transfusion safety [9]. Transfusion transmitted-DENV (TT-DENV) has been reported in Singapore, Hong Kong, Puerto Rico, and Brazil [10–13]. To date, no cases of TT-DENV have occurred in Australia. The incidence of TT-DENV is likely to be higher than what has been published, due to underreporting. Moreover, DENV viremia has been detected in blood donors from Honduras, Puerto Rico, and Brazil, reinforcing the potential risk of TT-DENV [12, 14, 15].

To help mitigate the risk of TT-DENV in Australia, donors are unable to donate fresh blood components for 4 weeks upon their return from countries endemic for DENV or areas in northern Australia experiencing dengue outbreaks [16]. Plasma may still be collected during this 4-week restrictive period if destined for fractionation, as the manufacturing process includes viral inactivation steps that have been shown to effectively inactivate DENV, allowing plasma derivatives to be considered safe with respect to this virus [17]. Currently, there is no approved DENV test in Australia for blood screening, and although some pathogen inactivation (PI) technologies have been demonstrated to effectively inactivate DENV in plasma and platelet components [18–21] these methods are not approved for use in Australia at present. Our approach of restricting donations from "at-risk" individuals results in fresh component losses and considerable cost, which may potentially impact on the ability to meet clinical demand [22]. However, this approach is deemed suitable in the absence of other approved risk mitigation strategies.

It is clear that DENV poses a risk to the safety of Australia's blood supply, which may justify these relatively high-cost risk-reduction strategies. However, alternative testing technologies for DENV detection may be utilised for donor screening, if deemed appropriate and licenced for such use. Therefore, this study aimed to determine the rate of viremia in Australian blood donors during local dengue outbreaks, by testing plasma samples for the presence of DENV RNA and DENV antigen (nonstructural protein 1, NS1).

2. Materials and Methods

2.1. Sample Collection. Samples were collected from donors in North Queensland during two dengue outbreaks: 2008/2009 (n = 973) and 2012/2013 (n = 5,518). For samples collected during the 2008/2009 outbreak, an extra sample was collected from all donations during the outbreak. These samples were collected in plasma preparation tubes (PPT, BD Vacutainer Plasma Preparation Tubes, Becton Dickinson, Plymouth, UK) and centrifuged at a relative centrifugal force (RCF) of 1,100 for 10 minutes as per routine procedure. Demographic data were obtained for all donations to allow identification of donors at "higher risk" of exposure to DENV, defined as residence in areas of Cairns that reported more than 20 laboratory confirmed dengue cases. Samples from 2012/2013 were collected from both Cairns and Townsville during the dengue outbreak. Additional control samples (n = 1,601) were obtained from Melbourne in southern Australia in 2012/2013, where transmission of DENV does not occur. Samples collected in 2012/2013 were recovered after routine testing was completed, representing a convenience sample. Samples from 2012/2013 were collected into ethylenediaminetetraacetic acid (EDTA) spray-coated tubes (BD Vacutainer Whole Blood Collection Tube with Spray-Coated K_2EDTA, Becton Dickinson) and centrifuged at 1,258 RCF for 10 minutes as per routine procedure. All samples were stored at −20°C until testing. This study was carried out under approval by the Blood Service Human Research Ethics Committee.

2.2. Dengue NS1 Testing. Samples from donors residing in "higher-risk" areas during the 2008/2009 DENV outbreak (n = 973) were tested for the presence of DENV NS1 using both the PanBio Dengue Early ELISA (Alere, Brisbane, Queensland, Australia) and the Platelia Dengue NS1 Ag Kit (Bio-Rad, Hercules, CA, USA) as per the manufacturer's instructions, which included positive, negative, and internal controls. Both kits utilised a one-step sandwich format ELISA for the detection of DENV NS1 in either plasma or serum. Samples were first tested in singlicate on both assays, with initial reactive or equivocal samples being retested in duplicate. Samples were only classified positive if they were reactive 2 or 3 times on both assays. Results from the PanBio Dengue Early ELISA Kit were calculated in "PanBio units" and considered negative if the results were <9, equivocal if 9–11, and positive if ≥11 (sensitivity: 72.3% and specificity: 100% [23]). The Platelia Dengue NS1 Ag Kit results were calculated in ratios and considered negative if results were <0.5, equivocal if between 0.5 and 1, and positive if ≥1 (sensitivity: 83.6% and specificity: 98.7% [23]).

2.3. Dengue RNA Testing. The following samples were tested for the presence of DENV RNA: 664 samples from higher-risk areas during the 2008/2009 DENV outbreak (representing all samples remaining with an adequate volume); 5,518 samples from the 2012/2013 DENV outbreak; and 1,601 control samples from southern Australia. Samples were tested with a Procleix DENV assay on a Procleix Panther System (Grifols Diagnostic Solutions, Inc., Emeryville, CA, USA, and Hologic, San Diego, CA, USA) as per manufacturer's instructions, which included positive, negative, and internal controls, at the American Red Cross laboratories in Charlotte, North Carolina. The Procleix DENV assay is based on transcription mediated amplification (TMA) and can detect all four DENV serotypes [4]. The 95% limit of detection is reported to be approximately 15 copies/mL (95% CI, 11.5–20.9 copies/mL), with a specificity of >99.91% [4].

2.4. Analyses. Data were stored using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) databases and analyses were also performed using this software. Individual proportions were calculated, along with the corresponding exact 95% confidence intervals (CI) using a standard method [24]. Specifically, for zero-risk estimates, the 95% CIs were calculated as follows:

Upper 95% CI = $1 - 0.025^{(1/n)}$, where n = number of samples tested.

Lower 95% CI = 0.

Table 1: Detection of DENV NS1 in donations from Australian blood donors collected during local DENV outbreaks.

Sample	Platelia Dengue NS1 (# positive, # tested)	PanBio Dengue Early ELISA (# positive, # tested)	Overall result
1	N (0, 3)	P (3, 3)	Negative
2	N (0, 3)	P (3, 3)	Negative
3	N (0, 3)	P (3, 3)	Negative
4	E (1, 3) N (2, 3)	P (3, 3)	Negative
5	N (0, 3)	P (3, 3)	Negative
6	N (0, 3)	P (3, 3)	Negative
7	N (0, 3)	P (3, 3)	Negative
8	N (0, 3)	P (3, 3)	Negative
9	N (0, 3)	P (3, 3)	Negative
10	N (0, 3)	E (1, 3) P (2, 3)	Negative
11	N (0, 3)	P (3, 3)	Negative
12	N (0, 3)	P (3, 3)	Negative
13	N (0, 3)	P (3, 3)	Negative
14	N (0, 3)	P (3, 3)	Negative
15	N (0, 3)	P (3, 3)	Negative
16	N (0, 3)	P (3, 3)	Negative
17	N (0, 3)	E (1, 3) P (2, 3)	Negative
18	N (0, 3)	E (1, 3) P (2, 3)	Negative
19	N (0, 3)	P (3, 3)	Negative
20	N (0, 3)	P (3, 3)	Negative
21	N (0, 3)	P (3, 3)	Negative
22	N (0, 3)	P (3, 3)	Negative
23	N (0, 3)	E (1, 3) P (2, 3)	Negative
24	N (0, 3)	P (3, 3)	Negative
25	N (0, 3)	P (3, 3)	Negative
26	N (0, 3)	P (3, 3)	Negative
27	N (0, 3)	P (3, 3)	Negative
28	N (0, 3)	P (3, 3)	Negative
29	N (0, 3)	P (3, 3)	Negative
30	N (0, 3)	P (3, 3)	Negative
31	N (0, 3)	P (3, 3)	Negative
32	N (0, 3)	E (1, 3) P (2, 3)	Negative

N = negative, P = positive, and E = equivocal.

3. Results

Samples from areas of Cairns with higher numbers of confirmed DENV cases during the 2008/2009 outbreak were selected for DENV NS1 antigen testing ($n = 973$). Of the samples tested, 32 were positive (overall either 2/3 or 3/3) with the PanBio Dengue Early ELISA (Table 1). Using the Platelia Dengue NS1 Ag Kit, only one sample tested initially equivocal; however, it was negative on duplicate repeat testing (Table 1). As no samples tested positive on both assays, all samples were deemed negative for DENV antigen.

Samples collected from the 2008/2009 and 2012/2013 DENV outbreaks, as well as control samples, were tested for the presence of DENV RNA by TMA. None of the samples collected during local dengue outbreaks were positive for DENV RNA (zero estimate, with a one-sided 95% CI: 0–0.06%), despite a subset being collected from "higher-risk" areas (Table 2). All of the control samples were also negative for DENV RNA (zero estimate, with a one-sided 95% CI: 0–0.23%).

Table 2: Detection of DENV RNA, by TMA, in donations from Australian blood donors.

Samples	# tested	DENV RNA positive		
		#	%	95% CI
Dengue outbreak (2008/2009 and 2012/2013)	6,182	0	—	0–0.06
2008/2009 DENV epidemic	664	0	—	0–0.55
2012/2013 DENV outbreak	5,518	0	—	0–0.07
Control region	1,601	0	—	0–0.23

4. Discussion

Dengue is an emerging disease of global significance and a current concern for the international transfusion community given the increasing number of transfusion transmitted (TT) cases [10–13]. DENV viremia has been detected in blood donors from Honduras, Puerto Rico, and Brazil, highlighting the potential risk for TT-DENV [25]. Countries have different risk mitigation approaches for managing the risk of TT-DENV, which in part depend on the level of dengue endemicity, the "risk appetite" of local clinicians and the public, and the size of their healthcare budget. These and additional factors should be considered in risk-based decision-making for blood safety. Currently, in Australia, donations of fresh blood components are restricted from "at-risk" donors travelling from areas where DENV transmission occurs, both within Australia and overseas. In this study, we were unable to detect DENV viremia in blood donors residing in areas of Australia experiencing local DENV outbreaks. Given that the upper confidence interval from this study was 0.06% (1 in 1,667), the current precautionary strategy of restricting "at-risk" donors to donating plasma for fractionation only is reasonable to mitigate the risk of a viremic donation.

None of the donors tested had detectable DENV infection, as evidenced by the absence of detecting either DENV RNA or DENV antigen. These results are concordant with earlier studies during previous outbreaks [4]. We previously estimated the risk of collecting a viremic donation during the 2008/2009 DENV outbreak to be 1 in 7,147 (95% CI: 1 in 2,218 to 1 in 50,021) [22], and modelling based on notification data obtained during a DENV outbreak in 2004 estimated the overall transmission risk to be 1 in 19,759 (95% CI: 1 in 3,404 to 75,486) with a peak of 1 in 5,968 (95% CI: 1 in 1,028 to 22,800) [26]. While this previous data suggests a low likelihood of finding a viremic sample in our study the absence of detectable evidence of DENV infection in donors, despite the higher number of reported cases during these outbreaks [6, 8], provides reassurance that our existing risk

modelling does not substantially underestimate the risk of TT-DENV. While future studies using a larger sample size would refine the risk estimate, the small donor population in areas of Australia with DENV transmission means that such studies would not be practicable.

In Australia TT-DENV risk is mitigated through donation restrictions for "at-risk" donors, which has the potential to impact on availability of blood components for clinical use. Therefore, a blood donation screening assay that is capable of detecting donations containing an infectious virus could be used as an alternative. DENV antibody assays (IgM/IgG) would not be suitable for such a purpose, as DENV IgM is typically not present within blood until 3–5 days after the presentation of clinical symptoms and DENV IgG is not present for 1–14 days [27]. Detection of DENV antigen could be suitable for detecting asymptomatic and early infections in blood donors, as high levels of the antigen NS1 have been shown to be detectable within 72 hours of disease onset [28] and the assay format can be applied for high-throughput use. In this study two DENV NS1 antigen detection assays were used to determine the efficacy of detecting early DENV infection. We found discordant results between the two assays used, which is consistent with other studies. The Platelia Dengue NS1 Ag kit has been shown to have a higher sensitivity compared to the PanBio Dengue Early ELISA [23, 29, 30]. Although NS1 has been suggested as a useful tool in early DENV screening, sensitivity and specificity remain a major concern. Blood donor samples collected during a DENV outbreak in 2010/2012 in Puerto Rico were initially tested for NS1 antigen and later tested for DENV RNA (with TMA) to assess the possibility of TT-DENV from blood donors [25]. This study found that only 20% of RNA positive donor samples were positive on the Platelia Dengue NS1 Ag Kit, resulting in 42 patients being transfused with DENV RNA positive components [25]. This highlights the limitations of using DENV NS1 detection as a basis for blood donation screening assays for detecting DENV [25]; however, it should be noted that DENV NS1 still has a place in diagnostics. Therefore, detection of DENV RNA appears to be the most suitable for blood donation screening for DENV; however, testing may become costly. With the absence of detectable DENV RNA in any of the samples tested in this study, our current strategy of restricting fresh components from "at-risk" donors but continuing to collect plasma for fractionation appears an effective method for reducing the risk of TT-DENV in Australia. The strategy is also cost-effective because of the increasing demand in Australia for plasma to manufacture plasma derived immunoglobulin products [22]. Such an approach may also be suitable in other nonendemic areas, particularly those that experience episodic outbreaks and where source plasma is collected.

The levels of DENV viremia in blood donors during DENV outbreaks in Puerto Rico, Brazil, and Honduras (all considered endemic for dengue) were 0.19% [12], 0.04% [4], and 0.3% [4], respectively. Interestingly, DENV viremia was lower, 0.07%, in Puerto Rico in blood donors in a nonoutbreak period but during the seasonally heightened peak of dengue activity [41]. In contrast, no DENV viremic samples were found in Australian blood donors during the 2003 outbreak, although this study involved a relatively small number of donations from outbreak-affected areas [4]. The rate of dengue viremia in blood donors differs between areas endemic for DENV and also those considered episodic or nonendemic. Therefore, blood operators around the world require different risk-based decision-making strategies for dengue, depending on a number of factors including but not limited to the degree of dengue endemicity (endemic, episodic, and nonendemic); the acceptance of TT risk among local clinicians, as well as the public; the perceived severity of DENV infection among clinicians as well as the public compared to other infections; the proportion of the donor population travelling abroad and subject to travel-related blood donation restrictions; and, perhaps most importantly, the size of the healthcare budget. There are several approaches for reducing the risk of TT-DENV utilised by various blood operators across the world (Table 3). For example, in Hong Kong, where there is a constant high risk of dengue introduction from nearby mainland China [33], TT-DENV is mitigated through deferral of donors for 6 months who have previously had a dengue infection and a 2-week deferral for history of fever; however, no travel-related deferrals are in place [31]. However, in countries with minimal risk of DENV infection, such as New Zealand, TT-DENV is minimised through deferral of donors for 4 weeks who have previously had a DENV infection. In the future, other approaches may be used, for example, PI, particularly in areas endemic for dengue. The Theraflex UV-Platelets System (MacoPharma) has been shown to inactivate DENV in spiked platelet units to the limit of detection of the assay used [19], as has the Theraflex-MB Plasma System (MacoPharma) for DENV spiked into plasma [20]. Similarly the Intercept Plasma Inactivation System (Cerus Corporation) demonstrated inactivation of DENV in spiked plasma to the limit of detection [21]. In contrast the Mirasol PRT System (TerumoBCT) only partially inactivated DENV in spiked platelet units [42]. PI has the potential to assist with managing the TT-DENV risk in areas endemic for dengue or with episodic transmission and also has the potential to replace travel-related donation restrictions [43]. However, current technologies are not available for all blood products and therefore limit their application.

5. Conclusion

In this study we did not find evidence of DENV infection in the blood donors tested [6, 8]. The upper confidence interval of our blood donor viremia estimate of 1 in 1,667 suggests that the risk of collecting a viremic donation may be significant and supports the current precautionary strategy of restricting "at-risk" donors to donating plasma for fractionation only. It is clear that there is no overarching approach for the management of TT-DENV that is suitable for all countries. Each area should therefore assess TT-DENV risk based on local epidemiology and perform region-specific cost and risk analyses, which should collectively be considered in any future risk-based decision-making. The latter should consider that, as well as directly addressing transfusion risk, routine surveillance for infectious diseases in the blood donor population,

Table 3: Dengue endemicity and approaches used by blood operators for managing TT-DENV risk. Adapted from Teo et al., 2009 [31].

