International blood collection and storage: clinical use of blood products

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Running Title: clinical use of blood products

Keywords: blood / transfusion / plasma / serum / standard operating procedures / biomarkers

Abbreviations: ACD, acid-citrate-dextrose; ARCBS, Australian Red Cross Blood Service; CLIA, Clinical Laboratory Improvement Amendments; EDRN, Early Detection Research Network; HIV, human immunodeficiency virus; HTLV, human T-cell lymphotrophic virus; HUPO, Human Proteome Organisation; OSHA, Occupational Safety and Health Administration; PPP, Plasma Proteome Project; PRP, platelet-rich plasma; PRT, Pathogen reduction technology; TA-GVHD, transfusion-associated graft-versus-host disease; TRALI, transfusion-related acute lung injury; TTD, transfusion-transmitted disease.

Abstract

Human blood transfusion is the process of transferring blood or blood-based products from an individual into the circulatory system of another. From the theory of circulation of blood to the early practice of blood transfusion, transfusion medicine has been an important concept for many centuries. The practicality of transfusion, however, only became a possibility during and shortly after the Second World War. Today, blood and its derivatives play a critical role in worldwide health care systems, with blood components having direct clinical indications. Over the past several years worldwide organizations including the World Health Organization (WHO) have made a number of substantial improvements to the regulation of the worlds blood supply. This continuous supply plays a critical role throughout health care systems worldwide, with procedures for blood collection, processing, and storage now complex, standardised processes. As the areas of clinical validation of different disease states from blood-derived sources (i.e., disease biomarkers) move towards validation stages, the importance of controlled - and standardised-protocols is imperative.

1 Introduction

The practice of blood transfusion, that is the transference of blood from the circulation of one individual to that of another for practical therapeutic purposes, is of relatively recent origin. Although the practical possibility of blood transfusion coincided with World War II and the resultant need for massive blood replacement, the concept of transfusion has a much longer history. Transfusion medicine history is one of continued progress from a primitive, invasive, harmful, and often deadly procedure to an organised, complete specialty [1]. Today the United States utilises approximately 14 million units of whole blood, drawn from about 8 million volunteer donors to make products that are transfused into more than 3.5 million Americans. Some of this blood, and an additional 12 million units of source plasma, is further processed into products referred to as derivatives. Improvements in the safety of the blood supply and the increasing costs associated with transfusion therapies have led to a re-evaluation of the clinical practices of blood transfusion and blood conservation. In this review we focus on the history and practice of blood transfusion, advances in the use of blood products and current worldwide practices of blood transfusion, and blood products as components of clinical research.

2 Blood – History and practice of blood transfusion

Blood has always held a mysterious fascination and is considered a living force of the body. The idea of the theoretical beneficial effects of blood has been recognised for centuries. For example, ancient Egyptians, Romans and Greeks recognised the

mysterious properties of blood in bathing and resuscitating the sick, injured, and elderly. Most ancient and medieval references of blood administration practices refer most likely to the ingestion of blood, rather than to its infusion [1, 2]. The theory of blood circulation and the human circulatory system was first described in detail by Harvey in 1613, and during 1628, the transfusion of blood [2]. Blood was used for transfusion as a therapeutic measure as early as 1667 when Boyle, Lower and Denis administered animal blood to humans [2, 3]. Although initially successful, these series of direct and exchange transfusions often resulted in haemolytic transfusion reactions, with members often dying soon after the transfusion [2]. Importantly, these practices of blood transfusion experiments were ruled criminal acts if performed throughout France and England, and led to the abandonment of the study of the physiology of circulation for approximately 150 years [2]. It wasn't until the 19th century that the era of human blood transfusion was pioneered by an English obstetrician, James Blundell. He is credited not only with rekindling interests in a national approach towards blood transfusion, but in 1818 was the first to transfuse human blood [2]. Of interest, Blundell noted that vein-to-vein direct transfusions were impractical due to the major problem of blood clotting. Considerable effort was invested in blood anticoagulation and defibrillated blood (platelet clot depleted), although it would be half a century before a practical means of anticoagulation was devised and accepted. By 1900 it was recognised that the reactions observed during initial transfusion therapies were the result of destruction of transfused red cells by immunological mechanisms due to individual differences in blood group antigens (ABO blood group system, devised by Landsteiner [4]). The presence of these antibodies, together with the variable frequencies of the different blood groups in European

