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Testing of buffy coat platelet concentrate pathogen inactivation by Intercept double-dose system in North Estonia Medical Centre´s Blood Centre

Master´s Thesis

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Testing of buffy coat platelet concentrate pathogen inactivation by Intercept double-dose system in North Estonia Medical Centre´s Blood Centre

The aim of this thesis was to develop a method for production of buffy coat platelet concentrates suitable for use with Intercept platelet processing set with dual-storage containers and to assess the conformity of pathogen inactivated platelet products with the required and recommended quality parameters.

The validation of platelet concentrate production using six buffy coats proved the conformity to requirements for platelet content, volume, plasma content, and red blood cell concentration set for platelet concentrates suitable to be processed with Intercept system. After processing with Intercept system, the platelet and white blood cell content were assessed to be appropriate. A little concern was the lower than recommended amount of volume per platelet content in about half of the products, but as those were the units containing about average or higher platelet content and storage analysis showed good platelet recovery and normal pH values at the end of shelf-life, the overall results for volume were also considered to be acceptable. Both the platelet concentrate production and Intercept treatment process were evaluated to be efficient in terms of platelet recovery.

Keywords: platelets, blood processing, blood safety, pathogen inactivation, Intercept

CERCS code: T115 Medical technology

BC trombotsüütide kontsentraadi patogeeninaktiveerimise katsetamine Intercept kahe säilituskoti süsteemiga Põhja-Eesti Regionaalhaigla Verekeskuses

Antud magistritöö eesmärgiks oli välja töötada tootmismeetod, mis võimaldaks valmistada BC trombotsüütide kontsentraate, mis sobiksid töötlemiseks kahe säilituskotiga Intercept trombotsüütide patogeeninaktiveerimise süsteemiga ning vastaksid töötlemise järgselt kohustuslikele ning soovituslikele kvaliteedinõuetele.

Trombotsüütide kontsentraadi valmistamise valideerimisega tõestati, et saadud tooted vastavad patogeeninaktiveerimissüsteemi tootja poolt kehtestatud trombotsüütide sisalduse, mahu, plasma sisalduse ja erütrotsüütide kontsentratsiooni kriteeriumitele ning sobivad seega töötlemiseks Intercept süsteemiga. Intercept patogeeninaktiveerimise süsteemiga töötlemise järgselt hinnati toote trombotsüütide ja leukotsüütide sisaldus sobivaks. Kuigi kõigis toodetes maht ei vastanud soovitatud nõuetele, hinnati see siiski sobivaks kuna antud toodetes oli trombotsüütide sisaldus pigem kõrgem kui keskmiselt ning säilivusanalüüsid näitasid häid tulemusi nii trombotsüütide sisalduse kui ka pH suhtes. Nii trombotsüütide kontsentraadi tootmise kui ka Intercept süsteemiga töötlemise protsess hinnati efektiivseks trombotsüütide saagise koha pealt.

Märksõnad: trombotsüüdid, vere töötlemine, vereohutus, patogeeninaktiveerimine, Intercept

CERCS kood: T115 Meditsiinitehnika

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Abbreviations

BC	buffy coat
CAD	compound adsorption device
DNA	deoxyribonucleic acid
HBC	hepatitis B virus
Hct	hematocrit
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HTLV	human T-lymphotropic virus
NAT	nucleic acid testing
NEMC	North Estonia Medical Centre
PC	platelet concentrate
PI	pathogen inactivation
PLT	platelet
PRP	platelet-rich plasma
RBC	red blood cell
RCF	relative centrifugal force
RNA	ribonucleic acid
RPM	revolutions per minute
SCD	sterile connecting device
TTI	transfusion-transmissible infection
vCJD	variant Creutzfeldt-Jakob disease
WBC	white blood cell

1. Introduction

Though saving lives, transfusion of donor blood also poses risk of various adverse reactions in patients. Since the beginning of transfusion therapy, a lot of effort has been put to enhance the safety of blood products through the development and implementation of screening and processing procedures. The newest concept in blood transfusion safety can be considered the pathogen inactivation technology – a promising advancement for managing the risk of both well-known and emergent transfusion transmissible infections, as well as bacterial contamination.