Country	Endemicity	Management approach
Australia	Nonendemic/episodic outbreaks in Queensland [6, 8]	(i) 4-week deferral for history of dengue infection [16] (ii) 4-week deferral for persons returning from dengue affected areas [16]
Canada	Nonendemic	3-week travel-related deferral for travel outside of Canada, continental USA, or Europe [32]
Hong Kong	Nonendemic [33]	(i) 6-month deferral for history of dengue infection [31] (ii) 2-week deferral for history of fever [31] (iii) No travel-related deferral for dengue [31]
Netherlands	Nonendemic	(i) 2-week deferral for history of dengue infection [34] (ii) 2-week deferral for history of fever [34] (iii) 4-week travel-related deferral for donors returning from dengue risk areas [34]
New Zealand	Nonendemic	(i) 4-week deferral for history of dengue infection [35] (ii) No travel-related deferral for dengue [35]
Puerto Rico	Endemic [36]	Pathogen inactivation (Intercept) recently implemented for use on plasma and platelet products [37]
Singapore	Endemic [38]	(i) 6-month deferral for history of dengue infection [31] (ii) 3-week deferral for history of fever [31] (iii) No travel-related deferral for dengue [31]
Sri Lanka	Endemic [39]	(i) No specific deferral for history of dengue infection [31] (ii) 2-week deferral for history of fever [31] (iii) No travel-related deferral for dengue [31]
United Kingdom	Nonendemic	(i) 2-week deferral for history of dengue infection [31] (ii) No travel-related deferral for dengue [31]
United States of America	Nonendemic/episodic outbreaks in some states [8, 40]	(i) 4-week deferral for history of dengue infection [31] (ii) No travel-related deferral for dengue [31]

including DENV, also serves as an important disease surveillance tool.

Competing Interests

Jerry A. Holmberg is Director, Scientific Development, Grifols Diagnostic Solutions Inc. All other authors have disclosed no conflict of interests.

Acknowledgments

The authors thank Donor Services staff involved in collecting additional samples; members of the Brisbane Manufacturing Team, especially Kathryn Van Der Merwe for assistance with processing samples; Blood Service staff in Melbourne for the supply of interstate samples; and staff at the American Red Cross for performing TMA. Australian governments fund the Australian Red Cross Blood Service to provide blood, blood products, and services to the Australian community. This work was partly supported by Grifols, in the form of reagent supply.

References

[1] S. Bhatt, P. W. Gething, O. J. Brady et al., "The global distribution and burden of dengue," *Nature*, vol. 496, no. 7446, pp. 504–507, 2013.

[2] C. P. Simmons, J. J. Farrar, N. Van Vinh Chau, and B. Wills, "Dengue," *The New England Journal of Medicine*, vol. 366, no. 15, pp. 1423–1432, 2012.

[3] World Health Organization, *Dengue: Guidelines for Treatment, Prevention and Control*, World Health Organization, Geneva, Switzerland, 2009.

[4] M. P. Busch, J. M. Linnen, E. Vinelli et al., "Dengue viremia in blood donors from Honduras, Brazil, and Australia," *Transfusion*, vol. 48, no. 7, pp. 1355–1362, 2008.

[5] WHO Regional Office for South-East Asia, *Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Revised and Expanded Edition*, World Health Organization, New Delhi, India, 2011.

[6] E. Viennet, S. A. Ritchie, H. M. Faddy, C. R. Williams, and D. Harley, "Epidemiology of dengue in a high-income country: a case study in Queensland, Australia," *Parasites & Vectors*, vol. 7, no. 1, article 379, p. 1, 2014.

[7] G. J. Fitzsimmons, P. Wright, C. A. Johansen, P. I. Whelan, and National Arbovirus and Malaria Advisory Committee, "Arboviral diseases and malaria in Australia, 2008-09: annual report of the National Arbovirus and Malaria Advisory Committee," *Communicable Diseases Intelligence*, vol. 34, no. 3, pp. 225–240, 2010.

[8] E. Viennet, S. A. Ritchie, C. R. Williams, H. M. Faddy, and D. Harley, "Public health responses to and challenges for the control of dengue transmission in high-income countries: four case studies," *PLoS Neglected Tropical Diseases*, vol. 10, no. 9, Article ID e0004943, 2016.

[9] S. L. Stramer, F. B. Hollinger, L. M. Katz et al., "Emerging infectious disease agents and their potential threat to transfusion safety," *Transfusion*, vol. 49, no. 2, pp. 1S–29S, 2009.

[10] P. A. Tambyah, E. S. C. Koay, M. L. M. Poon, R. V. T. P. Lin, and B. K. C. Ong, "Dengue hemorrhagic fever transmitted by blood transfusion," *New England Journal of Medicine*, vol. 359, no. 14, pp. 1526–1527, 2008.

[11] V. W. M. Chuang, T. Y. Wong, Y. H. Leung et al., "Review of dengue fever cases in Hong Kong during 1998 to 2005," *Hong Kong Medical Journal*, vol. 14, no. 3, pp. 170–177, 2008.

[12] S. L. Stramer, J. M. Linnen, J. M. Carrick et al., "Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico," *Transfusion*, vol. 52, no. 8, pp. 1657–1666, 2012.

[13] E. C. Sabino, P. Loureiro, M. Esther Lopes et al., "Transfusion-transmitted dengue and associated clinical symptoms during the 2012 epidemic in Brazil," *Journal of Infectious Diseases*, vol. 213, no. 5, pp. 694–702, 2016.

[14] M. C. Lanteri and M. P. Busch, "Dengue in the context of 'safe blood' and global epidemiology: to screen or not to screen?" *Transfusion*, vol. 52, no. 8, pp. 1634–1639, 2012.

[15] J. E. Levi, A. Nishiya, A. C. Félix et al., "Real-time symptomatic case of transfusion-transmitted dengue," *Transfusion*, vol. 55, no. 5, pp. 961–964, 2015.

[16] Service, A.R.C.B., Guidelines for the Selection of Blood Donors, pp. 384, 2016.

[17] Y.-W. Xie, P. K. S. Chan, C. K. Szeto et al., "Clearance of dengue virus in the plasma-derived therapeutic proteins," *Transfusion*, vol. 48, no. 7, pp. 1342–1347, 2008.

[18] H. Faddy et al., "The effect of pathogen reduction technology (Mirasol) on the infectivity of dengue viruses," in *Vox Sanguinis*, Wiley-Blackwell, 2012.

[19] H. M. Faddy, J. J. Fryk, N. A. Prow et al., "Inactivation of dengue, chikungunya, and Ross River viruses in platelet concentrates after treatment with ultraviolet C light," *Transfusion*, vol. 56, no. 6, pp. 1548–1555, 2016.

[20] J. J. Fryk, D. C. Marks, J. Hobson-Peters et al., "Dengue and chikungunya viruses in plasma are effectively inactivated after treatment with methylene blue and visible light," *Transfusion*, vol. 56, no. 9, pp. 2278–2285, 2016.

[21] D. Musso, V. Richard, J. Broult, and V.-M. Cao-Lormeau, "Inactivation of dengue virus in plasma with amotosalen and ultraviolet A illumination," *Transfusion*, vol. 54, no. 11, pp. 2924–2930, 2014.

[22] H. M. Faddy, C. R. Seed, J. J. Fryk et al., "Implications of dengue outbreaks for blood supply, Australia," *Emerging Infectious Diseases*, vol. 19, no. 5, pp. 787–789, 2013.

[23] M. D. R. Q. Lima, R. M. R. Nogueira, H. G. Schatzmayr, and F. B. dos Santos, "Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of Dengue in Brazil," *PLoS Neglected Tropical Diseases*, vol. 4, no. 7, article e738, 2010.

[24] A. M. Mood, F. A. Graybill, and D. C. Boes, *Introduction to the Theory of Statistics*, vol. 3, McGraw Hill, New York, NY, USA, 1974.

[25] D. Matos, K. M. Tomashek, J. Perez-Padilla et al., "Probable and possible transfusion-transmitted dengue associated with NS1 antigen–negative but RNA confirmed-positive red blood cells," *Transfusion*, vol. 56, no. 1, pp. 215–222, 2016.

[26] C. R. Seed, P. Kiely, C. A. Hyland, and A. J. Keller, "The risk of dengue transmission by blood during a 2004 outbreak in Cairns, Australia," *Transfusion*, vol. 49, no. 7, pp. 1482–1487, 2009.

[27] S. Schilling, D. Ludolfs, L. Van An, and H. Schmitz, "Laboratory diagnosis of primary and secondary dengue infection," *Journal of Clinical Virology*, vol. 31, no. 3, pp. 179–184, 2004.

[28] D. H. Libraty, P. R. Young, D. Pickering et al., "High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever," *Journal of Infectious Diseases*, vol. 186, no. 8, pp. 1165–1168, 2002.

[29] P. Dussart, L. Petit, B. Labeau et al., "Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum," *PLoS Neglected Tropical Diseases*, vol. 2, no. 8, article e280, 2008.

[30] M. G. Guzman, T. Jaenisch, R. Gaczkowski et al., "Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis," *PLoS Neglected Tropical Diseases*, vol. 4, no. 8, article e811, 2010.

[31] D. Teo, L. C. Ng, and S. Lam, "Is dengue a threat to the blood supply?" *Transfusion Medicine*, vol. 19, no. 2, pp. 66–77, 2009.

[32] C. B. Service, *Surveillance Report*, 2015.

[33] C. M. Poon and S. S. Lee, "Has dengue found its home in Hong Kong?" *Hong Kong Medical Journal*, vol. 21, no. 1, pp. 85–87, 2015.

[34] Sanquin, "May I Give?" 2015.

[35] J. R. Hess, R. L. Sparrow, P. F. Van Der Meer, J. P. Acker, R. A. Cardigan, and D. V. Devine, "Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions," *Transfusion*, vol. 49, no. 12, pp. 2599–2603, 2009.

[36] T. M. Sharp, E. Hunsperger, G. A. Santiago et al., "Virus-specific differences in rates of disease during the 2010 Dengue epidemic in Puerto Rico," *PLoS Neglected Tropical Diseases*, vol. 7, no. 4, Article ID e2159, 2013.

[37] A. S. B. Centers, *Puerto Rico's Largest Blood Bank to use Pathogen Reduction Technology*, 2016.

[38] H. C. Hapuarachchi, C. Koo, J. Rajarethinam et al., "Epidemic resurgence of dengue fever in Singapore in 2013-2014: a virological and entomological perspective," *BMC Infectious Diseases*, vol. 16, no. 1, article 300, 2016.

[39] W. B. Messer, U. T. Vitarana, K. Sivananthan et al., "Epidemiology of dengue in Sri Lanka before and after the emergence of epidemic dengue hemorrhagic fever," *The American Journal of Tropical Medicine and Hygiene*, vol. 66, no. 6, pp. 765–773, 2002.

[40] G. Añez and M. Rios, "Dengue in the United States of America: a worsening scenario?" *BioMed Research International*, vol. 2013, Article ID 678645, 13 pages, 2013.

[41] H. Mohammed, J. M. Linnen, J. L. Muhoz-Jorddn et al., "Dengue virus in blood donations, Puerto Rico, 2005," *Transfusion*, vol. 48, no. 7, pp. 1348–1354, 2008.

[42] H. M. Faddy, J. J. Fryk, D. Watterson et al., "Riboflavin and ultraviolet light: impact on dengue virus infectivity," *Vox Sanguinis*, vol. 111, no. 3, pp. 235–241, 2016.

[43] H. M. Faddy, N. A. Prow, J. J. Fryk et al., "The effect of riboflavin and ultraviolet light on the infectivity of arboviruses," *Transfusion*, vol. 55, no. 4, pp. 824–831, 2015.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 9647675, 6 pages
http://dx.doi.org/10.1155/2016/9647675

Research Article

A Comparative Study of Assay Performance of Commercial Hepatitis E Virus Enzyme-Linked Immunosorbent Assay Kits in Australian Blood Donor Samples

Ashish C. Shrestha,[1,2] Robert L. P. Flower,[1] Clive R. Seed,[3] Susan L. Stramer,[4] and Helen M. Faddy[1,2]

[1]*Research and Development, Australian Red Cross Blood Service, Brisbane, QLD, Australia*
[2]*School of Medicine, The University of Queensland, Brisbane, QLD, Australia*
[3]*Medical Services, Australian Red Cross Blood Service, Perth, WA, Australia*
[4]*American Red Cross, Gaithersburg, MD, USA*

Correspondence should be addressed to Helen M. Faddy; hfaddy@redcrossblood.org.au

Received 18 August 2016; Accepted 19 October 2016

Academic Editor: Sandra Ramirez-Arcos

Hepatitis E virus (HEV) is transfusion-transmissible and therefore poses a risk to blood transfusion safety. Seroprevalence studies are useful for estimating disease burden and determining risk factors. Considerable variability in the sensitivity of HEV antibody detection assays exists. This study aimed to compare the performances of commercially available HEV enzyme-linked immunosorbent assays (ELISA) in Australian blood donor samples. Plasma samples that tested positive ($n = 194$) or negative ($n = 200$) for HEV IgG (Wantai HEV IgG ELISA) were selected. Of the 194 HEV IgG positive samples, 4 were positive for HEV IgM (Wantai HEV IgM ELISA). All samples were tested with the MP Diagnostics: HEV IgG ELISA, total (IgG, IgM, and IgA) HEV antibody ELISA, and HEV IgM ELISA. Of the 194 Wantai HEV IgG positive samples, 92 (47%) tested positive with the MP Diagnostics HEV IgG ELISA ($\kappa = 0.47$) and 126 (65%) with MP Diagnostics total HEV antibody assay ($\kappa = 0.65$). There was poor agreement between Wantai and MP Diagnostics HEV IgM assays. This study demonstrated poor agreement between the assays tested. These observations are consistent with previous reports demonstrating significant variability between HEV ELISAs, highlighting that results of HEV serology should be interpreted with caution.

1. Introduction

Hepatitis E virus (HEV) is a nonenveloped, RNA virus, classified in the genus *Hepevirus* of the Hepeviridae family [1]. There are 4 genotypes of HEV [1–4], representing a single serotype, which infect humans [2]. This classification into genotypes is based on variation in the nucleotides within open reading frame-2 (ORF-2) [3, 4]. HEV was first observed under immune electron microscopy in stool samples from a volunteer experimentally infected with non-A, non-B hepatitis [5]. Isolation of cDNA identified this virus as being different from hepatitis A [6] and facilitated the development of serological assays for HEV.

HEV causes self-limited acute phase disease with known cases of chronic hepatitis [7]. The incubation period on average is 40 days [8]. Clinical features include anorexia, nausea, vomiting, diarrhoea, epigastric pain, fever, jaundice, elevation of serum transaminase, and hepatomegaly [5, 7, 9–11]. Chronic HEV infections have been reported in solid-organ transplant recipients [12] and in immune suppressive conditions [13, 14]. A case fatality rate of 0.5–4% has been reported in developing countries [7], which is as high as 10–25% in pregnant women during the third trimester [2, 15, 16].

HEV is transfusion-transmissible and causes chronic infections in immunocompromised individuals [17]. The risk of transfusion-transmission from a donor with asymptomatic

viraemia can be identified through the detection of HEV RNA. However, the detection of HEV antibodies provides useful information on the immune status or stages of HEV infection in blood donors and may assist with the identification of risk factors for exposure. Seroprevalence is also important for assessing the overall disease burden in a population, and studies have shown that HEV exposure in blood donors varies widely between geographical regions [18, 19]. For example, 6% of Australian blood donors have been shown to be HEV IgG positive, while 52% of donors in southwestern France were HEV IgG positive with the same assay [20, 21].

Serology-based HEV tests for the detection of viral-specific antibodies include the detection of HEV IgG, HEV IgM, and HEV IgA in serum or plasma. Antibody testing assays are generally based on the detection of antibodies against epitopes of the gene products from ORF2 and ORF3 [22]. Many enzyme immunoassays with antigens derived from one HEV genotype are able to detect antibodies against a different genotype [23]. Detection of HEV IgG in an individual indicates a previous HEV infection. This antibody may persist in an infected individual for more than 12 years [24]. The acute phase of HEV infection can be detected by the detection of HEV IgM. This class of antibody is detectable after the onset of acute hepatitis and can last for up to 6 months following infection [25].

Studies with different commercial HEV IgG enzyme immunoassays have shown variability in sensitivity [26–28]. A study using anti-HEV reference serum (from the World Health Organisation) and including known HEV cases has shown 98% seropositivity with the Wantai IgG assay compared to 56% with the Genelabs IgG assay [27]. In a Korean study, HEV IgG seroprevalence was measured to be 23.1% with the Wantai assay, compared to 14.3% with the Genelabs assay [29]. Moreover, a study in HEV infected individuals has shown positivity of 83.3%, 100%, and 96.7% with the MP Diagnostics assay, Axiom Diagnostics assay (developed by Wantai), and Mikrogen assay, respectively [30]. Seroprevalence determined with different assays therefore needs to be interpreted with caution. Evaluation of HEV IgM commercial assays has also shown variability in sensitivity and specificity [31]. Given the importance of reliable seroprevalence estimates, this study aimed to compare the performances of commercially available HEV antibody detection assays (IgG and/or IgM) using a panel of Australian blood donor samples, made up of preselected positive and negative samples by one widely used assay.