populations, explained why blood transfusion from one person to another, historically, had been so capricious. During the ensuing 50 years, evolving serologic techniques including the direct antiglobulin (Coombs) test led to the discovery of numerous new red-cell antigens and antibodies [5]. The practical problem of anticoagulation was discovered by Lewisohn in 1924, who introduced sodium citrate as a means of inducing anticoagulation [6, 7]. Weil demonstrated in 1915 the feasibility of refrigerated storage of anticoagulated blood for short periods. Citrate-glucose was further introduced by Rous and Turner in 1916 as an anticoagulant to permit storage of blood for several days post-collection. These discoveries led to the basic blood depots during World War I by Robertson [8], and the genesis of today's blood banking systems. The identification of the 'Rhesus' (Rh) blood group system by Levine in 1940 was recognised as the cause for the continued reactivity of blood during transfusion. Freeze-dried plasma was developed in 1940, acid-citrate-dextrose (ACD) anticoagulant solution was developed for the storage of blood by Loutit and Mollison in 1943, and this was soon followed by the development of a method of freezing blood using glycerol cryoprotectant in 1950. Plasma fractionation was developed by Cohn in 1944 based on cold ethanol fractionation [9]. The Cohn Process utilizes the differential solubility of albumin and other plasma proteins based on pH, ethanol concentration, temperature, ionic strength, and protein concentration [10]. The Cohn Process and its variations have served as a foundation for the field of blood fractionation.

With the advent of voluntary blood donation across the United States and Europe (Soviet Union), a national system of blood banks were devised in the 1920s-1940s. The

International Committee of the Red Cross announced that the Australian Red Cross be recognised part of the international movement in 1928. The finding in the early 1940s that blood separated into plasma and red blood cells could be stored for longer time periods without contamination increased the blood supply and facilitated resource exchange between blood banks. In 1947 the formation of the American Association of Blood Banks (AABB) and in 1948 the American [National] Red Cross, led to the development of a comprehensive program to collect and distribute blood on a national scale in the United States. By 1949, 69 Red Cross and Red Crescent National Societies were in existence worldwide. Over the next 30 years the standardisation of blood collection, processing, and storage of human blood and blood components has become a focus of the WHO and FDA, with the computerization of blood-related establishments in constant use by the early 1990s (http://www.who.int/biologicals/publications/en/).

Clinical practice of blood transfusion has changed considerably throughout history as a result of our increased knowledge of the biology of the circulatory system, haemostasis, and development of new technologies for the collection, processing, storage and testing of blood. An important and continued area of focus worldwide is blood transfusion safety, particularly in regard to strategies to minimize transfusion transmissible diseases (TTDs) and other transfusion risks, including human error resulting in the inadvertent transfusion of incompatible blood, and adverse reactions such as acute and delayed transfusion reactions, transfusion-related acute lung injury (TRALI), transfusion-associated graft-versus-host disease (TA-GVHD), and transfusion-induced immunomodulation [11] (http://www.transfusion.com.au/Consent-and-Risk.aspx).

Blood, whether whole blood or components thereof, can potentially cause adverse reactions or TTDs. The International Committee of the Red Cross has employed several steps to minimise TTDs by insisting on - (i) voluntary blood donations, - (ii) identifying donor-related high risk behaviours, - (iii) donor screening for TTDs, - (iv) inclusion of viral inactivation processes of manufactured plasma products, and - (v) public awareness with regard to screening and the delayed onset of blood-based infections (e.g., malaria).