Since the development of different pathogen inactivation systems, a great number of studies have been conducted to assess the toxicity, pathogen inactivation capability, impact on blood product, and the subsequent clinical outcome for patient. The findings of those studies can be considered more or less universally applicable, provided that the pathogen inactivation system has been implemented according to the requirements set by the producer. The proper validation and subsequent correct usage of the system is the responsibility of the blood establishment and ensures the maximum efficacy and safety the technology can provide.

North Estonia Medical Centre´s Blood Centre is the biggest blood establishment in Estonia, collecting, processing, and providing over half of the country´s blood supply. The author of this thesis is working as a production specialist in the abovementioned organization and had an opportunity to test the pathogen inactivation of buffy coat platelet concentrates by Intercept platelet processing set from October 2014 to March 2015. Testing comprised of two validations and subsequent limited production of pathogen inactivated platelet products. The aim of the testing and this thesis was to develop a method for production of buffy coat platelet concentrates that would meet two sets of requirements: firstly the criteria for platelet products suitable for use with Intercept system and subsequently, after Intercept treatment, the required and recommended quality characteristics for pooled buffy coat platelets in additive solution.

In chapter 2 the composition and functions of blood are briefly introduced, followed by the explanation of main principles for collecting donor blood and processing it into blood components. The main indications for platelet concentrate transfusion are mentioned, as well as the transfusion safety concerns brought out with an emphasis on risks regarding pathogen transmission. Also an overview of pathogen inactivation technologies on market is given and the methods for platelet concentrate pathogen inactivation explained. The experimental work is described in chapter 3 and the results are presented and discussed in chapter 4.

2. Literature overview

2.1 Composition of blood

Blood is a living tissue composed of liquid called plasma and cellular components: red blood cells (erythrocytes), white blood cells (leucocytes), and platelets (thrombocytes). Blood accounts approximately 6 – 8% of adult body weight. Normal adult blood volume for men is 5.0 – 6.0 and for women 4.5 – 5.5 liters. The usual count of cells in one micro liter of blood is 4.2 – 6.2 million for red blood cells (men have usually higher concentrations than women), 7000 – 10 000 for white blood cells, and 150 000 – 350 000 for platelets [1].

Plasma forms about 55% of the total volume of blood and consists of water (about 91.5%) and dissolved organic and inorganic substances such as plasma proteins (albumins, globulins, fibrinogen), organic materials (sugars, amino acids, lipids), inorganic electrolytes, hormones, blood gases, and waste products (urea, creatinine, uric acid, bilirubin). Main functions of plasma include transport of substances, release of heat from the body core, maintenance of osmotic pressure, as well as contribution to the acid-base balance, blood coagulation, and immune responses [2].

More than 99% of blood cells are red blood cells. The hematocrit is defined as the percentage of blood volume that is occupied by red blood cells. The normal hematocrit in men is about 45% and in women 42%. Red blood cells have a size of 7 μm in diameter and a shape of biconcave disk that produces large surface area and makes them very flexible, allowing to be squeezed in small capillaries without rupture. The main functions of red blood cells are to carry oxygen taken in by lungs and carbon dioxide produced by cells. Red blood cells contain large amount of the protein hemoglobin, with which oxygen and, to a lesser extent, carbon dioxide reversibly combine [3].

The site of red blood cell production and differentiation is bone marrow. During the maturation, red blood cells lose their nuclei and other organelles before entering the circulation. After that, they can neither reproduce themselves nor maintain their normal structure for very long. The average lifespan of a red blood cell is approximately 120 days. Red blood cell destruction normally occurs in the spleen or in the liver, and most of the iron released in the process is conserved [3].

White blood cells are a versatile group of blood cells (neutrophils, basophils, eosinophils, monocytes, B and T lymphocytes) that contribute to the immune response in case of foreign

invaders, such as microorganisms. White blood cells differ in size (smallest are lymphocytes, 8 – 10 μm in diameter, and largest are monocytes, 15 – 20 μm in diameter) and cellular composition. They all have nucleus and some of them contain large cytoplasmic granules. All classes of white blood cells are produced in the bone marrow but some types undergo further development and cell division in lymphoid tissues outside bone marrow. White blood cells are part of a system of defensive cells that phagocytize material, detoxify poisons, produce antibodies, and release chemical messengers, enzymes, and other substances. Each type of white blood cells has a different function contributing to an integrated and effective body defense [1].