2. Materials and Methods

2.1. Samples. Plasma samples from individual donors (n = 394) selected from a previous HEV seroprevalence study [20] were included in this study. These included samples (n = 194) that tested positive for HEV IgG with the Wantai HEV IgG ELISA (Beijing Wantai Biological Pharmacy, Beijing, China). These positive samples were all of the HEV IgG positive samples obtained from the previous seroprevalence study, which included 3,237 donors randomly selected for sex and age group [20]. Of the HEV IgG positive samples, 4 were also positive for HEV IgM with Wantai HEV IgM ELISA. In addition, age-matched negative samples (n = 200) were also sourced from the same seroprevalence study. Blood samples were collected in EDTA tubes (BD Vacutainer® Whole Blood Collection tube with spray-coated K2EDTA 6 mL, Becton Dickinson, Plymouth, UK), centrifuged at 1,258 g for 5 minutes and stored at −20°C until testing. Convenience samples no longer required after routine viral screening were utilised for this study and all samples were collected between August and September, 2013. The age of the donor was obtained from Blood Service records. This study was approved by Blood Service Human Research Ethics Committee.

2.2. Sample Testing: Wantai HEV ELISAs. The above-selected samples were tested for HEV IgG with the Wantai HEV IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). Samples reactive for HEV IgG were tested for HEV IgM with the Wantai HEV IgM ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). Samples were tested as per the manufacturer's instructions and absorbance was measured using a Hybrid Multimode Microplate Reader (BioTek Instruments, Inc., Winooski, USA) at 450 nm. Samples initially reactive for HEV IgG or HEV IgM were retested in duplicate with the respective assay and considered positive if reactive at least twice. After testing, samples were aliquoted into microtubes (Axygen Inc., USA) and stored at −20°C prior to testing with secondary commercial assays.

The Wantai HEV IgG assay is based on a recombinant HEV PE2 protein containing 211 amino acids of ORF2 derived from HEV genotype 1 [26, 27]. Sensitivity and specificity of the HEV IgG assay have been shown to be 97.96% and 99.6%, respectively [32, 33]. The Wantai HEV IgM assay is also based on a recombinant protein derived from HEV ORF2 [34]. Sensitivity of HEV IgM assay has been shown to be 97.10% [34]. Both the assays required 10 μL of sample, which was diluted with diluent (1 : 11) [32, 34].

2.3. Sample Testing: MP Diagnostics ELISAs. The above-selected samples were tested in singlet for HEV IgG with the MP Diagnostics HEV ELISA (MP Biomedicals Asia Pacific, Singapore), total (IgG, IgM, and IgA) HEV antibody with the MP Diagnostics HEV ELISA 4.0 (MP Biomedicals); and HEV IgM with the MP Diagnostics HEV IgM ELISA 3.0 (MP Biomedicals). Samples were tested as per the manufacturer's instructions and absorbance was measured using a Hybrid Multimode Microplate Reader (BioTek Instruments, Inc.) at 450 nm. Samples initially reactive with each assay were retested in duplicate with the same assay and considered positive if reactive at least two out of three times.

The MP Diagnostics HEV IgG assay uses three recombinant proteins, consisting of 42-amino acid sequence derived from ORF2 of genotype 2, 33-amino acid sequence from ORF3 of genotype 3, and ORF3 sequence from genotype 1 [26]. The assay has a reported sensitivity of 98% and specificity of 97% [35]. The assay required 10 μL of sample and was diluted with diluent (1 : 21).

MP Diagnostic HEV ELISA 4.0 detects IgG, IgM, and IgA antibodies. The assay uses highly conserved HEV ORF2.1

Table 1: Comparison of test results between the Wantai HEV IgG ELISA and MP Diagnostics HEV ELISA (IgG).

Wantai (HEV IgG)	MP Diagnostics (HEV IgG)		Total
	Positive	Negative	
Positive	92 (47.4%)	102	194
Negative	1	199 (99.5%)	200
Total	93	301	394

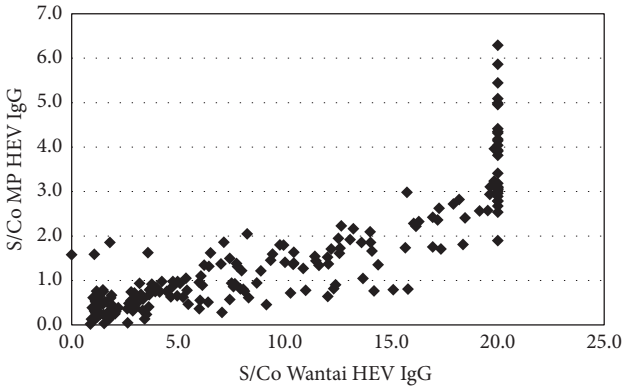


Figure 1: S/Co Wantai HEV IgG versus MP Diagnostics HEV IgG.

antigen, which is able to detect all antibody isotypes [36]. The test required 20 μL of sample and was diluted with diluent (1 : 50). The assay has a reported sensitivity of 99.2% and specificity of 99.2% [35].

MP Diagnostic HEV IgM ELISA is based on genotype 1 and 2 antigens derived from ORF2 and ORF3 [37]. The assay has a reported sensitivity of 98% and specificity of 96.7% [35]. The assay used 10 μL of sample and was diluted with diluent (1 : 21).

2.4. Data Analysis. Sample to cut-off ratio was calculated, and results were interpreted based on criteria from the manufacturers' instructions. Concordance between assays was determined by calculating Kappa (κ) correlation, which measures the agreement between two assays, using IBM SPSS Statistics 23 (IBM Centre, NSW, Australia).

3. Results

Of the 194 Wantai HEV IgG reactive samples, 92 were reactive with the MP Diagnostics HEV IgG ELISA. One of the 200 negative samples with the Wantai HEV IgG assay tested positive with MP Diagnostics HEV IgG ELISA. There was a poor agreement between these assays (κ = 0.47) (Table 1, Figure 1). However, the agreement between MP Diagnostics total HEV antibody assay and Wantai HEV IgG was higher (κ = 0.65) with 126/194 testing positive (Table 2, Figure 2). All the Wantai HEV IgG negative samples were also negative with MP Diagnostics total HEV antibody assay. Of the 4 Wantai HEV IgM positive samples, none tested positive for HEV IgM on the MP Diagnostics HEV IgM ELISA (Table 3).

Table 2: Comparison of test results between the Wantai HEV IgG ELISA and MP Diagnostics HEV ELISA 4.0 (IgG, IgM, and IgA).

Wantai (HEV IgG)	MP Diagnostics (HEV IgG, IgM, and IgA)		Total
	Positive	Negative	
Positive	126 (64.94%)	68	194
Negative	0	200 (100%)	200
Total	126	268	394

Table 3: Comparison of test results between the Wantai HEV IgM ELISA and MP Diagnostics HEV IgM ELISA 3.0.

Wantai (HEV IgM)	MP Diagnostics (HEV IgM)		Total
	Positive	Negative	
Positive	0	4	4
Negative	5	385 (98.7%)	390
Total	5	389	394

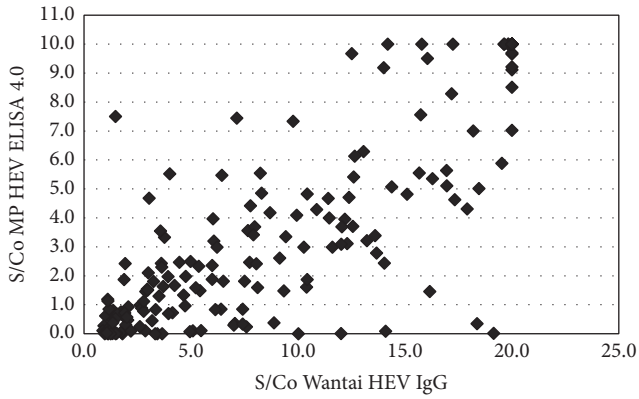


Figure 2: S/Co Wantai HEV IgG versus MP Diagnostics HEV ELISA 4.0 (IgG, IgM, and IgA).

All Wantai HEV IgM positive samples were positive with MP Diagnostics total HEV antibody assay.

Comparing the test results between the MP Diagnostics total HEV antibody ELISA and MP Diagnostics HEV IgG ELISA, 82 of 126 (88.17%) tested positive with the latter (κ = 0.65). However, 11 of the samples that tested negative with MP Diagnostics total HEV antibody ELISA were positive with MP Diagnostics HEV IgG ELISA (Table 4, Figure 3). Of these, 10 samples were positive with Wantai HEV IgG ELISA.

4. Discussion

HEV is a causative agent of acute hepatitis. The majority of HEV cases in developed countries are in travellers returning from developing countries endemic for HEV [25]; however, autochthonous HEV related to zoonotic transmission [2] and transfusion-transmission [17] have also been reported. HEV serological assays have allowed seroprevalence studies, which provide useful surveillance data on the distribution of this virus, and have also assisted with identifying risk factors for exposure to HEV. However, studies have shown variability in

Table 4: Comparison of test results between the MP Diagnostics HEV ELISA (IgG) and MP Diagnostics HEV ELISA 4.0 (IgG, IgM, and IgA).

MP Diagnostics (HEV IgG)	MP Diagnostics (HEV IgG, IgM, and IgA)		Total
	Positive	Negative	
Positive	82 (88.17%)	11	93
Negative	44	257 (85.38%)	301
Total	126	268	394

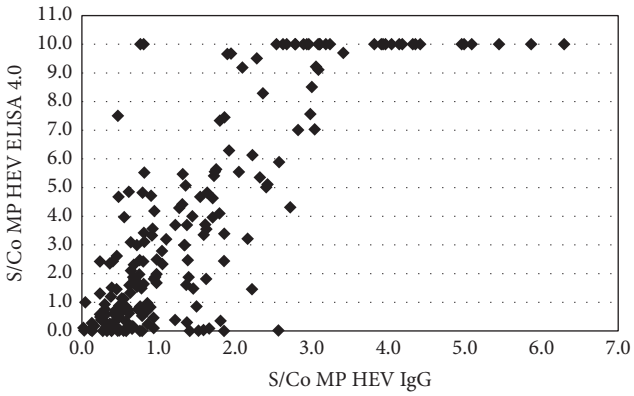


Figure 3: S/Co MP Diagnostics HEV IgG versus MP Diagnostics HEV ELISA 4.0 (IgG, IgM, and IgA).

estimates with different commercial assays [21, 28], and the results presented herein are consistent with such findings.

In this study, a poor concordance of test results between the two tested commercial HEV IgG ELISAs was observed. Only 47% (92/194) of Wantai HEV IgG positive samples were positive with the MP Diagnostics HEV IgG ($\kappa = 0.47$). This observation is similar to a Korean study, which also compared Wantai and Genelabs (now MP Diagnostics) HEV IgG assays ($\kappa = 0.31$) [29]. One of the samples negative with Wantai HEV IgG assay was positive with the MP Diagnostic HEV IgG assay (0.50%), similar to an observation in a French study (0.69%) using a Fortress Diagnostics assay that uses Wantai recombinant proteins [26]. Previous studies have shown that the Wantai HEV IgG ELISA is one of the most sensitive commercial assays available for the detection of HEV IgG [27, 29, 30].

Our study also showed a higher agreement between the Wantai HEV IgG and MP Diagnostic HEV total antibody assay ($\kappa = 0.65$). The total antibody ELISA is more recently developed (compared to the MP Diagnostics HEV IgG assay) incorporating an improved antigen with the ability to detect total antibodies (IgG, IgM, and IgA) against all HEV genotypes [36]. It is possible that the Wantai HEV IgG could have given nonspecific results, but the majority of Wantai HEV IgG negative samples still tested negative with the MP Diagnostic HEV IgG assay (199 of 200). In addition, there was also nonconcordance between MP Diagnostic total and IgG assays. The proportion of samples positive with the MP Diagnostics IgG assay compared to the proportion positive with the MP Diagnostics total assay was unexpected (88%) and therefore questions the performance of the IgG assay assuming that the samples represented true positives.

Comparison of the Wantai HEV IgM and MP Diagnostics HEV IgM assays also showed poor agreement between these assays. A prior study has shown good specificity of the MP Diagnostic HEV IgM assay (99.5%) [37]. However, in our study, one HEV IgM positive sample with the MP Diagnostics HEV IgM assay was negative with MP Diagnostic total antibody assay. All four samples positive with Wantai HEV IgM assay were also positive with MP Diagnostic total antibody assay, demonstrating agreement between these assays.

The observed variability in assay performance could be explained by differences in recombinant proteins, assay formats, or other components (e.g., diluents) used in each assay, as well as sample selection given they were primarily preselected Wantai IgG-positive. Additional studies are required to elucidate the exact mechanism; however, it is clear that a "gold standard" for HEV antibody detection is desperately needed. The validity of serological assays for use in a particular study should be assessed prior to their use, and control samples from individuals diagnosed with HEV should be included wherever possible. Given that neither the infection history nor the exact serostatus (positive or negative based on confirmatory assays) of the samples was known, sensitivity and specificity of these assays could not be assessed in the present study. Thus, the findings of this study should be interpreted considering this limitation. Further studies including pedigreed seropositive/negative samples or those from individuals with a known history of HEV infection are clearly required.

5. Conclusion

In this study, a poor concordance of test results between the Wantai and MP Diagnostics HEV ELISAs was observed. Variability in results was likely due to differences in antigens, assay format, or other components used in each assay, as well as the fact that assumed seropositive samples were primarily preselected Wantai IgG-positive samples. These observations are consistent with previous reports demonstrating significant variability between HEV ELISAs, highlighting that due caution is required when interpreting the results of HEV serology. There is still a need for the development of sensitive, specific, and cost-effective HEV antibody assays, including confirmatory tests, to aid in estimating disease burden and determining risk factors for HEV exposure.

Competing Interests

All authors have disclosed no conflict of interests.

Acknowledgments

The authors thank Australian Red Cross Blood Service staff in Donor Services and Manufacturing for assisting with sample collection and J. Fryk for technical assistance. Australian governments fund the Australian Red Cross Blood Service to

provide blood, blood products, and services to the Australian community.

References

[1] X. Meng, D. Anderson, V. Arankalle et al., "Hepeviridae," in *Virus Taxonomy, 9th Report of the ICTV*, pp. 1021–1028, Elsevier Academic Press, London, UK, 2012.

[2] N. Kamar, R. Bendall, F. Legrand-Abravanel et al., "Hepatitis E," *The Lancet*, vol. 379, no. 9835, pp. 2477–2488, 2012.

[3] D. B. Smith, M. A. Purdy, and P. Simmonds, "Genetic variability and the classification of hepatitis E virus," *Journal of Virology*, vol. 87, no. 8, pp. 4161–4169, 2013.

[4] H. C. Worm, W. H. M. van der Poel, and G. Brandstätter, "Hepatitis E: an overview," *Microbes and Infection*, vol. 4, no. 6, pp. 657–666, 2002.

[5] M. S. Balayan, A. G. Andjaparidze, S. S. Savinskaya et al., "Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route," *Intervirology*, vol. 20, no. 1, pp. 23–31, 1983.

[6] G. R. Reyes, M. A. Purdy, J. P. Kim et al., "Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis," *Science*, vol. 247, no. 4948, pp. 1335–1339, 1990.

[7] R. Aggarwal, "Clinical presentation of hepatitis E," *Virus Research*, vol. 161, no. 1, pp. 15–22, 2011.

[8] E. H. Teshale, D. J. Hu, and S. D. Holmberg, "The two faces of hepatitis E virus," *Clinical Infectious Diseases*, vol. 51, no. 3, pp. 328–334, 2010.

[9] A. Chauhan, J. B. Dilawari, Y. K. Chawla, S. Jameel, U. Kaur, and N. K. Ganguly, "Hepatitis E virus transmission to a volunteer," *The Lancet*, vol. 341, no. 8838, pp. 149–150, 1993.

[10] I. K. Mushahwar, "Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention," *Journal of Medical Virology*, vol. 80, no. 4, pp. 646–658, 2008.

[11] A. C. Shrestha, H. M. Faddy, R. L. P. Flower, C. R. Seed, and A. J. Keller, "Hepatitis E virus: do locally acquired infections in Australia necessitate laboratory testing in acute hepatitis patients with no overseas travel history?" *Pathology*, vol. 47, no. 2, pp. 97–100, 2015.

[12] N. Kamar, J. Selves, J.-M. Mansuy et al., "Hepatitis E virus and chronic hepatitis in organ-transplant recipients," *The New England Journal of Medicine*, vol. 358, no. 8, pp. 811–817, 2008.

[13] H. R. Dalton, R. P. Bendall, F. E. Keane, R. S. Tedder, and S. Ijaz, "Persistent carriage of hepatitis E virus in patients with HIV infection," *The New England Journal of Medicine*, vol. 361, no. 10, pp. 1025–1027, 2009.