Identification of the pathogens that cause known TTDs (e.g., hepatitis B, hepatitis C, malaria, syphilis) as well as the emergence of new TTDs and pathogens (e.g., human immunodeficiency virus-1/2 (HIV-1/2), human T-lymphotropic virus (HTLV-1/2), West Nile Virus, Chagas disease) have lead to the development and implementation of screening tests for these pathogens. Many of these screening tests have been widely implemented as mandatory tests (HIV-1/2, HTLV-1/2, Hepatitis B, Hepatitis C, syphilis), whilst others have been implemented in regions where the pathogen is known to be present in the community (e.g., West Nile Virus in North America and Chagas in the United States of America). Tests may also be performed for various common pathogens which, although widely prevalent in the community and/or cause mild/asymptomatic illness in otherwise healthy individuals (e.g., Cytomegalovirus, parvovirus B19), may cause more severe illnesses in susceptible patients. Other new pathogens that have emerged over the past decade in which high-throughput screening tests are not yet available, such as prion-mediated variant Creutzfeldt-Jacob Disease (vCJD), have

resulted in the implementation of wide-ranging and costly measures to prevent transmission of these infections.

Other technological developments include the formulation of anticoagulants, additives and replenishing fluids and cryobiology has enhanced blood preservation and the storage period by improving cell viability and survival. Most blood collection and processing steps now utilize a closed sterile system to minimize potential opportunities for contamination of the blood components [12]. Multiple donor pools of plasma can be processed to yield albumin and other derivatives, such as immune globulin fractions for therapeutic use. The emergence and application of numerous laboratory techniques spanning the fields of haematology, cell biology and biochemistry has enhanced and modernised the diagnostic field of transfusion medicine (for review see [1]).

3 Blood collection for transfusion purposes

In developed countries, blood for clinical transfusion is collected by transfusion services (such as those overseen by the Red Cross), the management of which varies in different jurisdictions. In some countries the transfusion service is directly affiliated with hospitals, whilst other countries have one or more independently operated transfusion services. Transfusion services operate in an environment of increasing regulatory control, designed to ensure maximum quality and safety of blood transfusion products. Codes of practice similar to those that apply to the manufacture of medicinal products and devices (i.e., the code of good manufacturing practice) apply to transfusion services.

These regulations also apply to companies that specialise only in the collection of plasma and/or manufacture of fractionated plasma products. Regulatory control covers all aspects of the steps involved in blood collection, processing and storage conditions, ensuring that blood products are manufactured according to standardised procedures to achieve consistency of individual products [13]. Individual jurisdictions have their own regulatory agencies and codes of practice, which overall are similar, but vary in certain aspects.

The primary blood transfusion products are red cell concentrates, platelet concentrates, plasma, cryoprecipitate and cryo-depleted plasma (Figure 1). The clinical uses of these products are shown in Table 1. Plasma can be further fractionated into a range of speciality protein products, including albumin, concentrated coagulation factors and immunoglobulin products (Figure 1), all of which have defined and specific clinical applications.

<Insert Figure 1>

In Australia, the blood supply is managed by the Australian Red Cross Blood Service (ARCBS). Fractionation of therapeutic plasma protein products is provided by CSL (www.csl.com.au/) Bioplasma from plasma collected by ARCBS (Figure 1). In Australia, blood is donated by non-remunerated, volunteer donors. Donors undergo a rigorous screening process that involves a questionnaire and an interview to identify relevant medical history and risk factors that could influence the quality or safety of the

donated blood, or the health of the donor. Every unit of donated blood is also screened for HIV 1 and 2, hepatitis B and C, human T-cell lymphotrophic virus (HTLV) I and II, syphilis and the blood group (ABO system, Rhesus antigen and red cell antibodies). In addition, a certain percentage (a minimum of 1%) of blood products undergo additional, quality assurance testing including volume and cell counts in addition to haemolysis and haematocrit for red cell concentrates, pH for platelet concentrates and clotting factors for plasma products. Donations are also screened for bacteria.