A platelet is a cell fragment split from a large cell called megakaryocyte. One megakaryocyte gives rise to about 6000 platelets. Megacaryocytes develop in bone marrow and most of them stay there releasing platelets into bloodstream. Some megacaryocytes enter blood and travel to other organs (particularly the lung) where they remain to produce platelets. Circulating platelets have a lifespan of approximately 1 – 2 weeks. If not consumed in the process of blood coagulation, platelets are destroyed by macrophages in the liver and spleen [1].

Platelets are small, about 2 – 4 μm in diameter, membrane-bounded bodies without nuclei. They play several important roles in hemostasis. Following injury, platelets release chemicals to the lining of blood vessels, where they stimulate the contraction of the vessel wall in order to minimize blood loss. Because of their adhesive properties, platelets clump together forming a plug to the vessel wall. In addition, they participate in the formation of factors that initiate coagulation of blood and they release growth factors that promote repair after vascular injury [1].

2.2 Blood collection and processing

By the data collected from 179 countries for the period of 2011 – 2013 by World Health Organization, it was estimated that globally about 112.5 million blood donations are collected in a year [4]. A safe and sufficient supply of blood is an essential part of medical services. In many medical cases the transfusion of blood collected from voluntary donors can be the only life-saving treatment as, despite of an effort to develop artificial blood, no effective alternative to completely replace the donated blood have been found as of today [5, 6].

Blood products are considered to be medication and all the activities required to maintain the sufficient blood supply are the responsibility of blood establishments acting according to the

legislation and recommendations regulating collection, testing, processing, distribution, and quality issues of donated blood [5, 7]. In Estonia, the activity license for the blood establishment is given and the regular surveillance conducted by the State Agency of Medicines [7, 8]. From 2003 there are four blood establishments belonging to hospitals and working independently from each other: North-Estonia Medical Centre´s Blood Centre, Tartu University Hospital´s Blood Centre, Pärnu Hospital´s Blood Centre, and East-Viru Central Hospital´s Blood Centre. Slightly more than half of the donated blood is collected, processed and issued by NEMC Blood Centre [9].

2.2.1 Blood collection

Blood is collected from voluntary donors. Before the donation, the medical condition of the donor is examined (for instance weight taken, hemoglobin and blood pressure measured) and questions asked about the medical history, travel, and lifestyle. All that is done to ensure the donation will be safe for the donor and the blood collected suitable for transfusion [5, 10]. The blood from donor can be taken by whole blood collection or apheresis procedure [11].

In whole blood collection, 450 ml ± 10% of blood is collected by phlebotomy into the primary bag of sterile blood collection system, where it is mixed with anticoagulant to prevent clotting. The volume of blood has to be in the abovementioned range, otherwise the blood cells may be damaged or anticoagulation may not be satisfactory. The actual time for phlebotomy and bleeding is usually less than 10 minutes, but the maximum of 15 minutes is mostly allowed, otherwise clots may form in the tubing before the blood mixes with the anticoagulant in container. The blood collection system is made of plasticized material that is biocompatible with blood cells and can be used only once [10]. After collection, the blood is cooled down to 20 – 24º C and kept at that temperature for up to 24 hours, until further processing [12]. The temperature around 22º C is required to maintain the activity of platelets. The cooling and maintenance of collected blood at room temperature is done by placing the units after collection into specific containers [10].

During apheresis procedure, blood is passed through specific device that separates out one particular constituent of whole blood and the remainder is returned to the donor. Usually the red blood cells are returned as that is the portion of blood that takes the longest to replace and apheresis procedure is used to collect either plasma, platelets, or both. Using this method an individual can donate plasma or platelets much more frequently than they can safely donate whole blood [5, 11].