[14] P. Le Coutre, H. Meisel, J. Hofmann et al., "Reactivation of hepatitis E infection in a patient with acute lymphoblastic leukaemia after allogeneic stem cell transplantation," *Gut*, vol. 58, no. 5, pp. 699–702, 2009.

[15] U. Navaneethan, "Seroprevalence of hepatitis E infection in pregnancy—more questions than answers," *Indian Journal of Medical Research*, vol. 130, no. 6, pp. 677–679, 2009.

[16] M. S. Khuroo, M. R. Teli, S. Skidmore, M. A. Sofi, and M. I. Khuroo, "Incidence and severity of viral hepatitis in pregnancy," *The American Journal of Medicine*, vol. 70, no. 2, pp. 252–255, 1981.

[17] P. E. Hewitt, S. Ijaz, S. R. Brailsford et al., "Hepatitis e virus in blood components: a prevalence and transmission study in southeast England," *The Lancet*, vol. 384, no. 9956, pp. 1766–1773, 2014.

[18] J. M. Mansuy, K. Saune, H. Rech et al., "Seroprevalence in blood donors reveals widespread, multi-source exposure to hepatitis E virus, southern France, October 2011," *Euro Surveillance*, vol. 20, no. 19, pp. 27–34, 2015.

[19] F. Ren, C. Zhao, L. Wang et al., "Hepatitis E virus seroprevalence and molecular study among blood donors in China," *Transfusion*, vol. 54, no. 3, pp. 910–917, 2014.

[20] A. C. Shrestha, C. R. Seed, R. L. P. Flower et al., "Hepatitis E virus and implications for blood supply safety, Australia," *Emerging Infectious Diseases*, vol. 20, no. 11, pp. 1940–1942, 2014.

[21] J.-M. Mansuy, R. Bendall, F. Legrand-Abravanel et al., "Hepatitis E virus antibodies in blood donors, France," *Emerging Infectious Diseases*, vol. 17, no. 12, pp. 2309–2312, 2011.

[22] T. M. Ghabrah, S. Tsarev, P. O. Yarbough, S. U. Emerson, G. T. Strickland, and R. H. Purcell, "Comparison of tests for antibody to hepatitis E virus," *Journal of Medical Virology*, vol. 55, no. 2, pp. 134–137, 1998.

[23] R. Bendall, V. Ellis, S. Ijaz, P. Thurairajah, and H. R. Dalton, "Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response," *Journal of Medical Virology*, vol. 80, no. 1, pp. 95–101, 2008.

[24] M. Sultan Khuroo, S. Kamili, M. Yousuf Dar, R. Moecklii, and S. Jameel, "Hepatitis E and long-term antibody status," *The Lancet*, vol. 341, no. 8856, p. 1355, 1993.

[25] H. R. Dalton, R. Bendall, S. Ijaz, and M. Banks, "Hepatitis E: an emerging infection in developed countries," *The Lancet Infectious Diseases*, vol. 8, no. 11, pp. 698–709, 2008.

[26] A. Schnegg, P. Bürgisser, C. André et al., "An analysis of the benefit of using HEV genotype 3 antigens in detecting anti-HEV IgG in a European Population," *PLoS ONE*, vol. 8, no. 5, article e62980, 2013.

[27] R. Bendall, V. Ellis, S. Ijaz, R. Ali, and H. Dalton, "A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries," *Journal of Medical Virology*, vol. 82, no. 5, pp. 799–805, 2010.

[28] B. H. M. Meldal, F. Sarkodie, S. Owusu-Ofori, and J.-P. Allain, "Hepatitis E virus infection in Ghanaian blood donors—the importance of immunoassay selection and confirmation," *Vox Sanguinis*, vol. 104, no. 1, pp. 30–36, 2013.

[29] H. K. Park, S. Jeong, J. Kim et al., "Seroprevalence of anti-hepatitis E virus (HEV) in a Korean population: comparison of two commercial anti-HEV assays," *BMC Infectious Diseases*, vol. 12, no. 1, article 142, 2012.

[30] J. J. Wenzel, J. Preiss, M. Schemmerer, B. Huber, and W. Jilg, "Test performance characteristics of anti-HEV IgG assays strongly influence hepatitis e seroprevalence estimates," *Journal of Infectious Diseases*, vol. 207, no. 3, pp. 497–500, 2013.

[31] J. Drobeniuc, J. Meng, G. Reuter et al., "Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances," *Clinical Infectious Diseases*, vol. 51, no. 3, pp. e24–e27, 2010.

[32] Wantai HEV-IgG ELISA, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., http://www.ystwt.cn/IFU/HEV/HEV-IgG_CE.pdf.

[33] Q. Yan, H. Du, Y. Wang et al., "Comparison of two diagnostic reagents to detect anti-hepatitis E virus IgG antibodies," *Chinese Journal of Zoonoses*, vol. 24, pp. 1087–1089, 2008.

[34] Wantai HEV-IgM ELISA, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, http://www.ystwt.cn/IFU/HEV/HEV-IgM_CE.pdf.

[35] MP Biomedicals, USA, http://www.mpbio.com/featured.php?fid=25&country=13.

[36] M. K. Yong, E. K. Paige, D. Anderson, and J. F. Hoy, "Hepatitis E in Australian HIV-infected patients: an under-recognised pathogen?" *Sexual Health*, vol. 11, no. 4, pp. 375–378, 2014.

[37] F. Legrand-Abravanel, I. Thevenet, J. M. Mansuy et al., "Good performance of immunoglobulin M assays in diagnosing genotype 3 hepatitis E virus infections," *Clinical and Vaccine Immunology*, vol. 16, no. 5, pp. 772–774, 2009.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 2920514, 11 pages
http://dx.doi.org/10.1155/2016/2920514

Research Article

A New Proof of Concept in Bacterial Reduction: Antimicrobial Action of Violet-Blue Light (405 nm) in *Ex Vivo* Stored Plasma

Michelle Maclean,[1,2] John G. Anderson,[1] Scott J. MacGregor,[1] Tracy White,[1] and Chintamani D. Atreya[3]

[1] *The Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST), Department of Electronic and Electrical Engineering, University of Strathclyde, 204 George Street, Glasgow, UK*
[2] *Department of Biomedical Engineering, University of Strathclyde, Wolfson Centre, 106 Rottenrow, Glasgow, UK*
[3] *Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA*

Correspondence should be addressed to Chintamani D. Atreya; chintamani.atreya@fda.hhs.gov

Received 11 July 2016; Accepted 28 August 2016

Academic Editor: William P. Sheffield

Bacterial contamination of injectable stored biological fluids such as blood plasma and platelet concentrates preserved in plasma at room temperature is a major health risk. Current pathogen reduction technologies (PRT) rely on the use of chemicals and/or ultraviolet light, which affects product quality and can be associated with adverse events in recipients. 405 nm violet-blue light is antibacterial without the use of photosensitizers and can be applied at levels safe for human exposure, making it of potential interest for decontamination of biological fluids such as plasma. As a pilot study to test whether 405 nm light is capable of inactivating bacteria in biological fluids, rabbit plasma and human plasma were seeded with bacteria and treated with a 405 nm light emitting diode (LED) exposure system (patent pending). Inactivation was achieved in all tested samples, ranging from low volumes to prebagged plasma. 99.9% reduction of low density bacterial populations ($\leq 10^3$ CFU mL^{-1}), selected to represent typical "natural" contamination levels, was achieved using doses of 144 Jcm^{-2}. The penetrability of 405 nm light, permitting decontamination of prebagged plasma, and the nonrequirement for photosensitizing agents provide a new proof of concept in bacterial reduction in biological fluids, especially injectable fluids relevant to transfusion medicine.

1. Introduction

Bacterial contamination of *ex vivo* stored injectable biological fluids such as blood and blood components preserved in plasma is a major complication for transfusion medicine, resulting in both wasteful discarding of valuable blood products and, more significantly, health risks for recipients of contaminated donor blood [1, 2]. Major progress has been made in the provision of a safe supply of blood components, and measures such as more effective donor screening, extensive laboratory testing protocols, and the application of bacterial reduction methods have significantly reduced the risk of transfusion-transmitted bacterial infections [1–3]. Nevertheless, the risk of bacterial transmission has not been completely eliminated and there is a recognised need for continued research to improve the efficacy of these methods and to minimise incidental adverse changes in biological fluids, such as cellular blood components preserved in plasma, which can compromise product quality and safety [4–6].

A number of bacterial reduction methods have been developed for plasma treatment, and pathogen reduced plasma is routinely used [7], with several of these methods now licensed for use in North America and Europe [5]. The original methods developed for plasma treatment included the use of solvent/detergent and methylene blue in combination with visible light [8–11]. More recently, developed methods have employed ultraviolet (UV) light. Exposure to amotosalen (S-59) plus long-wave ultraviolet (UVA) light [12, 13] and treatment with riboflavin and UV light [7, 14] have been developed to treat both plasma and platelets.

Whilst light-based processes have typically used photosensitive chemicals to generate microbicidal effects, a UV-C-based pathogen reduction system without a requirement for photoactive substances has been developed and is undergoing clinical efficacy and safety testing [15–17].

It is generally accepted that all these methods have limitations [5, 7], and because the full extent of future microbiological challenges cannot be predicted, pathogen reduction technologies will remain an active area of investigation in transfusion medicine well into the future [1, 4].

Here, we report the first proof-of-concept results on the use of a novel visible violet-blue light method that does not require the addition of photosensitive chemicals for inactivation of bacterial pathogens in plasma. This method utilises light with a peak wavelength of 405 nm, which causes photoexcitation of endogenous microbial porphyrin molecules and oxidative damage through reactive oxygen species [18]. 405 nm light has previously been shown to inactivate a wide range of bacterial pathogens in laboratory tests [19–28] as well as in hospital settings with use as an environmental disinfection technology [29–31] and also potential for wound decontamination applications in clinical settings [32–34]. An advantage of this technology over UV light for certain applications is that, even at irradiance values and dose levels that are bactericidal, it can be applied safely for human exposure. Therefore, we envisioned that this feature makes 405 nm light of potential interest for decontamination of injectable stored biological fluids such as blood plasma or plasma containing cellular blood components. Tests on bacterial-seeded plasma were carried out on both small-scale liquid samples and artificially contaminated prebagged plasma. Direct treatment of prebagged plasma was facilitated by the highly transmissible properties of 405 nm light, and the bacterial inactivation results obtained using this novel approach are described for the first time in this paper.

2. Materials and Methods

2.1. Bacterial Cultures. The organisms used in this study were *Staphylococcus aureus* NCTC 4135, *Staphylococcus epidermidis* NCTC 11964, and *Escherichia coli* NCTC 9001. Cultures were obtained from the National Collection of Type Cultures (NCTC), Colindale, UK. For experimental use, bacteria were cultured in 100 mL nutrient broth at 37°C under rotary conditions (120 rpm) for 18 h. Broths were centrifuged at 3939 ×g for 10 minutes and the pellet was resuspended in 100 mL phosphate buffered saline (PBS) and serially diluted to obtain the required cell density (colony-forming units per millilitre, CFU mL^{-1}) for experimental use. All culture media were sourced from Oxoid Ltd. (UK).

2.2. Plasma. Lyophilised rabbit plasma (LRP020, E&O Laboratories, UK) was reconstituted using sterile distilled water. Fresh frozen human plasma (approximately 300 mL bag volume) was obtained from the Scottish National Blood Transfusion Service (SNBTS, UK) and defrosted before experimental use. Study involving human subjects protocol was approved by FDA Risk Involved in Human Subjects Committee (RIHSC, Exemption Approval # 11-036B) and by the University of Strathclyde Ethics Committee (letter dated 10 February 2011). Rabbit plasma and human plasma suspensions were seeded with known concentrations of bacterial contaminants by adding bacterial-PBS suspension to the plasma.

2.3. 405-nm Light Source. The 405 nm light sources used were rectangular arrays of 99 LEDs in an 11 × 9 matrix (Opto Diode Corp., USA). The array had a centre wavelength close to 405 nm, with a bandwidth of approximately 10 nm at full width at half maximum (FWHM). The LED array was powered by a direct current supply, and, for thermal management, the LED array was bonded to a heat sink and fan, thus ensuring that heating had no effect on the test samples exposed to the 405 nm light (device patent pending [35]).

2.4. 405 nm Antimicrobial Light Treatment. Three arrangements were employed for exposure of three different sample volumes: 3 mL, 30 mL, and approximately 300 mL (whole plasma transfusion bags). For exposure of 3 mL sample volumes, the samples were held in the well of a 12-well microplate (without the lid), and the LED array was mounted in a polyvinyl chloride (PVC) housing which positioned the array approx. 3 cm directly above the sample. Irradiance at the sample surface was measured to be approximately 100 $mWcm^{-2}$ (measured by using a radiant power meter and photodiode detector; LOT-Oriel Ltd.).

For exposure of 30 mL sample volumes, the human plasma was held in a sterile 90 mm Petri dish with the lid on. The LED array was positioned 8 cm directly above the closed sample dish, providing irradiance of approximately 8 $mWcm^{-2}$, through the lid, at the centre of the sample dish.

For exposure of plasma bags, a test rig was constructed which held two 405 nm LED arrays at a distance of 12 cm above the horizontally positioned plasma bag. This arrangement provided irradiance of approximately 5 $mWcm^{-2}$ at the centre position of the plasma bag, taking into account a 20% reduction in irradiance as the light transmits through the bag layer. In order to investigate the influence of higher irradiance on bacterial inactivation, plasma bags were also exposed using irradiance of 16 and 48 $mWcm^{-2}$. This higher irradiance was achieved by using two high-power 405 nm LED arrays (PhotonStar Technology, UK), with 14 nm FWHM.

All experimental systems were held in a shaking incubator (72 rpm; 25°C) to allow continuous sample agitation and maintain exposure conditions. Samples seeded with bacterial contamination were treated with increasing exposures of 405 nm light. Control samples were held in identical conditions but shielded from the 405 nm light.

The optical profiles of the light distribution across the Petri dishes and transfusion bags (plotted using MATLAB R2012b software) demonstrate the nonuniform irradiance of the plasma (Figures 2(a) and 3(a)); however, continuous agitation of the plasma samples during treatment ensures uniform mixing of the plasma contaminants. Negligible variation was recorded across the 22 mm diameter of the 3 mL samples.

2.5. Determination Whether Light Induces Toxicity within Human Plasma. To ensure that bacterial inactivation was not the result of the plasma becoming toxic upon exposure to 405 nm light, *S. aureus* (1×10^3 CFU mL^{-1}) was seeded into 3 mL plasma that had been preexposed to 1.08 kJcm^{-2} 405 nm light at irradiance of 100 mWcm^{-2} (the highest dose employed in the present study) and samples were taken at 30 min intervals for up to 3 hr.

2.6. Bacterial Enumeration. Following 405 nm light exposure, samples were either plated onto nutrient agar using an automatic spiral plater (Don Whitley Scientific, UK) or manually spread by using sterile L-shaped spreaders, depending on the expected population density of the samples. Sample plates were incubated at 37°C for 24 hours and then enumerated with the surviving bacterial load reported as colony-forming units per millilitre (CFU mL^{-1}).

2.7. Inactivation Data Analysis. Results are reported as surviving bacterial load ($\log_{10}$ CFU mL^{-1}) as a function of dose and are presented as mean values from triplicate independent experiments ($n = 3$). Dose (J cm^{-2}) is calculated as the product of the irradiance (W cm^{-2}) multiplied by the exposure time (sec), with the irradiance value being the maximum measured at the centre position of the sample dish/bag. Significant differences in the results were identified using 95% confidence intervals and one-way analysis of variance (ANOVA) with Minitab software Release 16. For dose response curves the dose that reduces $\log_{10}$ CFU count at 0 dose by 50% was estimated. This 50% $\log_{10}$ reduction was estimated using curve fitting software (GraphPad Prism V6) and quadratic or 5PL variable slope sigmoidal curves with R-squared fits in excess of 90%.

2.8. Optical Analysis of Plasma. The transmission values for rabbit plasma and human plasma, PBS, and the blood bag material were measured by using a BioMate 5 UV-Visible Spectrophotometer (Thermo Spectronic). Analysis was carried out in the wavelength range of 220–700 nm. Fluorescence spectrophotometry (RF-5301 PC spectrofluorophotometre; Shimadzu, US) was used to determine whether plasma or PBS contained photosensitive components which could be excited by 405 nm light. Excitation was carried out at 405 nm and emission spectra were recorded between 500 and 700 nm.