3.1 The Blood Donation Process

Whole blood donation - Blood donation can be divided into two distinct donation processes, namely whole blood donation and apheresis donation. Whole blood donation involves collection of 450 mL (± 10%) blood from the antecubital vein via a 16-gauge needle into the attached specialised PVC plastic blood collection bag typically containing 63 mL anticoagulant. Whole blood donation usually takes 5-10 min. Whole blood donors can donate every twelve weeks providing the donor is healthy and their haemoglobin level is within normal limits. The donated whole blood is then processed into components including red cell concentrate, platelet concentrate and plasma, via centrifugation (*Section 4*). Processing of whole blood must commence within 24 h of blood donation in order to maintain maximum quality of the components.

Apheresis blood donation - Apheresis donation uses a specialised programmable machine that processes over 3.5 L of the donors' blood and separates whole blood into the blood

component that is required (either up to 750 mL plasma or 100-400 mL platelets), returning the remainder to the donor (sometimes with saline fluid replacement). Blood is withdrawn and returned via the 16-gauge apheresis needle (single arm/needle procedure). Whole blood is mixed with anticoagulant and the required blood component (plasma or platelets) is collected into the attached plastic blood bag. Red cell products can also be collected via apheresis, although this is less common than plasma and platelet apheresis. Apheresis donation takes an average of 1 h. Plasma apheresis donors can donate every two to three weeks. Upon completion of the apheresis collection, the blood component requires no additional processing and has been depleted of the majority of white blood cells during the collection via filtration and/or centrifugal conditions built in to the apheresis procedure.

4 Processing of whole blood to separate components

Sedimentation of blood cells is determined by cell size and density. Upon centrifugation (minimum of 1000 × *g*), red cells sediment to the bottom, white cells form a 'buffy coat' layer on top of the red cells and the plasma forms the upper layer. Depending on the centrifugation force utilised, platelets being the smallest and lightest of the blood elements, will either remain suspended in the plasma (soft spin, 2,000-2,800 × *g*), which produces platelet-rich plasma (PRP), or will co-sediment with the white cell-buffy coat layer (hard spin, approximately 5,000 × *g*) resulting in cell-free/platelet-poor plasma [14]. For the preparation of transfusion components, whole blood is collected into an inter-

connected series of sterile collection bags and processed by a 'closed-system' method. For standard processing, following centrifugation the whole blood bag is placed in a pressing device that pushes the plasma into an attached empty bag, and the red cells remaining in the original collection bag are resuspended in an additive solution for storage and preservation (see below for further details). The buffy coat method involves a moderate spin (approximately 3,000 × *g*), which results in a plasma layer, a 'buffy coat' layer comprised of platelets and white cells and a red cell layer. Each layer can be then be fractionated into a separate collection bag by either manual or semi-automated pressing devices. Platelet concentrates prepared from whole blood donations must be pooled in order to achieve a therapeutic dose. Generally, platelets from 4-5 whole donors are pooled. Further details about the methods used to prepare platelet concentrates are discussed by Vassallo & Murphy [15].

Anticoagulants - Anticoagulants prevent blood from clotting. Citrate-based anticoagulants are used for the collection of blood for transfusion (Table 2). Citrate binds calcium, preventing the activation of plasma coagulation factors. EDTA, which binds calcium more strongly compared to citrate, is not used for the collection of blood transfusion products. Heparin is rarely used for the collection of blood transfusion components as its effective anticoagulant half-life (approximately an hour) is limited.

Whole blood is collected into a primary blood bag containing a citrate based anticoagulant (CPD, CPDA-1 or CP2D) and adequate mixing is ensured. The

anticoagulant solution may contain nutrients such as glucose and adenine (Table 2). Additional cell specific nutrients are supplied to the stored red cells [16] and platelets [17] by the additive solutions in the respective red cell and platelet satellite bags (Tables 3 and 4).