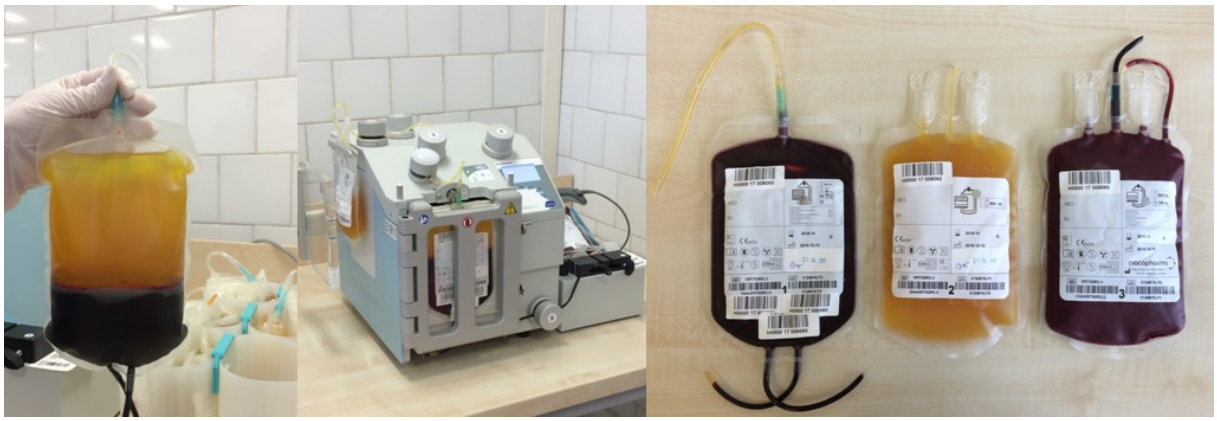
2.2.2 From whole blood to blood components

Starting from collection, some coagulation factors, especially V and VIII, decrease rapidly in quantity and platelets start losing viability in stored whole blood. Therefore, to retain most of the functionality of whole blood constituents and to enable the optimum utilization of limited donor blood, the whole blood is separated into components – plasma, red blood cells, and platelets. As mostly the condition of the patient requires transfusion of just one or few components of blood, and in some situations transfusion of components in ratios not ordinarily found in whole blood is necessary, the whole blood in developed countries is rarely transfused. Despite of the general move to blood component therapy, a widespread transfusion of whole blood has continued in resource poor developing countries [10, 11].

Component production from whole blood consists of centrifugation to separate out plasma and cells of different density, followed by manual or automated transfer of components from the primary collection bag of the blood collection system to transfer packs. The blood collection system contains multiple bags connected by tubing so that the components can be transferred between bags in a closed system to maintain sterility [5, 10].

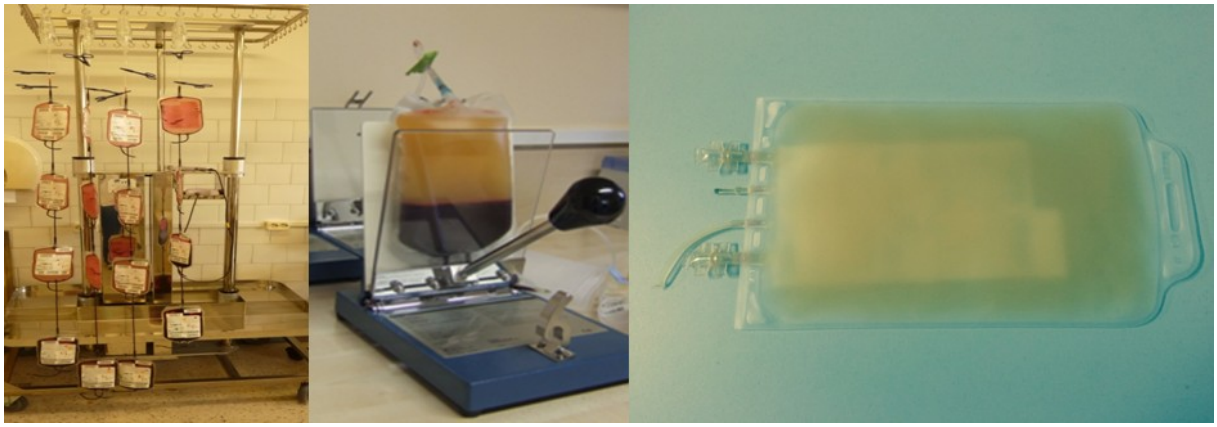
There are two general methods for whole blood processing – buffy coat (BC) method long favored in Europe and adopted recently in Canada and platelet-rich plasma (PRP) method still standard in USA. These methods differ by whole blood centrifugation program, first separation step, and further platelet processing [5, 10, 13].