3. Results

3.1. Inactivation of Microbial Contaminants in 3 mL PBS and Plasma. Results from the exposure of PBS, rabbit plasma, and human plasma seeded with bacterial contamination (10^5 CFU mL^{-1}) to 100 mWcm^{-2} 405 nm light are presented in Figure 1. Results demonstrate that bacterial inactivation in PBS is achieved using the lowest dose. Data for *S. aureus* (Figure 1(a)) show that near complete inactivation (<10 CFU mL^{-1} surviving) of the organism in PBS was achieved after exposure to a dose of 60 Jcm^{-2}. To achieve a comparable reduction in rabbit plasma and human plasma, 4.5 times the dose was required (270 Jcm^{-2} compared to 60 Jcm^{-2}). 50% $\log_{10}$ reductions were estimated to occur at doses of 23, 224, and 181 Jcm^{-2} for PBS, rabbit plasma, and human plasma, respectively.

Similar inactivation kinetics was observed for *S. epidermidis* (Figure 1(b)), although this species showed comparatively greater susceptibility to 405 nm light when exposed in plasma. The 50% $\log_{10}$ reductions were obtained in PBS, rabbit plasma, and human plasma at 36, 121, and 174 Jcm^{-2} respectively. Reduction of *E. coli* contamination required markedly increased doses (Figure 1(c)). The 50% $\log_{10}$ reductions required doses of 328, 585, and 742 Jcm^{-2} for PBS, rabbit serum, and human serum, respectively. For inactivation in PBS, 450 Jcm^{-2} was required for near complete inactivation (<10 CFU mL^{-1} surviving): 7.5 times more than observed with the staphylococci. Inactivation of *E. coli* contamination in plasma again required increased doses compared to suspension in PBS, with a 5-$\log_{10}$ reduction in human plasma achieved after a dose of 1.08 kJcm^{-2}.

3.2. Determination of Light Induced Toxicity within Human Plasma. No significant change in the seeded 10^3 CFU mL^{-1} population [$P = 0.663$] was evident in the bacterial contamination added to plasma after exposure, thus indicating no residual toxicity in 405 nm light-exposed plasma which could induce the inactivation of microbial contaminants.

3.3. Inactivation of Contaminants in Larger Volumes of Human Plasma

3.3.1. 30 mL Volume in Covered Sample Dish. Figure 2 demonstrates the inactivation of low density *S. aureus* contamination in 30 mL plasma in a closed Petri dish using irradiance of ~8 mWcm^{-2}. Results for a seeding density of 10^3 CFU mL^{-1} (Figure 2(b)) demonstrate that exposure to doses of greater than 100.8 Jcm^{-2} caused significant inactivation of the contamination [$P = 0.030$], with near complete inactivation achieved with 230.4 Jcm^{-2}. Control contamination levels rose significantly by approximately 1-$\log_{10}$ over the course of the experiment [$P < 0.001$]. Similar results were observed for inactivation of the 10^2 CFU mL^{-1} contamination levels (Figure 2(c)): significant inactivation became evident after exposure to a dose of 115.2 Jcm^{-2} [$P = 0.009$], with near complete inactivation achieved with 187.2–230.4 Jcm^{-2}. Control contamination levels remained relatively unchanged [$P = 0.255$]. Significant inactivation of a 10^1 CFU mL^{-1} seeding population was shown after a dose of 115.2 Jcm^{-2} [$P = 0.031$], with near complete inactivation achieved by exposure to doses of 201.6–230.4 Jcm^{-2} (Figure 2(d)). Control contamination levels showed no significant change compared to the exposed samples [$P = 0.054$].

3.3.2. Decontamination of Plasma in a Blood Bag. Inactivation of low density (10^1–10^2 CFU mL^{-1}) bacterial contaminants within plasma transfusion bags was achieved using irradiance as low as 5 mWcm^{-2} (Figure 3(b)). A notable downward trend in contamination was observed after exposure to 108 Jcm^{-2}, with a significant 0.6 $\log_{10}$ reduction

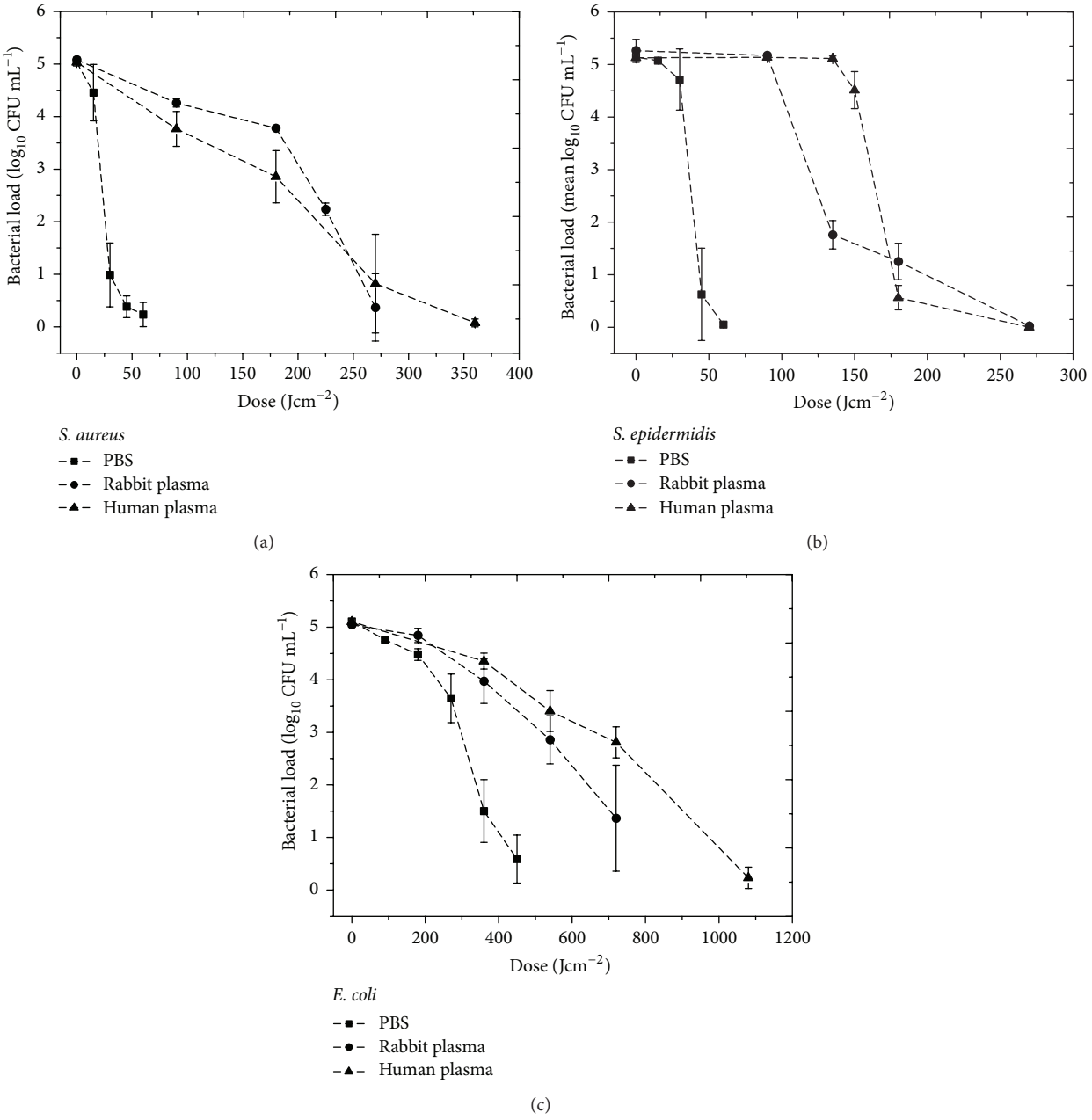


Figure 1: Inactivation of bacterial contamination: (a) *S. aureus*, (b) *S. epidermidis*, and (c) *E. coli*, in phosphate buffered saline (PBS) rabbit plasma and human plasma by exposure to 405 nm light with irradiance of approximately 100 mWcm^{-2} ($n = 3 \pm$ SD). Nonexposed control samples for all experiments demonstrated no significant change in population over the exposure period [$P \geq 0.05$].

in contamination [$P \leq 0.001$]. Complete/near complete inactivation was achieved after exposure to 144 Jcm^{-2} [$P = 0.017$]. This slightly reduced inactivation rate, compared to that found within the sample dishes, is due to the lower irradiance light being used for exposure. Contamination levels in the control plasma bags rose by approximately 0.5-$\log_{10}$ [$P = 0.052$]. Similar inactivation kinetics was obtained for seeded transfusion bags exposed to irradiance of 16 and 48 mWcm^{-2}, with contamination levels decreasing upon exposure to increasing treatment. Comparison of the results for the three irradiance levels used demonstrated that when looking at exposure time (Figure 4(a)) the decontamination effect observed with 16 and 48 mWcm^{-2} is relatively comparable, with inactivation being slightly slower when using the lowest irradiance of 5 mWcm^{-2}. However, when looking at the actual dose levels applied (Figure 4(b)), it is apparent that the germicidal efficiency (defined as $\log_{10}$ reduction of a bacterial population [$\log_{10}(N/N_0)$] by inactivation per unit dose in Jcm^{-2} [23]) of the 5 mWcm^{-2} irradiance is greater than that of the irradiance of 16 and 48 mWcm^{-2} ($P = 0.007$ and 0.013, resp.). Comparison of exposure to doses in the region of 140–180 Jcm^{-2} highlights this difference in efficacy,

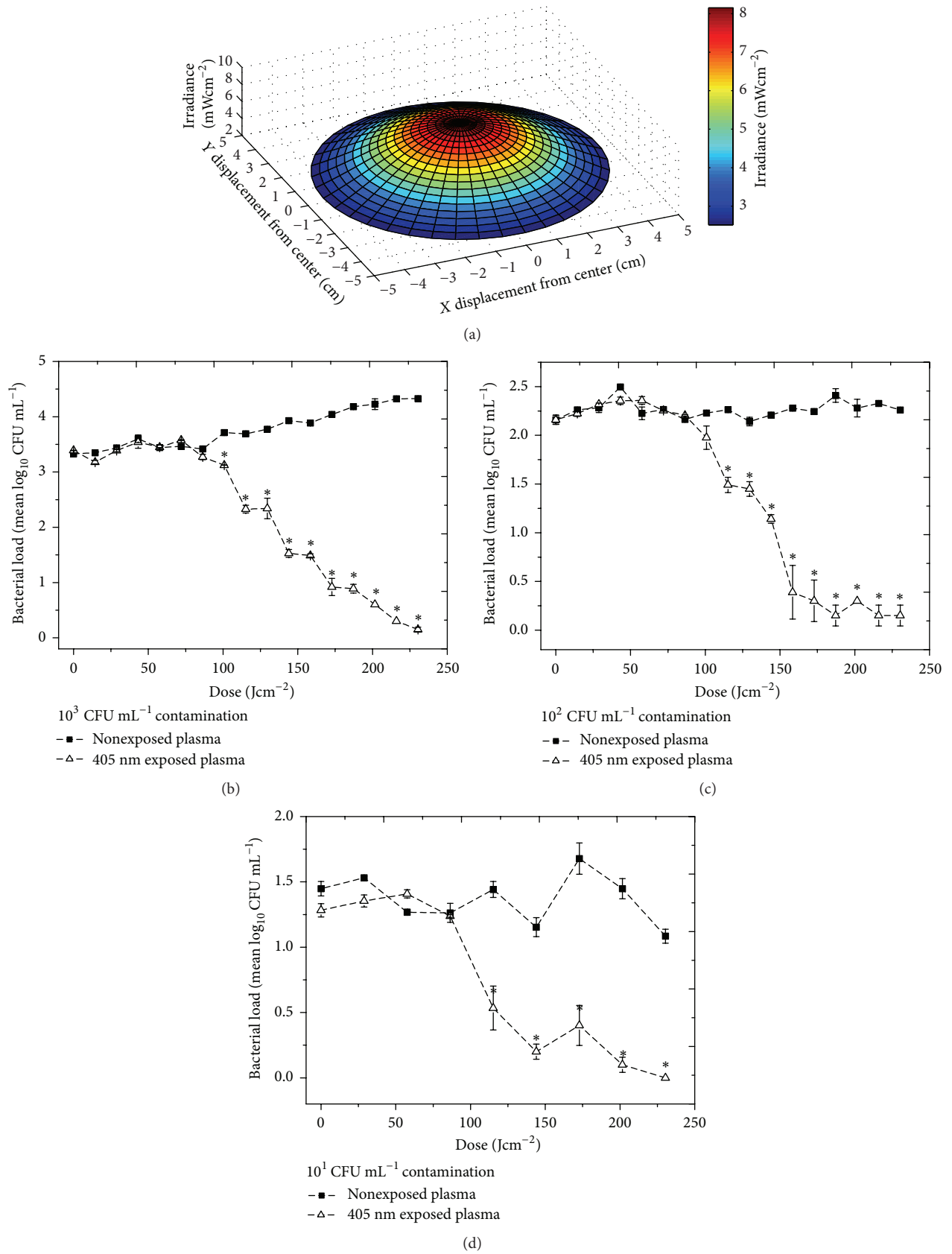


FIGURE 2: Inactivation of *S. aureus* contamination in 30 mL volumes of human plasma held in a closed sample dish by exposure to 405 nm light. (a) Three-dimensional model demonstrating the irradiance profile across the sample dish, with irradiance of ~8 mWcm^{-2} at the centre. Populations of (b) 10^3, (c) 10^2, and (d) 10^1 CFU mL^{-1} were used as the seeding densities ($n = 3 \pm$ SE). Asterisks (∗) represent data points, where the bacterial levels in light-exposed plasma were significantly different from the equivalent nonexposed control [$P \leq 0.05$].

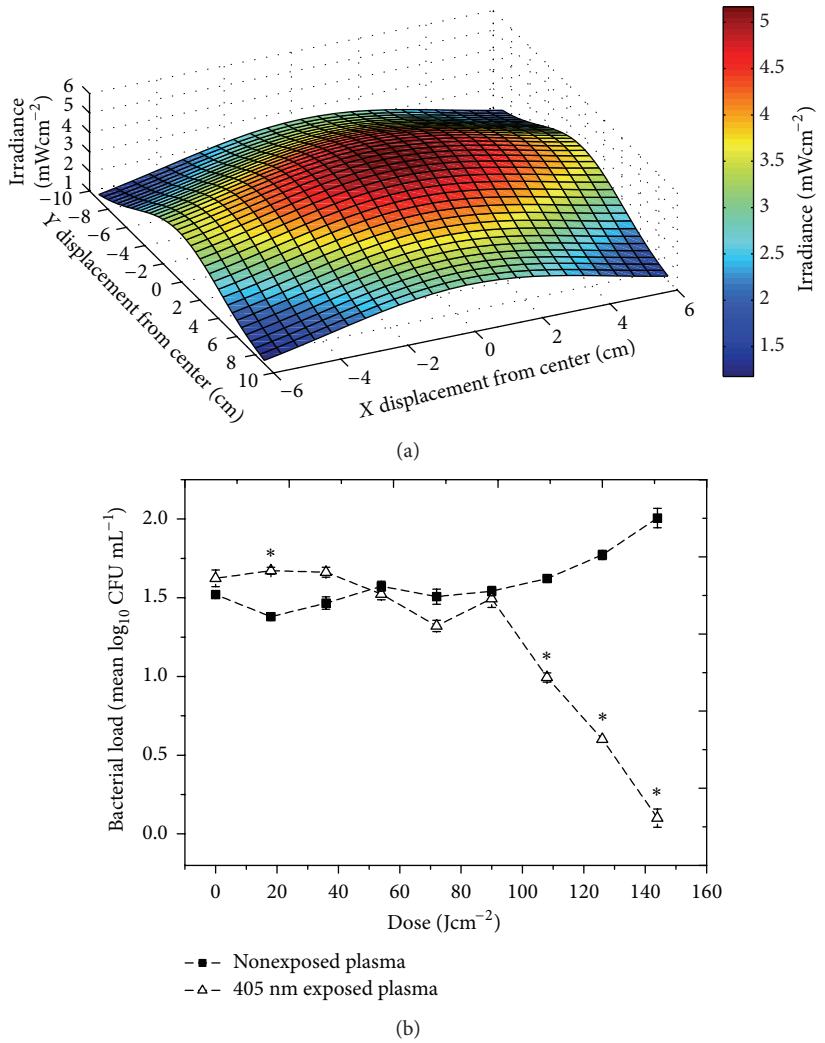


FIGURE 3: 405 nm light exposure of contaminated human plasma transfusion bags. (a) Three-dimensional model demonstrating the irradiance profile across the plasma bag, with irradiance of ~5 mWcm^{-2} at the centre. (b) Inactivation of *S. aureus* contamination in 300 mL bags of human plasma by exposure to 405 nm light ($n = 3 \pm$ SE). Asterisks ($*$) represent data points, where the bacterial levels in light-exposed plasma were significantly different from the equivalent nonexposed control [$P \leq 0.05$].

with a 1.91 log$_{10}$ reduction being achieved after exposure to 5 mWcm^{-2} for 8 h (144 Jcm^{-2}), a 1.14 log$_{10}$ reduction being achieved after exposure to 16 mWcm^{-2} for 3 h (172.8 Jcm^{-2}), but only a 0.08 log$_{10}$ reduction observed after 1 h exposure to 48 mWcm^{-2} (172.8 Jcm^{-2}).