Citrate is the most common anticoagulant used in apheresis blood collection. Sodium citrate (4%) is used for plasma apheresis and ACD-A is used for platelet apheresis (Table 2). During an apheresis blood donation, whole blood is mixed with the anticoagulant at a specific ratio as it is collected from the donor. The whole blood is then separated in the apheresis instrument and the required component is retained. The remainder of the whole blood is then returned to the donor.

Storage of blood components - Red cell concentrates and platelet concentrates are stored as fresh products ready for transfusion, although in special circumstances can be frozen using specific cryopreservation procedures. Red cell concentrates are stored refrigerated (2-6°C) for up to 42 days (maximum duration may vary depending on the type of additive solution used and/or local regulatory criteria). Platelet concentrates are stored at 20-24°C for up to 5 days, with continuous gentle agitation to maintain maximum biological function. During storage, red cells and platelets continue to metabolise and undergo a range of physiochemical changes, collectively referred to as the 'storage lesion' [18]. The storage lesion ultimately affects the *in vivo* function and survival of transfused red cells and platelets and thus limits their shelf life. Further details about the storage lesion

can be found in several important reviews [19-22]. Plasma is rapidly frozen to <-25°C in order to maintain maximum function of labile coagulation factors, such as Factor VIII.

4.1 Additional processing procedures

Removal of white blood cells, via filtration - Removal of the white blood cells (leucocytes) is beneficial in reducing alloimmunisation (whereby an individual develops immunity from another individual of the same species against their own cells) to white cell antigens, reducing febrile reactions to transfused blood and reducing white cell borne viruses [23]. White blood cells are typically removed via specialised filters, which reduce the residual white cells by an order of greater than 4 $\log_{10}$ [24] to less than one million white cells per unit of blood product transfused. White cell depleting filters can be used at the whole blood stage or the specialised for component filtration (either platelet or red cell component).

Irradiation of blood products - Gamma irradiation of blood products is beneficial for patients at risk of TA-GVHD, including immunocompromised patients and patients that partly share genetic tissue types with the transfusion donor (i.e. haploidentical) [25, 26]. Selected red cell and platelet concentrates may be gamma irradiated by 25-50 Gray, which inactivates any viable lymphocytes. Platelets are relatively unaffected by gamma irradiation at the prescribed dosage of 25-50 Gray, and their shelf-life is therefore unchanged. Red cells are sensitive to irradiation damage, which causes a range of changes to the red cell membrane, including increased leakage of potassium, and reduces

their survival and function when transfused. Consequently the shelf-life of irradiated red cell concentrates is reduced. Different guidelines apply in different jurisdictions.

Washing of blood products - In a limited number of circumstances, red cell concentrates or platelet concentrates must be washed prior to transfusion for patients with particular susceptibility to transfusion reactions. Washing involves separating the cellular portion of the blood product from the soluble portion, which contains the substances of concern. The cellular portion is then washed a number of times with saline before suspension in saline or other additive solution. For example, platelets require a certain amount of plasma to function normally and other blood products (e.g., red cells or platelets) have reduced shelf life after washing. Washed blood products are transfused to patients who have suffered adverse transfusion reactions previously, have antibodies against IgA, have allergies to particular plasma proteins or are sensitive to elevated potassium or anti-coagulant levels.

Pathogen reduction technology / pathogen inactivation - Pathogen reduction technology (PRT) is a relatively new concept in blood banking and transfusion medicine. The aim of PRT is to inactivate any transfusion transmissible infectious agents, such as viruses and bacteria in the blood product. The use of PRT could reduce the number of screening tests required for donated blood, may allow currently ineligible donors to donate and combats the issue of emerging pathogens. PRT techniques have been developed for pooled plasma, single donor plasma and platelets, and these techniques have been implemented in some countries (Table 5). PRT for red cells is currently in the development phase.

PRT for pooled plasma uses a combination of detergent, solvents, pasteurisation and/or nano-filtration to inactivate pathogens [27]. PRT for single donor plasma and platelets uses a combination of a photoactivating agent such as riboflavin or psoralen and UV light or methylene blue to disrupt the nucleic acid of the pathogen, preventing replication [27-29]. PRT treatment of blood components inevitably causes changes to the protein and cellular elements, the nature of which varies with the different technologies applied. The extent of these changes must be balanced to ensure both safety and clinically efficacy of the blood component.