For BC method, the centrifugation of whole blood uses relatively high centrifugal force and is therefore called hard spin centrifugation, resulting in formation of three distinctive layers – the denser red cells settle in the bottom, leaving plasma on top, while thin white layer called buffy coat, containing most of the platelets and white blood cells, forms in between. With the aid of blood separator, plasma is transferred into empty satellite bag connected to the top of primary bag. Red blood cells are transferred into the satellite bag connected to the bottom of primary bag, where they are mixed with appropriate storage solution, forming red blood cell suspension (see picture 1). The buffy coat layer, with a fraction of plasma and red blood cells, will stay in the primary bag, and those bags, called buffy coat bags, are further used for production of platelet concentrates [10].



Picture 1. Whole blood separation by BC method.

The initial step for platelet concentrate production by BC method is pooling together a number of buffy coats (usually 4 to 6) with platelet storage solution, followed by centrifugation of BC pool with lower centrifugal force (soft spin) program resulting in settlement of heavier cells (red blood cells and white blood cells), while leaving most of the platelets suspended in mixture of plasma and storage solution in the upper part of the BC pool bag. That mixture of plasma, storage solution, and platelets is then transferred either by manual or automated separation devices into the platelet concentrate storage container (see picture 2) [10, 12].



Picture 2. Preparation of platelet concentrate by BC method.

In PRP processing method, a soft spin is used for the centrifugation of whole blood, resulting in settlement of red blood cells and white blood cells in the bottom, leaving most of the platelets suspended in plasma on top. Platelet-rich plasma is then separated into satellite bag and subsequent centrifugation with hard spin is used, resulting settlement of platelets on bottom, allowing separation of almost cell free plasma from top of the container into the next satellite bag. The platelets are then resuspended in about 50 ml of plasma left [10] and 4 – 6

such single unit platelet concentrates can be connected and pooled together to form a therapeutically effective dose [12].

2.2.3 Storage of blood components

To preserve adequate amount of plasma proteins, plasma should be separated from whole blood and frozen within 24 hours of collection. Freezing must take place in a system that allows to bring the core temperature down to -30º C within 60 minutes. After freezing, plasma must be stored at temperature -25º C or below. At that temperature, storage for up to 3 years is allowed. Storing at temperatures from -18º C to -25º C is also allowed, but in this case plasma should be used within 3 months [12].

Red blood cells are stored at controlled temperature between 2º C and 6º C [12]. The storage time depends on anti-coagulant/preservative system but the common red blood cell suspension storage period is either 35 or 42 days counting from the day of collection [11, 12].

Platelet concentrates are stored with constant agitation at 22 ± 2º C in oxygen permeable plastic containers. Platelets should never be placed into refrigerator as this impairs the recovery and survival of platelets following transfusion [5]. The storage time of 5 days can be extended to 7 days if suitable additive solution and detection of bacterial contamination or pathogen inactivation is used [12]. During storage, platelets undergo a fall in pH due to accumulation of lactate and change from discoid to round. Clinical significance of changes in shape and function during platelet storage are debatable and difficult to routinely monitor [5, 14, 15]. Therefore pH remains the only adequate change that should be monitored routinely and must be above 6.4 at outdate [12].

2.3 Clinical use of platelet concentrates

Platelet transfusion therapy has made major contributions to the care of patients with a variety of medical conditions. Most platelet transfusions are used for the prevention of bleeding rather than for treatment of active bleeding [10, 16, 17]. The cause of thrombocytopenia should be established before the decision about the use of platelet concentrate is made as transfusion may not be necessarily indicated. The causes for thrombocytopenia that might require platelet transfusion are chemotherapy for hematologic cancers and solid tumors, hematopoietic stem-cell transplantation, massive blood loss during trauma or surgery, and inherited or acquired qualitative defects in platelets. The decision whether to transfuse platelets should depend on the clinical condition of the patient, the cause of

thrombocytopenia, the platelet count, and the functional ability of patient's own platelets [10, 12, 18, 19].

The widely used term "transfusion trigger" refers to the hematologic value at which a transfusion is given. For platelet transfusions the patient platelet count as transfusion trigger has been frequently used. Prophylactic transfusions are often given when the patient platelet count is less than 5 – 10 x 10^9 in liter. In presence of fever, local injuries or coagulation disorders the prophylactic transfusion in case of platelet count above 15 – 20 x 10^9 in liter may be required [10, 12].