3.4. Optical Analysis of Plasma. Spectrophotometric analysis shows that transmission of 405 nm light through plasma is low (1-2%) compared with transparent PBS (99%), and this correlates with the longer exposure times/increased doses required for comparative microbial inactivation in plasma compared to PBS. Figure 5(a) highlights the transmissibility of the Petri dish material and the blood bag, with results showing that 405 nm light can transmit through these materials, thus permitting decontamination of the blood plasma whilst being contained in the sample dish and blood bag. The fluorescence emission spectra of rabbit plasma and human plasma and PBS demonstrated that excitation of the suspensions at 405 nm produced no prominent fluorescence emission peaks between 500 and 700 nm (Figure 5(b)).

4. Discussion

In order to assess the potential of 405 nm light for decontamination of blood plasma, the penetrability and antimicrobial efficacy of 405 nm light in plasma required evaluation, and the aim of this study was to determine the antibacterial effects of 405 nm light at varying irradiance on bacteria seeded in blood plasma ranging from small volume samples up to prebagged plasma.

Initial investigation of the inactivation of bacterial contaminants in low volume (3 mL) plasma samples using 100 mWcm^{-2} 405 nm light demonstrated that successful inactivation could be achieved in both rabbit plasma and human plasma. Significantly greater doses were required for inactivation of bacterial contaminants when being suspended in plasma compared to PBS, and this is accredited to the

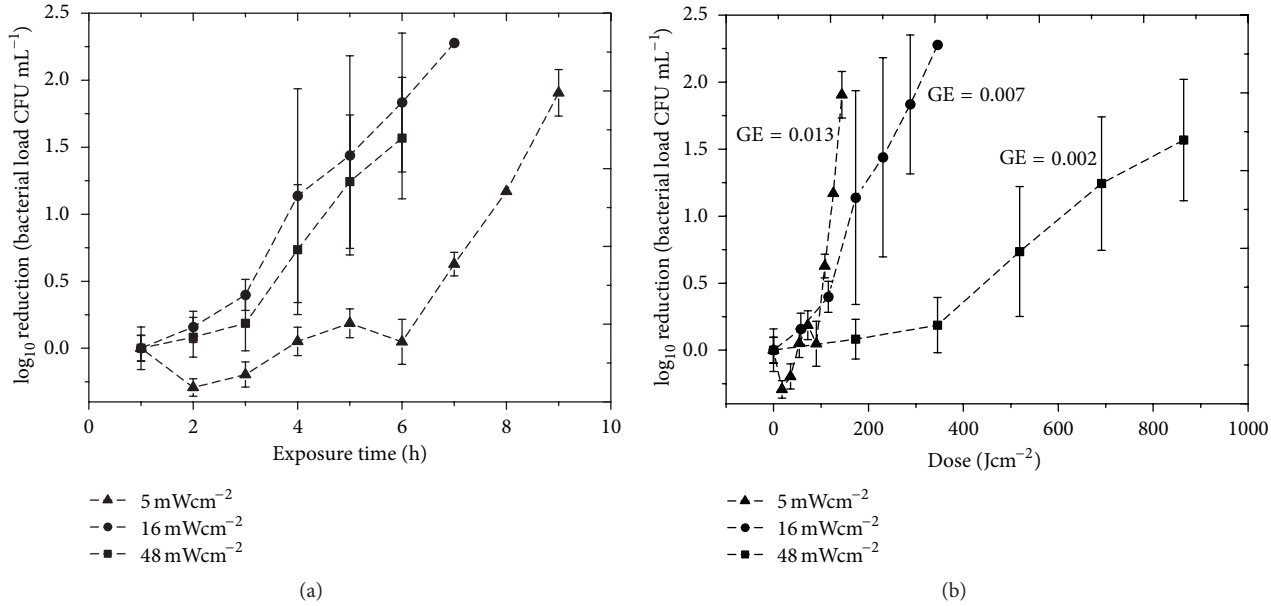


FIGURE 4: Comparison of the exposure times (a) and doses (b) required for inactivation of *S. aureus* contamination in human plasma transfusion bags. (a) Inactivation kinetics was achieved utilising irradiance of 5, 16, and 48 mWcm^{-2} at the centre of the bags. Results are presented as $\log_{10}$ reduction (CFU mL^{-1}) as compared to the equivalent nonexposed control samples ($n = 3 \pm$ SD). Germicidal efficiency (GE) values for each irradiance are shown in (b). (GE is defined as $\log_{10}$ reduction of a bacterial population $[\log_{10}(N/N_0)]$ by inactivation per unit dose in Jcm^{-2}).

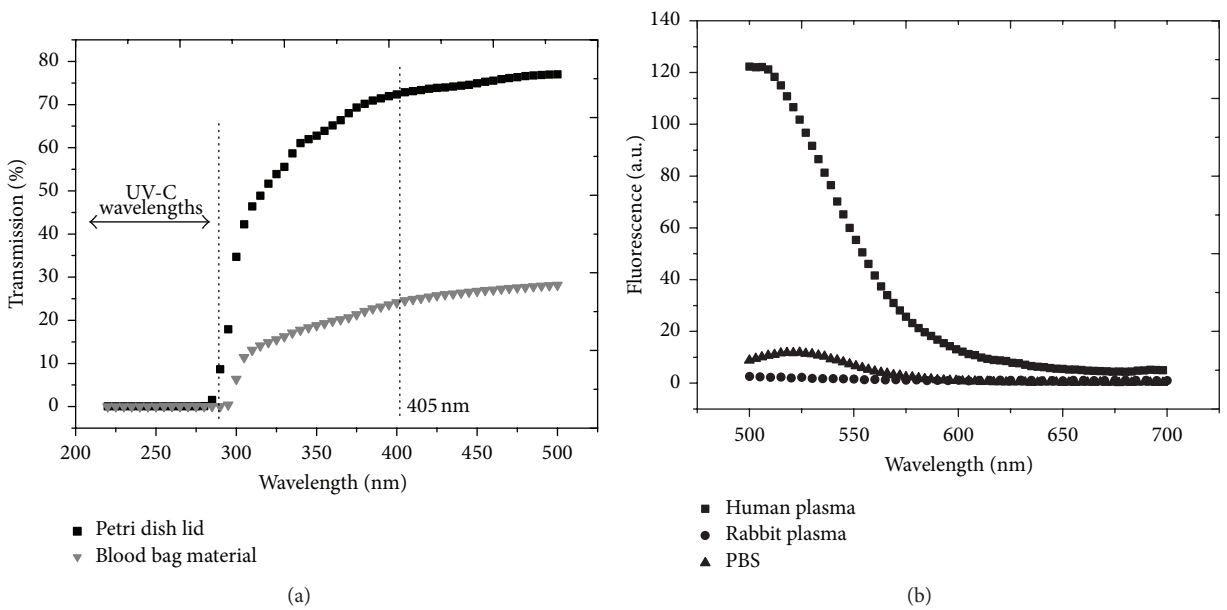


FIGURE 5: Optical analysis. (a) Transmission properties of the Petri dish and blood bag material, highlighting 405 nm and UV-C light wavelengths for reference. (b) Fluorescence emission spectra of PBS and plasma (500–700 nm), detected using an excitation wavelength of 405 nm.

differing optical properties of these suspending media. The opacity, and consequent low transmissibility of plasma (Figure 5(a)), reduces photon penetration through the suspension, resulting in the requirement for greater doses, compared with suspension in clear, transparent liquids such as PBS. Despite this, these proof-of-principle results demonstrate that significant inactivation of bacterial contaminants in human plasma can be achieved; and the higher the irradiance of light applied, the shorter the exposure time required for successful inactivation.

Despite the optical transmission properties of rabbit plasma and human plasma being relatively similar, slight

differences were recorded between the susceptibilities of the bacterial contaminants when seeded in these media. This is likely due to the batch-to-batch variation in color and opacity of the rabbit plasma and, in particular, the human plasma. Indeed, optical analysis of a number of human plasma bag samples ($n = 30$) demonstrated variation in transmission at 405 nm between 0.2 and 25% (Maclean, Anderson, MacGregor, and Atreya; unpublished data). This is likely the reason for the large standard deviation in some of the data points in the inactivation kinetics for the prebagged plasma.

The bacterial species used in this study were selected to represent significant contaminants associated with blood components [3]. Although only three organisms were utilised, the wide antimicrobial efficacy of 405 nm light has been reported in a number of publications [20, 22, 23, 25, 36]. It is therefore expected that these organisms would also be successfully inactivated by 405 nm light when suspended in plasma. Typically, Gram-positive bacteria tend to have greater susceptibility to 405 nm light than Gram-negative bacteria [23], and this is consistent with the results reported here, with approximately 4 times greater dose required to inactivate *E. coli* in plasma, compared to the staphylococci. Interestingly, the difference between the susceptibilities of the staphylococci and *E. coli* was less pronounced when suspended in plasma compared to in PBS (4 versus 7.5 times the dose required).

The initial exposure tests in this study to establish proof of principle utilised low volumes of plasma seeded with high population densities of bacterial contaminants at a level of 10^5 CFU mL^{-1}. A more realistic scenario involves larger volumes of plasma contaminated with low microbial densities. Indeed, it has been reported that the levels of naturally occurring bacterial contamination in plasma are likely to be as low as 10–100 bacterial cells per product at the beginning of storage [37]. Accordingly, experiments were scaled up 10-fold and 100-fold using larger plasma volumes seeded with bacterial contamination levels down to 10^1 CFU mL^{-1}, using *S. aureus* as the model organism. Results demonstrated that bacterial contamination levels, even less than 10 CFU mL^{-1}, can be significantly reduced in larger volumes of plasma by exposure to 405 nm light. It was interesting to note that when using similar irradiance values the bacterial inactivation rates in the 30 mL and 300 mL samples were very similar (~1.5 $\log_{10}$ reductions with a dose of 144 Jcm^{-2}—Figures 2(c) and 3(b)) despite the 10-fold difference in sample volume. Although the sample volumes were different, the depths of plasma were similar (~1-2 cm in both cases), thus indicating that when using similar irradiance values it is the depth of plasma that is likely to influence the light inactivation efficacy rather than the overall sample volume. Also, results demonstrated that use of lower irradiance is likely to be more efficient, in terms of both optical energy and antimicrobial activity, compared to higher irradiance. This is possibly due to the fact that there is a critical level of photons that can be involved in the photoexcitation of the bacterial porphyrin molecules, and above this irradiance level, there is provision of excess photons which, although exposing the cells, are unable to contribute to the reaction due to the fact that there is a limit on the free porphyrin to photon ratio.

In addition to demonstrating efficacy when applied to larger volumes of plasma, these experiments highlighted that the 405 nm light disinfection effect can be achieved through transparent packaging. A similar effect was reported in a recent study which highlighted the ability of 405 nm light to decontaminate biofilms on the underside of transparent materials [38]. The ability of 405 nm light to transmit through the PVC bag layer to treat the plasma is particularly advantageous as it opens up the possibility for prebagged plasma to be treated immediately prior to storage, without the need for addition of photosensitizers, and/or passing the plasma through external decontamination systems, which can potentially introduce new contamination into the plasma products [6]. The transmissibility of 405 nm light is also a significant advantage over UV-C light, which is blocked by the PVC bag material (Figure 5(a)). Measurements in the present study demonstrated that transmission of 405 nm light through the blood component bag material resulted in an approximate 20% loss in irradiance; however, light irradiance can be increased through the use of higher power light sources in order to compensate for this loss if required. Future developments would also look to improve the uniformity of the light systems used to treat the plasma.

Published studies have identified microbial endogenous porphyrin molecules as the key photosensitive targets which initiate the lethal oxidative damage exerted by 405 nm and other violet light wavelengths [19, 32]. Since human blood also contains porphyrins and porphyrin derivatives, it was important to establish that inactivation by 405 nm light in our study was a result of the photoexcitation reaction within the microbial contaminants and not a consequence of excitation of any photosensitive molecules within the plasma, and this was evidenced by the absence of antimicrobial toxicity to bacterial contaminants seeded into the 405 nm light-exposed plasma. Qualitative analysis of the rabbit plasma and human plasma also detected no notable fluorescence emission peaks between 500 and 700 nm when excited at 405 nm, thus indicating no significant levels of free porphyrins or other photoexcitation sources within the plasma which might have acted as exogenous photosensitizers for the inactivation of the microbial contaminants.

The 405 nm light doses required in this study for the decontamination of blood plasma have been in the region of 158 Jcm^{-2} and above. These doses are relatively high compared to those typically required for other light-based methods, and this is due to the higher germicidal efficacy of UV light compared to 405 nm light [39], and the involvement of photosensitizing compounds such as riboflavin, methylene blue, and amotosalen also accelerates the antimicrobial effects of light, with doses as low as 6.24 JmL^{-1} being reported as sufficient for use [7, 40], significantly lower than 83 JmL^{-1} used in the present study (calculated based on the 158 Jcm^{-2} dose, transfusion bag dimensions, and volume). This benefit, however, is counterbalanced by the fact that photosensitizers are added to the blood products, and significant care must be taken to ensure that there is

no toxicity to the blood components or the recipient due to the presence of residual photosensitizers [6]. Methods utilising UV-C light are currently under development and also demonstrate efficient microbial inactivation [16]. Although it does not require photosensitizers, UV-C is naturally more germicidal than 405 nm light; however, as mentioned, the limited penetrability of shortwave UV-C radiation means it is unable to decontaminate plasma packed in blood bags, as evidenced in the present study using 405 nm light (Figure 4(a)). The longer wavelength of 405 nm light also confers other benefits when compared to UV light, including reduced polymer degradation and increased human safety [41, 42].

Due to the absence of cells, solvent/detergent treatment, methylene blue and visible light, amotosalen and UV-A light, riboflavin and UV, and UV-C light are generally accepted as being suitable for plasma decontamination. This study has generated significant evidence of the efficacy of 405 nm light for decontamination of blood plasma as a model system to study injectable biological fluids. Since person-to-person variation in the activity of plasma proteins in healthy individuals is known to be significant, any loss in plasma integrity due to 405 nm light treatment is unlikely to have noticeable clinical impact. Further, since violet-blue light (405 nm) is relatively safer compared to already accepted UV light-based methods [39], its impact on plasma integrity has the potential to be reduced. Nonetheless, it is important in future studies to establish what effects are imparted onto plasma proteins when exposed to antimicrobial levels of 405 nm light relative to UV light exposure.

5. Conclusions

Overall, this study provides the first evidence that 405 nm light has the ability to inactivate bacterial contamination within biological fluids such as blood plasma. Significant inactivation of microbial contaminants was achieved in plasma samples of varying volumes held in different containers including prebagged plasma. The penetrability of 405 nm light and the nonrequirement for photosensitizing agents provide this antimicrobial method with unique benefits that could support its further development as a potential alternative to UV light-based systems. Further work is, however, required not only to extend the microbiological data but also to investigate the compatibility of 405 nm light with plasma components before its potential for plasma decontamination can be fully assessed. Although this study has focused on the antimicrobial effects of 405 nm light for the decontamination of plasma, it will also be of interest to establish whether bacterial reductions can be achieved in platelets stored *ex vivo* in plasma-based suspensions, which have a significantly greater risk of contamination due to the limitations of their storage conditions.

Disclosure

The views expressed in this article are an informal communication and represent the authors own best judgment. These comments do not bind or obligate FDA.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper. The authors have filed a joint US device patent application.

Acknowledgments

This work was supported by US FDA funding to Chintamani D. Atreya, with experimental work conducted at ROLEST through a collaborative research contract. The authors would like to thank J. Gillespie and HVT technical staff (Department of Electronic & Electrical Engineering (EEE), University of Strathclyde) for technical support with Matlab software and system construction, respectively, and Professor M. H. Grant, K. Henderson, and K. McKenzie for access and technical support with the fluorescence spectrophotometry (Departments of Biomedical Engineering and EEE, University of Strathclyde). The authors would also like to thank the Scottish National Blood Transfusion Service (SNBTS) for provision of blood components.

References

[1] T. Burnouf and M. Radosevich, "Reducing the risk of infection from plasma products: specific preventative strategies," *Blood Reviews*, vol. 14, no. 2, pp. 94–110, 2000.