5 Blood products as components of clinical research

The goal of modern transfusion therapy is to provide appropriate replacement therapy with blood components as opposed to whole blood for patients with specific hematologic deficiencies (Table 1). During the past 20 years significant progress has been made in the technology of blood component preparation and storage [1, 30]. However, variables that impact on analytical outcomes have also become an important focus in modern clinical laboratory tests. Specimens from subjects with various disease stages, as well as those without disease, are critical tools in the discovery and validation for biomarkers, or tests that will detect serious diseases earlier or more readily than current practices. Clinical Laboratory Improvement Amendments (CLIA) certification procedures (http://www.fda.gov/cdrh/clia) include detailed information regarding specimen collection and handling procedures that must be followed to achieve valid test results that can be used for diagnoses. Standardization of collection methodologies are imperative if

research testing results are to be translated to future clinical and diagnostic tests [31]. Multiple studies have begun determining standardised protocols for this important aspect of clinical proteomics, including the Human Proteome Organisation (HUPO) Plasma Proteome Project (PPP) [32] (detailed standard operating procedures for collection of plasma and serum, refer Supplementary Tables I and II). The HUPO report in 2005 detailed an extensive analysis of the variables including - (i) anticoagulant used in collection tube types (e.g., EDTA and ascorbate), - (ii) sample processing times and - (iii) temperatures at which blood specimens are processed and stored, - (iv) sample storage parameters, and - (v) thaw/refreeze cycles, associated with obtaining human plasma and serum samples for proteomic analyses directed towards clinical research. These studies concluded that the stability of blood for diagnostic use is a critical factor, from its collection as an anticoagulated or coagulated source, processing, handling and storage.

The variance of blood specimens may also be affected through differences associated with - (i) patient demographic, - (ii) sample haemolysis, and - (iii) enzymatic degradation over extended periods of time. These factors directly influence many biochemical markers, RNA and proteins that are particularly susceptible to haemolysis and enzymatic degradation, requiring special procedures to maintain their integrity during collection and processing. Several studies monitoring the stability of coagulation proteins during storage have been conducted [33-36], with most concluding that some degree of degradation occurs over time, as most enzymes keep an activity, although minimal, even at -80°C. Rouy *et al*. [37] demonstrated that plasma levels of active matrix metalloproteinase-9 (MMP-9), in contrast to MMP-2, were reduced by 90% after two

years at -80°C. A similar study on various lipid parameters [38] showed a sharp decrease in total cholesterol, triglycerides, and lipoprotein(a) after one year at -80 °C. These studies raise the issue of normalising plasma levels and monitoring their levels as a consequence of sample storage. For clinical assays, it must be determined if the blood or blood fraction sample is stable under the planned storage conditions (i.e., length of time and temperature).

In order to preserve blood and blood fractions in optimal condition appropriate for intended clinical laboratory analyses, all collection and processing steps must be conducted under a well-defined quality assurance program. It is also important to consider new or alternative methods and reagents that may offer longer term stability or increased efficiency in the collection and preservation of blood. Worldwide, the Red Cross is actively pursuing these means. The World Health Organisation also has developed extensive recommendations for control and regulation of human blood for clinical use, from blood products through to transfusion purposes (http://www.who.int/entity/bloodsafety/clinical_use/en/Handbook_EN.pdf).