Over the years, growing concerns about unnecessary overtransfusions have risen and a number of studies have been conducted to determine the optimal trigger, optimal dose, and the effectiveness of therapeutic only transfusion strategy for different medical conditions. Some of these trials have indicated that a smaller dose, lower transfusion triggers and a therapeutic rather than preventive strategy could be used with the same clinical outcome [10, 18, 20 - 23].

The number of platelet concentrates transfused in Estonia for the period 2010 - 2015 (separately for BC, apheresis, and all platelet concentrates together) are given in table 1.

Table 1. Transfused platelet concentrate doses in Estonia [24].

Year	2010	2011	2012	2013	2014	2015
BC PC	4134	4902	5712	5795	5279	5622
Apheresis PC	1282	1357	1273	1465	1451	1609
All PC	**5416**	**6259**	**6985**	**7260**	**6730**	**7231**

2.4 Blood transfusion safety

Though saving lives, blood transfusion also poses a variety of risks to the recipient´s health. Transfusion reactions are unintended responses in a patient that are associated with the transfusion of blood or blood products. Transfusion complications can be broadly categorized as immunologic and non-immunologic. Immunologic complications are due to introducing a non-self biologic matter to recipient´s immune system. It´s also possible that white blood cells or antibodies present in donor blood start acting against patient´s cells. Non-immunologic complications are the transmission of a pathogen or can be physiological effects in patient not related to immune system (cardiac overload, citrate toxicity, iron overload, hypothermia).

Adverse reactions from transfusion can be acute (occurring within 6 hours of transfusion) or may emerge days, months or years later [10, 11].

A well legislated and regulated blood transfusion service is a cornerstone for assuring safety of the blood supply within a country. The European Directive 2002/98/EC sets standards relating to blood collection, testing, processing, and storage [25]. These requirements from the EU Directive have been transposed into Blood Act that, together with specifying regulations (based on Blood Act and Communicable Diseases Prevention and Control Act) established by the minister responsible for the area, create the legislation regulating all aspects of blood service in Estonia [7, 26, 27]. In addition to legislation, blood establishments often follow non-binding recommendations such as "Guide to the preparation, use and quality assurance of blood components" to standardize the blood service practice [12].

The blood establishment must have an effective quality management system to control the whole operation. In addition, the hemovigilance system in a country must be set up to ensure the traceability from donor to the recipient and vice versa. Hemovigilance is a set of surveillance procedures covering the entire vein-to-vein transfusion chain (from the donation of blood or its components to the follow-up of recipients of transfusion) intended for collecting and assessing information on unexpected or undesirable effects from the use of blood products. Hemovigilance system allows recognition of risks and improvement of the transfusion system [5, 12, 27].

2.4.1 Transfusion-transmissible infections

A number of pathogens, including viruses, bacteria, protozoan parasites, and one prion are known to be transmitted by transfusion. Measures are in place to control such transmission, including blood donor selection, laboratory testing and component processing [5].

Protozoan infections are endemic mainly in tropical countries. Most notable transfusion-transmissible protozoan infections are malaria (caused by different species in genus *Plasmodium* transmitted by mosquitoes), babesiosis (caused by variety of species in genus *Babesia* transmitted by ticks) and Chagas disease (caused by *Trypanosoma cruzi* transmitted by reduviid bugs). In non-endemic areas like Europe, malaria, Chagas disease, and babesiosis are imported diseases for which prevention depends mainly on deferral of donors for few month after travel to endemic area [5, 11, 28].

It is now clear that at least one prion that causes the variant Creutzfeldt-Jakob disease (vCJD) can be transmitted by transfusion [29]. vCJD is the human form of bovine spongiform encephalopathy (mad cow disease), that can be transmitted to humans through ingestion of tissues from infected cattle. The detection of prion caused disease transmission is difficult, as the incubation period, before the symptoms emerge, is several years. The disease is a concern mainly in the United Kingdom, where domestic plasma was partly eliminated for transfusion and fractionation. Also the universal white blood cell reduction of blood components was implemented, as it was believed to reduce the risk of transmission [5]. Tests suitable for screening donors for vCJD infection and filters for prion protein removal are under development [30 - 32]. In NEMC Blood Centre the donors who have been living in the United Kingdom or Ireland between 1980 – 1996 for more than 6 month or who have had blood transfusion there since 1980 are permanently deferred [33].