[2] E. Vasconcelos and J. Seghatchian, "Bacterial contamination in blood components and preventative strategies: An overview," *Transfusion and Apheresis Science*, vol. 31, no. 2, pp. 155–163, 2004.

[3] M. E. Brecher and S. N. Hay, "Bacterial contamination of blood components," *Clinical Microbiology Reviews*, vol. 18, no. 1, pp. 195–204, 2005.

[4] Y. Y. Wu and E. L. Snyder, "Safety of the blood supply: role of pathogen reduction," *Blood Reviews*, vol. 17, no. 2, pp. 111–122, 2003.

[5] M. A. Blajchman, H. G. Klein, S. A. Glynn, and P. M. Ness, "Research opportunities for pathogen reduction/inactivation of blood components: summary of an NHLBI workshop," *Transfusion*, vol. 49, no. 6, pp. 1262–1268, 2009.

[6] B. G. Solheim and J. Seghatchian, "Update on pathogen reduction technology for therapeutic plasma: an overview," *Transfusion and Apheresis Science*, vol. 35, no. 1, pp. 83–90, 2006.

[7] V. S. Hornsey, O. Drummond, A. Morrison, L. McMillan, I. R. MacGregor, and C. V. Prowse, "Pathogen reduction of fresh plasma using riboflavin and ultraviolet light: effects on plasma coagulation proteins," *Transfusion*, vol. 49, no. 10, pp. 2167–2172, 2009.

[8] B. Horowitz, R. Bonomo, A. M. Prince, S. N. Chin, B. Brotman, and R. W. Shulman, "Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma," *Blood*, vol. 79, no. 3, pp. 826–831, 1992.

[9] P. Hellstern and B. G. Solheim, "The use of solvent/detergent treatment in pathogen reduction of plasma," *Transfusion Medicine and Hemotherapy*, vol. 38, no. 1, pp. 65–70, 2011.

[10] B. Lambrecht, H. Mohr, J. Knuver-Hopf, and H. Schmitt, "Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light," *Vox Sanguinis*, vol. 60, no. 4, pp. 207–213, 1991.

[11] J. Seghatchian, W. G. Struff, and S. Reichenberg, "Main properties of the THERAFLEX MB-plasma system for pathogen reduction," *Transfusion Medicine and Hemotherapy*, vol. 38, no. 1, pp. 55–64, 2011.

[12] P. Schlenke, T. Hervig, H. Isola et al., "Photochemical treatment of plasma with amotosalen and UVA light: process validation in three European blood centers," *Transfusion*, vol. 48, no. 4, pp. 697–705, 2008.

[13] R. P. Goodrich, R. A. Edrich, J. Li, and J. Seghatchian, "The Mirasol™ PRT system for pathogen reduction of platelets and plasma: an overview of current status and future trends," *Transfusion and Apheresis Science*, vol. 35, no. 1, pp. 5–17, 2006.

[14] P. H. Ruane, R. Edrich, D. Gampp, S. D. Keil, R. L. Leonard, and R. P. Goodrich, "Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light," *Transfusion*, vol. 44, no. 6, pp. 877–885, 2004.

[15] H. Mohr, U. Gravemann, A. Bayer, and T. H. Müller, "Sterilization of platelet concentrates at production scale by irradiation with short-wave ultraviolet light," *Transfusion*, vol. 49, no. 9, pp. 1956–1963, 2009.

[16] A. Seltsam and T. H. Müller, "UVC irradiation for pathogen reduction of platelet concentrates and plasma," *Transfusion Medicine and Hemotherapy*, vol. 38, no. 1, pp. 43–54, 2011.

[17] A. Seltsam and T. H. Müller, "Update on the use of pathogen-reduced human plasma and platelet concentrates," *British Journal of Haematology*, vol. 162, no. 4, pp. 442–454, 2013.

[18] P. Ramakrishnan, M. Maclean, S. J. MacGregor, J. G. Anderson, and M. H. Grant, "Cytotoxic responses to 405 nm light exposure in mammalian and bacterial cells: involvement of reactive oxygen species," *Toxicology in Vitro*, vol. 33, pp. 54–62, 2016.

[19] M. R. Hamblin, J. Viveiros, C. Yang, A. Ahmadi, R. A. Ganz, and M. J. Tolkoff, "*Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 7, pp. 2822–2827, 2005.

[20] J. S. Guffey and J. Wilborn, "*In vitro* bactericidal effects of 405-nm and 470-nm blue light," *Photomedicine and Laser Surgery*, vol. 24, no. 6, pp. 684–688, 2006.

[21] M. Maclean, S. J. MacGregor, J. G. Anderson, and G. A. Woolsey, "High-intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*," *FEMS Microbiology Letters*, vol. 285, no. 2, pp. 227–232, 2008.

[22] C. S. Enwemeka, D. Williams, S. Hollosi, D. Yens, and S. K. Enwemeka, "Visible 405 nm SLD light photo-destroys methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro," *Lasers in Surgery and Medicine*, vol. 40, no. 10, pp. 734–737, 2008.

[23] M. Maclean, S. J. MacGregor, J. G. Anderson, and G. Woolsey, "Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array," *Applied and Environmental Microbiology*, vol. 75, no. 7, pp. 1932–1937, 2009.

[24] L. E. Murdoch, M. MacLean, S. J. MacGregor, and J. G. Anderson, "Inactivation of *Campylobacter jejuni* by exposure to high-intensity 405-nm visible light," *Foodborne Pathogens and Disease*, vol. 7, no. 10, pp. 1211–1216, 2010.

[25] L. E. Murdoch, M. Maclean, E. Endarko, S. J. MacGregor, and J. G. Anderson, "Bactericidal effects of 405 nm light exposure demonstrated by inactivation of *Escherichia, Salmonella, Shigella, Listeria*, and *Mycobacterium* species in liquid suspensions and on exposed surfaces," *The Scientific World Journal*, vol. 2012, Article ID 137805, 8 pages, 2012.

[26] E. Endarko, M. Maclean, I. V. Timoshkin, S. J. MacGregor, and J. G. Anderson, "High-intensity 405 nm light inactivation of *Listeria monocytogenes*," *Photochemistry and Photobiology*, vol. 88, no. 5, pp. 1280–1286, 2012.

[27] C. J. Wasson, J. L. Zourelias, N. A. Aardsma et al., "Inhibitory effects of 405 nm irradiation on Chlamydia trachomatis growth and characterization of the ensuing inflammatory response in HeLa cells," *BMC Microbiology*, vol. 12, article 176, 2012.

[28] M. MacLean, L. E. Murdoch, S. J. MacGregor, and J. G. Anderson, "Sporicidal effects of high-intensity 405 nm visible light on endospore-forming bacteria," *Photochemistry and Photobiology*, vol. 89, no. 1, pp. 120–126, 2013.

[29] M. Maclean, S. J. MacGregor, J. G. Anderson et al., "Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light," *Journal of Hospital Infection*, vol. 76, no. 3, pp. 247–251, 2010.

[30] S. E. Bache, M. MacLean, S. J. MacGregor et al., "Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings," *Burns*, vol. 38, no. 1, pp. 69–76, 2012.

[31] M. Maclean, M. Booth, J. Anderson et al., "Continuous decontamination of an intensive care isolation room during patient occupancy using 405 nm light technology," *Journal of Infection Prevention*, vol. 14, no. 5, pp. 176–181, 2013.

[32] T. Dai, A. Gupta, Y.-Y. Huang et al., "Blue light rescues mice from potentially fatal pseudomonas aeruginosa burn infection: efficacy, safety, and mechanism of action," *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 3, pp. 1238–1245, 2013.

[33] R. McDonald, S. J. MacGregor, J. G. Anderson, M. MacLean, and M. H. Grant, "Effect of 405-nm high-intensity narrow-spectrum light on fibroblast-populated collagen lattices: an *in vitro* model of wound healing," *Journal of Biomedical Optics*, vol. 16, no. 4, Article ID 048003, 2011.

[34] R. S. McDonald, S. Gupta, M. Maclean et al., "405 nm light exposure of osteoblasts and inactivation of bacterial isolates from arthroplasty patients: potential for new disinfection applications?" *European Cells and Materials*, vol. 25, pp. 204–214, 2012.

[35] C. D. Atreya, M. Maclean, J. G. Anderson, and S. J. MacGregor, "Inactivation of pathogens in ex vivo blood products in storage bags using visible light," US Patent Application no. 62/236,706, 2015.

[36] L. E. Murdoch, K. McKenzie, M. Maclean, S. J. MacGregor, and J. G. Anderson, "Lethal effects of high-intensity violet 405-nm light on *Saccharomyces cerevisiae*, *Candida albicans*, and on dormant and germinating spores of *Aspergillus niger*," *Fungal Biology*, vol. 117, no. 7-8, pp. 519–527, 2013.

[37] M. E. Brecher, P. V. Holland, A. A. Pineda, G. E. Tegtmeier, and R. Yomtovian, "Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage," *Transfusion*, vol. 40, no. 11, pp. 1308–1312, 2000.

[38] K. McKenzie, M. Maclean, I. V. Timoshkin, E. Endarko, S. J. Macgregor, and J. G. Anderson, "Photoinactivation of bacteria attached to glass and acrylic surfaces by 405 nm light: potential application for biofilm decontamination," *Photochemistry and Photobiology*, vol. 89, no. 4, pp. 927–935, 2013.

[39] R. Yin, T. Dai, P. Avci et al., "Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond," *Current Opinion in Pharmacology*, vol. 13, no. 5, pp. 731–762, 2013.

[40] D. J. Bihm, A. Ettinger, K. A. Buytaert-Hoefen et al., "Characterization of plasma protein activity in riboflavin and UV light-treated fresh frozen plasma during 2 years of storage at −30°C," *Vox Sanguinis*, vol. 98, no. 2, pp. 108–115, 2010.

[41] A. L. Andrady, S. H. Hamid, X. Hu, and A. Torikai, "Effects of increased solar ultraviolet radiation on materials," *Journal of Photochemistry and Photobiology B: Biology*, vol. 46, no. 1-3, pp. 96–103, 1998.

[42] Y. Matsumura and H. N. Ananthaswamy, "Toxic effects of ultraviolet radiation on the skin," *Toxicology and Applied Pharmacology*, vol. 195, no. 3, pp. 298–308, 2004.

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2016, Article ID 9316385, 5 pages
http://dx.doi.org/10.1155/2016/9316385

Research Article

Quantification of Cell-Free DNA in Red Blood Cell Units in Different Whole Blood Processing Methods

Andrew W. Shih,[1,2] Vinai C. Bhagirath,[3,4] Nancy M. Heddle,[2,3,5] Jason P. Acker,[6,7] Yang Liu,[2,3] John W. Eikelboom,[3] and Patricia C. Liaw[1,3,4]

[1]*Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada*
[2]*McMaster Centre for Transfusion Research, McMaster University, Hamilton, ON, Canada*
[3]*Department of Medicine, McMaster University, Hamilton, ON, Canada*
[4]*Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, ON, Canada*
[5]*Centre for Innovation, Canadian Blood Services, Hamilton, ON, Canada*
[6]*Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada*
[7]*Centre for Innovation, Canadian Blood Services, Edmonton, AB, Canada*

Correspondence should be addressed to Andrew W. Shih; andrew.shih@medportal.ca

Received 11 August 2016; Accepted 7 September 2016

Academic Editor: Rajendra K. Chaudhary

Background. Whole blood donations in Canada are processed by either the red cell filtration (RCF) or whole blood filtration (WBF) methods, where leukoreduction is potentially delayed in WBF. Fresh WBF red blood cells (RBCs) have been associated with increased in-hospital mortality after transfusion. Cell-free DNA (cfDNA) is released by neutrophils prior to leukoreduction, degraded during RBC storage, and is associated with adverse patient outcomes. We explored cfDNA levels in RBCs prepared by RCF and WBF and different storage durations. *Methods*. Equal numbers of fresh (stored ≤14 days) and older RBCs were sampled. cfDNA was quantified by spectrophotometry and PicoGreen. Separate regression models determined the association with processing method and storage duration and their interaction on cfDNA. *Results*. cfDNA in 120 RBC units (73 RCF, 47 WBF) were measured. Using PicoGreen, WBF units overall had higher cfDNA than RCF units ($p = 0.0010$); fresh WBF units had higher cfDNA than fresh RCF units ($p = 0.0093$). Using spectrophotometry, fresh RBC units overall had higher cfDNA than older units ($p = 0.0031$); fresh WBF RBCs had higher cfDNA than older RCF RBCs ($p = 0.024$). *Conclusion*. Higher cfDNA in fresh WBF was observed compared to older RCF blood. Further study is required for association with patient outcomes.

1. Introduction

Transfusion of red blood cells (RBCs) is one of the most widely used therapies in clinical medicine. In Canada, approximately 1.5 million transfusions were given each year from 2006 to 2012, with the majority being red blood cells (RBC). Emerging data suggests that there is variability in RBC product quality depending on the method used to process the whole blood donations and the duration of RBC storage [1]. Since 2008, Canadian Blood Services (CBS) produce RBCs using two different methods in an approximately 1 : 1 ratio, the red cell filtration (RCF) (also called buffy coat) method and the whole blood filtration (WBF) method [2]. In the RCF method, whole blood is held at room temperature for a maximum 20 hours before separation into platelets, plasma, and RBCs and red cell leukoreduction occurs at room temperature. In the WBF method, the whole blood is cooled within 8 hours of collection to 4°C and then leukoreduced in the cold and processed into plasma and RBCs any time within 72 hours of the collection.

Differences in processing method may have a negative impact on patient outcomes [1, 3]. A retrospective review of over 23,000 patients receiving approximately 92,000 RBC transfusions over a six-year period in three tertiary care

centers demonstrated higher in-hospital mortality in patients who received WBF products with a shorter storage duration (less than 8 days) compared to patients who received RCF products with a longer storage duration [4]. One difference between the two methods of whole blood processing is the timing and temperature of leukoreduction. In blood products that have not undergone leukoreduction, there are significant levels of cfDNA and associated histones that increase with time [5]. Both are released in the form of neutrophil extracellular traps (NETs) by neutrophils in the presence of microbial or inflammatory stimuli [6]. cfDNA activates coagulation via the contact pathway [7]. Interactions with platelets and neutrophils can result in microvascular thrombosis, leading to tissue hypoxia and endothelial damage. Histones activate platelets [8], induce neutrophil accumulation in organs [9], and cause endothelial cell toxicity [10]. cfDNA can also be released from mitochondria, in which case it is not associated with histones. Mitochondrial DNA (mtDNA) has similar procoagulant and platelet-stimulating potential as nuclear cfDNA but also has distinct proinflammatory properties [11, 12]. In animal models, reducing NETs with a DNA-digesting enzyme or inhibiting NETs with anti-histone antibodies results in improved survival in an animal model of transfusion-associated lung injury [13], and neutralizing histones with antibodies can rescue mice from lethal sepsis [14]. In humans, circulating cfDNA levels have been associated with deep vein thrombosis, increased risk of mortality in septic patients [15], increased severity in trauma patients, and thrombosis in cancer patients [16]. Thus, the cfDNA released from white blood cells is potentially harmful.

We hypothesized that the delay in leukoreduction in blood processed by the WBF method may lead to higher amounts of cfDNA released from leukocytes, thereby potentially explaining the observed association with increased mortality when the WBF product is transfused. We also hypothesized that DNases in blood may contribute to degradation of DNA over time and thus fresh blood will have higher amounts of cfDNA compared to older blood. To test these hypotheses, we measured cfDNA in RBC products and correlated these levels with the method of whole blood processing and duration of storage.

2. Materials and Methods

2.1. Sample Collection. Approximately 5 mL was sampled from packed RBC units in the Transfusion Medicine laboratory at the McMaster site of Hamilton Health Sciences using a sterile docking device. Samples were consecutively collected to meet a 1 : 1 ratio of fresh blood (defined as having a storage time of 14 days or less) or older (storage time greater than 14 days) blood. We utilized a cutoff of 14 days or less for fresh blood as it was the most common definition utilized for fresh blood [17]. The samples were immediately spun at 1700 g for 10 minutes, and the supernatant was aliquoted and frozen at −80°C. DNA from 200 μL of thawed supernatant was extracted into 200 μL of AE buffer (elution buffer) using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) as per the manufacturer's directions.

Anonymized data including product number and storage duration were recorded from the unit at the time of sampling. The unique product numbers were documented and sent to Canadian Blood Services who provided the method of processing for each unit. The protocol was approved by the Hamilton Integrated Research Ethics Board and the Research Ethics Board at Canadian Blood Services.