Bias is a fundamental problem in clinical research. Bias can be prevented at two levels: - (i) by choosing the appropriate study design for addressing the study hypothesis and - (ii) by carefully establishing the procedures of specimen collection and analysis and the definitions of exposures and outcomes [39]. Bias at this level can therefore be reduced through stringent use of standard operating procedures. This was demonstrated by McLerran *et al*. [40] whom acknowledged that patient age of the serum samples being

assayed for the detection of prostatic cancer was a direct source of bias. Even with identical sample processing and storage protocols the conditions of sample collection and processing can influence the diagnostic [41, 42]. A recent study by Thorpe *et al*. [43] investigated the effects of differences in blood collection conditions on clinical validity of three different ovarian cancer markers. This study demonstrated that biases between case and control populations can lead to false positive experimental results and that controlling for conditions of blood collection can reduce false discovery and false validation in biomarker experiments. The authors suggest that for effective diagnostics, validation studies should use standardised collection conditions, use multiple control groups, and/or collect samples from cases prior to influence of diagnosis whenever feasible to detect and correct for potential biases associated with sample collection. Recently, the Early Detection Research Network (EDRN), an initiative of the National Cancer Institute (NCI), established standardization of specimen collection protocols, from a variety of clinical specimens including blood (http://edrn.nci.nih.gov/resources/protocols/proto.xml).

Acknowledgements

We wish to thank Drs Rebecca Cardigan (National Blood Service, England), Pieter van der Meer (Sanquin Blood Service, The Netherlands) and Jason Acker (Canadian Blood Service, Canada) for information about blood component collection and processing in their countries. Funding was provided, in part, by the Australian National Health and Medical Research Council under Program Grant 487922 (RJS, DWG) and a University of Melbourne Post-Graduate Student Scholarship (DWG). We gratefully acknowledge the assistance of Jacqui Glenister in the preparation of Figure 1.

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Table 1. Blood products and their clinical applications and specifications.

The major blood products produced by ARCBS and their clinical indications, storage conditions, average volume and method of production.

Blood Product	Indications	Storage Conditions	Volume (mL)	Production method
Un-refrigerated whole blood, leukocyte depleted	Limited uses, shock	24 h at 20-24ºC.	450 ± 10%	Whole blood derived
Red cell concentrate	Anaemia, blood loss	42 days at 2-6ºC.	>240	Typically whole blood derived
Platelet concentrate	Bleeding due to platelet deficiency, decreased platelet production or decreased platelet function.	5 days at 20-24ºC.	100-400 (Apheresis) >160 (Buffy coat)	Apheresis or Buffy coat method
Fresh frozen plasma	Coagulopathy where the specific factor is not available. Replacement of particular plasma proteins.	12 months at ≤ 25ºC.	250-334 (can range up to 750)	Apheresis or whole blood separation
Cryodepleted plasma	Plasma exchange, particularly for thrombotic thrombocytopenic purpura.	12 months at ≤ 25ºC.	255 ± 10%	Apheresis or whole blood separation
Cryoprecipitate	Fibrinogen deficiency.	12 months at ≤ 25ºC.	30-40	Apheresis or whole blood separation

Adapted from [44-46].

Table 2. Anticoagulant composition.

Composition of the primary anticoagulants used in collection of blood for transfusion.

Constituent (g/L)	**CPD** (citrate-phosphate-dextrose)	**CPDA-1** (citrate-phosphate-dextrose-adenine-1)	**CP2D** (citrate-phosphate double dextrose)	**ACD-A** (acid citrate-dextrose formula 'A')
Trisodium citrate	26.3	26.3	26.3	22
Citric acid	3.27	3.27	3.27	8
Dextrose	25.5	31.90	51.5	24.5
Monobasic sodium phosphate	2.22	2.22	2.22	N/A
Adenine	N/A	0.28	N/A	N/A

Adapted from [45, 46].

Table 3. Red cell additive solution composition.

The composition of the major red cell additive solutions used in the storage of red cell concentrates for transfusion purposes.

	SAG-M (saline-adenine-glucose-mannitol)	**AS-1** (additive solution 1)	**AS-3** (additive solution 3)	**AS-5** (additive solution 5)
Dextrose (mM)	45.4	111	55.5	45.4
Adenine (mM)	1.26	2	2.22	2.22
Monobasic sodium phosphate (mM)	0	0	23	0
Mannitol (mM)	29	41.2	0	45.4
Sodium chloride (mM)	150	154	70	150
Citric acid (mM)	N/A	N/A	2	N/A
Sodium citrate (mM)	N/A	N/A	23	N/A
Manufacturer(s)	Baxter, Fresenius, MacoPharma, Pall.	Baxter	Haemonetics	Terumo
Trade or alternate names	N/A	Adsol	Nutricel	Optisol

Adapted from [45, 46].