Common bacterial infection known to be transfusion-transmissible is syphilis (caused by *Treponema pallidum*) and it was the first infection routinely tested in blood donors since 1950-s [10]. It´s questionable whether the Lyme disease (caused by bacteria in genus *Borrelia*) or other bacteria and viruses known to be transmitted to humans by ticks can be transmitted by blood products [34 - 37] but, for precautionary reasons, in Estonia donors are deferred for two month after tick bite [28].

The most numerous transfusion-transmissible infections are viruses, including human immunodeficiency virus (HIV), hepatitis A, B, C and E viruses (HAV, HBV, HCV, HEV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human T-lympotrophic virus (HTLV), parvovirus B19, etc. [5, 11]. Risk of transmission has become extremely low for HIV, HCV, and HBC infections due to testing of every donation. Residual risk is mostly attributable to window period – a period after infection when donor is already infectious but has a negative test result [5, 10]. Due to implementation of assays based on nucleic acid testing (NAT) technology, the window period has diminished to only few days for HIV and HCV and to two weeks for HBV if testing is done from single donations (not from pools of several donations) [38]. The residual risk for transmission of HIV, HCV, and HBV are usually less than one case per million components transfused [39 - 41].

In Estonia every blood donation is tested with immunoassays for antibodies against HIV, antibodies against HCV, HBV surface antigen, and antibodies against *Treponema palladium*. NAT testing is used to screen donor blood for HIV RNA, HCV RNA and HBV DNA [42].

Those are the most common infection markers screened in donor blood. Some countries additionally test donor blood for other infectious diseases like HTLV (USA, Canada, UK, France, and Australia), malaria for selected donors (France, UK, and Australia), Chagas disease for selected donors (USA, Canada, France, UK), etc. [5].

In addition to well-known transfusion-transmissible diseases, the emerging infections represent a serious challenge for blood transfusion safety: West Nile virus (USA), Chikungunya virus (Indian Ocean), Zika virus (Latin America), Dengue (Asia, Africa, Latin America), etc. [5, 11, 43 - 45]. The strategy to prevent the transmission of the emerging disease depends on the prevalence of the infection in the donor population, existence of the effective treatment for the disease, availability of a suitable screening assay, and recently also the possibility for inactivation of the pathogen through blood product treatment [5].

A possible, but rather unsatisfactory approach would be to focus on the recipient, by diagnosing and treating the infection in case of transmission. This, of course, works only for treatable infections. An example of this approach has been a management of transfusion transmitted babesiosis in USA, though screening assays are now in development to test blood donors in endemic areas [5, 46 - 49].

If the disease is not endemic in the area and is localized in certain regions, so that a possible infection risk can be assessed based on a travel of the donor, the deferral of possible infectious donors based on questionnaire can be a relatively inexpensive method to ensure blood supply safety. Nevertheless, donor´s failure to answer the questions correctly may lead to the collection of an infectious blood unit. It is also not generally possible to define the endemic area so that all those who are infected would be effectively deferred without an unreasonable loss of donors. Therefore, when formulating the restrictions for donors, the possible negative impact on blood availability also has to be considered [5].

Implementation of a screening test is a complex issue. It´s important to consider the prevalence of the disease in donor population, likelihood of the transmission, sensitivity and specificity of the test. Tests that perform well in patient population may result a considerable number of false-positive results in donor population. Strategy to deal with positive test results must be in place – confirmatory testing should be possible and procedures to communicate both the true and false positive test results to donors must be worked out [10].

In the past only serologic tests were available but now NAT testing is also a possibility. As usually considered more sensitive and specific, NAT testing may be a better solution in most cases but not always, as some diseases (particularly parasitic ones) result in a long-term infection with very low levels of infectious agent in the bloodstream. In those cases, a better option would be to test antibodies against the infectious agent in the donor blood as antibodies remain usually in detectable level [5].