2.2. Measurement of Cell-Free DNA Concentration. DNA concentration was determined by spectrophotometry, with concentration of DNA measured with UV absorbance at 260 nm on an Eppendorf Biophotometer Plus (Eppendorf, Hamburg, Germany). The PicoGreen assay (Life Technologies, Carlsbad, CA) was performed as per the manufacturer's directions, where a smaller volume of 100 μL per sample was used and read in 96-well opaque black plates.

2.3. Statistical Analysis. Statistical analysis was performed using computer software (SAS Version 9.3, Cary, North Carolina). Descriptive analyses of continuous variables were reported as mean and standard deviations. General linear regression models were conducted to determine the association between age of blood and whole blood processing method independently with cfDNA concentration by spectrophotometry or PicoGreen. Age of blood was analyzed both as a dichotomous variable (with fresh blood denoted as being stored for 14 days or less and older blood denoted as being stored for 15 days or more) and a continuous variable. The interacting effect between age of blood as a dichotomous variable and processing method on cfDNA measurements was also assessed in a separate regression model with an interaction term. Bonferroni adjustment was used for multiple comparisons and results were considered significant at p values of less than 0.025. As a secondary analysis, we also assessed if longer duration before leukoreduction predicted higher levels of cfDNA.

2.4. Sample Size Calculation. In another study analyzing cfDNA levels in stored blood [5], healthy control donors had a mean plasma cfDNA level of approximately 50 ng/mL. The mean level of cfDNA found in nonleukoreduced RBC units stored for 42 days was approximately 100 ng/mL (SD 30 ng/mL), an increase of 100%. We hypothesized that cfDNA will increase by approximately 50% in WBF units compared with RCF units. Calculating sample size using a two-sided test, an alpha of 0.05, and a desired power of 0.80, the sample size for each group was determined to be 48 samples. The method by which a unit of blood is processed is not known when the RBC arrives at the hospital; however, the ratio of RCF and WBF units produced by Canadian Blood Services is approximately 1 : 1; hence, we estimated that sampling 120 RBC units would provide a 95% probability of having at least 48 samples by each production method.

3. Results

120 units were sampled in total, with 60 being fresh units (≤14 days of storage duration) and 60 being older units. Of

Table 1: Differences in cfDNA between red blood cells (RBCs) processed by whole blood filtration (WBF) and red cell filtration (RCF).

	RBCs processed by WBF	RBCs processed by RCF	p value
cfDNA by PicoGreen (mean ng/mL ± SD)	1.08 ± 0.90 (n = 44)	0.50 ± 0.77 (n = 73)	0.0010
cfDNA by Spectrophotometry (mean μg/mL ± SD)	3.57 ± 1.99 (n = 47)	3.28 ± 1.28 (n = 73)	0.088

Table 2: The Interaction between storage duration and processing method on differences in cfDNA.

Assay	Red cell storage duration	Method of whole blood processing		p value
		Whole blood filtration (WBF)	Red cell filtration (RCF)	
cfDNA by PicoGreen (mean ng/mL ± SD)	Fresh (≤14 days)	1.16 ± 1.14 (n = 15)	0.37 ± 0.77 (n = 44)	0.0093
	Older (>14 days)	1.04 ± 0.78 (n = 29)	0.68 ± 0.74 (n = 29)	0.33
cfDNA by spectrophotometry (mean μg/mL ± SD)	Fresh (≤14 days)	4.15 ± 2.47 (n = 16)	3.63 ± 1.25 (n = 44)	0.67
	Older (>14 days)	3.27 ± 1.65 (n = 31)	2.75 ± 1.15 (n = 29)	0.57

the 60 fresh units, 48 (80%) had a storage duration of less than 8 days. After the method of processing was provided by Canadian Blood Services, it was determined that 73 units were made with the RCF method and 47 were made by the WBF method.

3.1. WBF Processed RBC Units Had Higher cfDNA Compared to RCF Units Processed Units by PicoGreen. To test our hypothesis that WBF processed RBC units have higher amounts of cfDNA, we compared cfDNA concentrations in WBF and RCF RBC units. cfDNA was significantly higher in WBF RBCs compared to RCF units when quantified by PicoGreen (1.08 ± 0.90 ng/mL versus 0.50 ± 0.77 ng/mL, $p = 0.0010$) (Table 1). When the interaction between storage duration and processing method was considered for cross-comparisons, fresh WBF RBCs had significantly higher levels of cfDNA than fresh RCF RBCs as measured by PicoGreen (1.16 ± 1.14 ng/mL versus 0.37 ± 0.77 ng/mL, $p = 0.0093$) (Table 2). No significant difference was seen between cfDNA in WBF and RCF RBCs when measured by spectrophotometry ($p = 0.088$), although the absolute values were concordant with the findings by PicoGreen. Similar results were obtained when age of blood was analyzed as a continuous variable. In this analysis, there was a trend towards increased cfDNA measured by spectrophotometry in WBF compared to RCF RBCs overall ($p = 0.063$).

3.2. Fresh RBC Units Had Higher cfDNA Compared to Older RBC Units by Spectrophotometry. Fresh RBCs were found overall to have a significantly higher concentration of cfDNA compared to older RBCs using spectrophotometry (3.77 ± 1.66 μg/mL versus 3.02 ± 1.44 μg/mL, $p = 0.0031$) (Table 3). This association strengthened when age of blood was analyzed as a continuous variable ($p = 0.00066$). When the interaction between storage duration and processing method was considered, fresh WBF RBCs had significantly higher cfDNA compared to older RCF RBCs ($p = 0.024$) (Table 2). No significant difference overall was seen when comparing cfDNA quantified by PicoGreen in fresh compared to older units ($p = 0.33$), even when age of blood was analysed as a continuous variable ($p = 0.39$).

We examined whether longer time to leukoreduction predicted higher levels of cfDNA but did not demonstrate an association, possibly because of relatively small numbers of products for which data was available ($n = 32$).

4. Discussion

Differences in RBC production method could affect clinical outcomes, with retrospective data suggesting that fresh blood produced by the WBF method could be associated with an increased risk of in-hospital mortality [18]. Retrospective studies of this nature could always be susceptible to confounding; however, if this association is true, biological mechanisms that could explain this finding need to be explored. In this study, we investigated the possibility that cfDNA levels could vary in different RBC products and possibly be an explanation for the clinical observations that have been observed. We found that RBC products produced by the WBF method have higher levels of cfDNA than products produced by the RCF method. We also found that fresh RBC products have higher levels of cfDNA than older products. Hence, the results of this study are consistent with the hypothesis that cfDNA in transfused RBC products could have an impact on patient outcomes.

The results of our study are consistent with other data showing that the in vitro quality of RBCs varies by method of processing and storage duration. RBCs processed by WBF have qualitative differences from RBCs processed by RCF such as higher residual plasma [19], smaller red cell

Table 3: Differences in cfDNA between fresh and older blood.

Assay	Duration of red blood cell storage		p value
	Fresh blood (≤14 days)	Older blood (>14 days)	
cfDNA by PicoGreen (mean ng/mL ± SD)	0.57 ± 0.93 (n = 59)	0.86 ± 0.78 (n = 58)	0.33
cfDNA by spectrophotometry (mean μg/mL ± SD)	3.77 ± 1.66 (n = 60)	3.02 ± 1.44 (n = 60)	0.0031

microvesicles [20], higher amounts of hemolysis at expiry, lower ATP levels [21], and higher MCV at expiry [1]. A previous study demonstrated higher mtDNA with RBCs processed by WBF, where our testing for cfDNA encompasses mtDNA as well as nuclear cfDNA [21]. mtDNA may potentially have immunomodulatory properties [11], where nuclear cfDNA may be more procoagulant [7]. The clinical significance of these differences is unclear.

There are several limitations to our study. While we demonstrated the correlation between WBF processed blood with a shorter duration of storage and increased cfDNA compared to RCF blood with a longer duration of storage, we cannot conclusively state that methods of blood preparation and storage duration are causative for our findings. How NETosis is affected by specific processing variables such as temperature, centrifugation force, extraction method, filter design, blood bags used, storage solution, and anticoagulant was not studied in our exploratory analysis. Other variables such as donor characteristics were also not assessed and could also be playing a role in patient outcomes [22]. However, consecutive sampling of units avoids selection bias and our sample size was appropriately conservative to account for potential variation amongst units. We also could not prove that cfDNA is linked to the pathobiology of fresh WBF units causing harm as we did not prospectively follow patients transfused with these units. A prospective study to link clinical outcomes would require a much larger sample size to demonstrate a conclusive effect.

A second issue is the difficulty in measurement of cfDNA. We found poor correlation between cfDNA levels measured by the two methods. This could be due to differences in sensitivities and specificities of each assay, where PicoGreen is specific for double-stranded DNA. The significance of single-stranded DNA and double-stranded DNA is unknown. Both assays are potentially affected by protein contamination, most notably with the spectrophotometry method. We also observed a high degree of variance with cfDNA measurements, where donor factors, specific parameters within whole blood processing, or poor precision in current methods of cfDNA measurement could be potential contributors. However, the higher levels of cfDNA in WBF and in fresher products by both methods, accounting for multiple tests of significance, suggest robustness of this finding. We did not perform testing for histones or nucleosomes. Our results do not differentiate between nuclear and mitochondrial DNA.

Our study has several strengths. We sampled a relatively large number of units in relation to our calculated sample size and explored the effect of duration of storage of blood as well as the method of whole blood processing. Concordance of findings in the quantification of cfDNA increases the robustness of our conclusion of increased cfDNA in fresh compared to older blood and in WBF compared to RCF blood. Our finding that older products had less cfDNA is consistent with results from recent randomized trials comparing fresh to standard issue (older) blood, as these studies have not shown that fresh blood is superior [23–25], with some trials suggesting a trend towards harm with fresher blood [18, 24, 26].

A prospective study with patients transfused blood produced via different methods of whole blood processing could link adverse patient outcomes to sampled transfused blood products and recipients. This would allow for testing of cfDNA levels as well as other biomarkers to elucidate the mechanisms by which transfusion of specific types of RBC products lead to adverse patient outcomes. Given its procoagulant nature, potential outcomes linked to cfDNA in RBC units that could be studied prospectively include cardiovascular events such as myocardial infarction, cerebrovascular accidents, or venous thromboses such as deep vein thrombosis and pulmonary embolism. In addition, presence of mtDNA in platelet concentrates has been linked to nonhemolytic transfusion reactions [27]. A prospective study may be able to confirm this finding in RBC units.

In conclusion, our study found that red blood cells processed by the WBF method and red blood cells with a shorter duration of storage were associated with increased concentrations of cfDNA. These findings are consistent with the clinical observations that fresh WBF blood may be associated with increased mortality in transfused patients. Further studies are required to confirm these observations and to understand the pathobiology.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] J. P. Acker, A. L. Hansen, J. D. R. Kurach, T. R. Turner, I. Croteau, and C. Jenkins, "A quality monitoring program for red blood cell components: in vitro quality indicators before and after implementation of semiautomated processing," *Transfusion*, vol. 54, no. 10, pp. 2534–2543, 2014.

[2] W. P. Sheffield, V. Bhakta, C. Jenkins, and D. V. Devine, "Conversion to the buffy coat method and quality of frozen plasma

derived from whole blood donations in Canada," *Transfusion*, vol. 50, no. 5, pp. 1043–1049, 2010.

[3] N. M. Heddle, D. M. Arnold, J. P. Acker et al., "Red blood cell processing methods and in-hospital mortality: a transfusion registry cohort study," *The Lancet Haematology*, vol. 3, no. 5, pp. e246–e254, 2016.

[4] N. M. Heddle, J. W. Eikelboom, Y. Liu, R. L. Barty, and R. J. Cook, "Mortality, blood storage duration, and method of red cell production: an exploratory analysis," *Transfusion*, vol. 52, pp. 51A–52A, 2012.

[5] T. A. Fuchs, J. J. Alvarez, K. Martinod, A. A. Bhandari, R. M. Kaufman, and D. D. Wagner, "Neutrophils release extracellular DNA traps during storage of red blood cell units," *Transfusion*, vol. 53, no. 12, pp. 3210–3216, 2013.

[6] V. Brinkmann, U. Reichard, C. Goosmann et al., "Neutrophil extracellular traps kill bacteria," *Science*, vol. 303, no. 5663, pp. 1532–1535, 2004.

[7] T. J. Gould, T. T. Vu, L. L. Swystun et al., "Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 34, no. 9, pp. 1977–1984, 2014.

[8] F. Semeraro, C. T. Ammollo, J. H. Morrissey et al., "Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4," *Blood*, vol. 118, no. 7, pp. 1952–1961, 2011.

[9] J. Xu, X. Zhang, M. Monestier, N. L. Esmon, and C. T. Esmon, "Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury," *Journal of Immunology*, vol. 187, no. 5, pp. 2626–2631, 2011.

[10] S. T. Abrams, N. Zhang, J. Manson et al., "Circulating histones are mediators of trauma-associated lung injury," *American Journal of Respiratory and Critical Care Medicine*, vol. 187, no. 2, pp. 160–169, 2013.

[11] Q. Zhang, M. Raoof, Y. Chen et al., "Circulating mitochondrial DAMPs cause inflammatory responses to injury," *Nature*, vol. 464, no. 7285, pp. 104–107, 2010.

[12] V. C. Bhagirath, D. J. Dwivedi, and P. C. Liaw, "Comparison of the proinflammatory and procoagulant properties of nuclear, mitochondrial, and bacterial DNA," *Shock*, vol. 44, no. 3, pp. 265–271, 2015.

[13] A. Caudrillier, K. Kessenbrock, B. M. Gilliss et al., "Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury," *The Journal of Clinical Investigation*, vol. 122, no. 7, pp. 2661–2671, 2012.

[14] J. Xu, X. Zhang, R. Pelayo et al., "Extracellular histones are major mediators of death in sepsis," *Nature Medicine*, vol. 15, no. 11, pp. 1318–1321, 2009.

[15] D. J. Dwivedi, L. J. Toltl, L. L. Swystun et al., "Prognostic utility and characterization of cell-free DNA in patients with severe sepsis," *Critical Care*, vol. 16, no. 4, article R151, 2012.

[16] M. Demers, D. S. Krause, D. Schatzberg et al., "Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 32, pp. 13076–13081, 2012.

[17] D. Wang, J. Sun, S. B. Solomon, H. G. Klein, and C. Natanson, "Transfusion of older stored blood and risk of death: a meta-analysis," *Transfusion*, vol. 52, no. 6, pp. 1184–1195, 2012.

[18] N. M. Heddle, R. J. Cook, D. M. Arnold et al., "The effect of blood storage duration on in-hospital mortality: a randomized controlled pilot feasibility trial," *Transfusion*, vol. 52, no. 6, pp. 1203–1212, 2012.

[19] A. Jordan and J. P. Acker, "Determining the volume of additive solution and residual plasma in whole blood filtered and buffy coat processed red cell concentrates," *Transfusion Medicine and Hemotherapy*, vol. 43, no. 2, pp. 133–136, 2016.

[20] B. Bicalho, A. S. Pereira, and J. P. Acker, "Buffy coat (top/bottom)- and whole-blood filtration (top/top)-produced red cell concentrates differ in size of extracellular vesicles," *Vox Sanguinis*, vol. 109, no. 3, pp. 214–220, 2015.

[21] S. Bakkour, J. P. Acker, T. R. Turner, D. M. Chafets, T.-H. Lee, and M. P. Busch, "Processing method affects mitochondrial DNA release in stored red blood cells," *Transfusion*, vol. 55, article 80A, 2015.

[22] R. A. Middelburg, E. Briët, and J. G. van der Bom, "Mortality after transfusions, relation to donor sex," *Vox Sanguinis*, vol. 101, no. 3, pp. 221–229, 2011.

[23] D. A. Fergusson, P. Hebert, D. L. Hogan et al., "Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the aripi randomized trial," *The Journal of the American Medical Association*, vol. 308, pp. 1443–1451, 2012.

[24] J. Lacroix, P. C. Hébert, D. A. Fergusson et al., "Age of transfused blood in critically ill adults," *The New England Journal of Medicine*, vol. 372, no. 15, pp. 1410–1418, 2015.

[25] M. E. Steiner, P. M. Ness, S. F. Assmann et al., "Effects of red-cell storage duration on patients undergoing cardiac surgery," *The New England Journal of Medicine*, vol. 372, no. 15, pp. 1419–1429, 2015.

[26] C. Aubron, G. Syres, A. Nichol et al., "A pilot feasibility trial of allocation of freshest available red blood cells versus standard care in critically ill patients," *Transfusion*, vol. 52, no. 6, pp. 1196–1202, 2012.

[27] K. Yasui, N. Matsuyama, A. Kuroishi, Y. Tani, R. A. Furuta, and F. Hirayama, "Mitochondrial damage-associated molecular patterns as potential proinflammatory mediators in post-platelet transfusion adverse effects," *Transfusion*, vol. 56, no. 5, pp. 1201–1212, 2016.