Table 4. Platelet additive solution composition.

The composition of the major platelet additive solutions used in the storage of platelet concentrates for transfusion purposes.

	PAS-II (platelet additive solution- II)	**PAS-III** (platelet additive solution- III)	**PAS-IIIM** (platelet additive solution-III-modified)	**Composol**
Sodium chloride (mM)	115.5	77	69.3	90
Sodium dihydrogen phosphate/ disodium hydrogen phosphate (mM)	N/A	26	28.2	N/A
Tri-sodium citrate (mM)	10.8	10	10.8	11
Sodium acetate (mM)	32.5	30	32.5	27
Sodium gluconate (mM)	N/A	N/A	N/A	23
Potassium chloride (mM)	N/A	N/A	5	5
Magnesium chloride/magnesium sulphate (mM)	N/A	N/A	1.5	1.5
Manufacturer(s)	Baxter	Baxter	Macopharma	Fresenius
Trade or alternate names	T-Sol	Intersol	SSP+	

Adapted from [17].

Table 5. Comparison of international blood collection and processing procedures.

A comparison of common blood collection, processing and storage practices in a number of countries and regions.

	Australia	**England**	**The Netherlands**	**Europe**	**Canada**	**United States**
Anti-coagulant used for whole blood collection	CPD	CPD	CPD	CPD	CPD	CPD, CP2D
Anti-coagulant used for apheresis collection	ACDA	ACDA	ACDA	ACDA	ACDA	ACDA
Predominant method for red cell production (i.e., plasma reduction; buffy coat poor; apheresis)	Buffy coat poor	Plasma reduction	Buffy coat-poor	Buffy coat-poor	Buffy coat poor	Plasma reduction
Red cell additive solution(s)	SAG-M	SAG-M	SAG-M	SAG-M	SAG-M	AS-1, AS-3, AS-5 [16]
Methods of platelet production (i.e., apheresis, buffy coat, PRP)	Pooled buffy coat Single donor apheresis	Single donor apheresis Pooled Buffy Coat [47]	Pooled buffy coat Single donor apheresis [47]	Pooled buffy coat Single donor apheresis	Pooled buffy coat, Single donor apheresis, Limited PRP [3]	PRP, single donor apheresis
Platelet suspending medium	SSP+ (buffy coat) Plasma (apheresis)	Plasma	Plasma (75% of buffy coat platelets), PASII (25% of buffy coat platelets); apheresis only in plasma	Plasma, PASII Composol, SSP+	Plasma	Plasma [48]
Prestorage leukoreduction prevalence	Universal	Universal [49]	Universal	Mostly universal [16]	Universal	Widely used [16]
Predominant type of leuko-depletion filter (ie. Whole blood or component)	Component	Whole blood	Component	Varies with country, some countries use whole blood filtration	Component	Whole blood and component
Pathogen reduction technology - plasma - platelets	Nil Nil	Methylene Blue FFP and solvent Detergent Treated FFP are used for particular patient groups.	Nil Nil	- plasma: Methylene Blue and psoralen in some countries; - platelets: psoralen in some regions	Nil Nil	Solvent detergent FFP withdrawn from market. No platelet PRT.

CPD, citrate-phosphate-dextrose; CPDA-1, citrate-phosphate-dextrose-adenine-1; CPD2, citrate-phosphate double dextrose; ACD-A, acid citrate-dextrose formula ‘A’; SAG-M, saline-adenine-glucose-mannitol; AS 1, 3, 5: additive solution 1, 3, 5; FFP, fresh frozen plasma; PAS-II, platelet additive solution-II.