A new approach to manage the risk of TTI-s is the pathogen inactivation technology (described in chapter 2.5), implemented in number of countries for today. An example of the use of novel technology to manage the epidemics of emerging virus was the implementation of pathogen inactivation for platelets in the island La Reunion during a large outbreak of chikungunya virus infection [5, 50].

2.4.2 Bacterial contamination

Collected blood may become contaminated with bacteria due to donor bacteremia or by introducing the bacteria from donor skin at the time of collection. The contamination from the environment during processing is rare but can occasionally happen. Bacterial contamination used to be a huge problem for the early blood transfusions but due to implementation of single use sterile needle and blood container, the incidence of contamination has decreased immensely but has not been eliminated completely. Other safety measures to avoid bacterial contamination are deferral of donors based on gastrointestinal disease or recent dental work, effective skin disinfection at phlebotomy site, diversion of the first 20 – 40 ml of collected blood, and maintaining a closed system during blood processing using devices that allow sterile connections and interruptions between blood containers [5, 10].

To further reduce the transfusion of blood contaminated with bacteria, it´s possible to test blood products for bacterial growth. Mostly platelet concentrates are tested due to their higher risk for rapid bacterial growth. But despite of the bacterial culture systems used, it´s been estimated that quite a lot of contamination is still unrecognized. One of the reasons being the low concentration of bacteria at the time of sampling but subsequent rapid growth during platelet concentrate storage. With the approach to let the possible bacteria contaminating the platelet concentrate to incubate a bit before sampling, the product cannot be issued for a day or two, which is a long time considering the short storage period for platelets. Another approach is to sample platelet concentrates on the day of production and incubate the sample for seven days in bacterial detection system. The product can be issued any time during

storage period as long as the bacterial growth has not been detected at the time of release [5, 10, 12, 51]. Due to possible false negative results, it´s been suggested that pathogen inactivation would be a better alternative for bacterial screening to manage the bacterial contamination in platelet concentrates [52].

The magnitude of the clinical problem of contaminated blood products is difficult to define, as mostly only more severe reactions are reported [53]. The severity of reaction usually depends on the patient´s underlying condition, the amount and type of bacteria, and presence of endotoxin in the blood component [10]. The predominant blood component bacterial contaminants are aerobic and anaerobic Gram-positive bacteria that belong to the normal skin flora and, more rarely, Gram-negative bacteria that originate from silent donor bacteremia or is part of the transient skin flora [5, 51]. It´s not possible to completely decontaminate skin and it´s been reported that normal skin bacteria like *Staphylococcus epidermidis* can survive the skin disinfection [54]. Low level bacteremia may occur in the incubation or recovery phase of acute infections after procedures such as tooth extraction [5].

Platelet concentrates are the main concern related to bacterial contamination, as the storage conditions (agitation in a storage solution at room temperature) are suitable for the growth of variety of bacteria. Gram-positive aerobic or facultative anaerobic bacteria are the predominant platelet concentrate contaminants, though strict anaerobic organisms have been found. Although able to survive and proliferate in platelet concentrates, most of them are not considered to be pathogenic [5, 10].

Propionibacteria and *Staphylococci* are the most dominant PC contaminants. *Staphylococcus epidermidis* is a commonly isolated bacteria and transfusions can have a fatal outcome [5, 53, 55]. Missed detection is attributed to low initial concentration and the ability of some strains of *Staphylococcus epidermidis* to form slimy bacterial aggregates that can attach to platelet concentrate storage container [56]. Anaerobe *Proprionibacterium acnes* is another common platelet contaminant but the clinical significance of this bacteria is still debatable [57]. Other bacteria often associated with PC include *Staphylococcus aureus, corynebacteria, Streptococcus, Bacillus,* etc. Although less common, Gram negative bacteria have been found in PC and can cause a severe infection due to endotoxins [5, 53].

Red blood cell suspensions are stored at refrigerator temperature where most of the bacteria will not proliferate [10, 11]. The contamination of red blood cell suspensions with cold-growing organisms is rare and usually involves *Y. enterocolitica* due to its ability to grow at

refrigerator temperatures. There is a rather long lag phase and most observations of contamination have been with units stored for 20 days or longer. Though the organism loses some of its virulence during growth, the endotoxins produced can have a severe clinical effect. As *Y. enterocolitica* infection in normal, healthy individuals is often asymptomatic or may be associated with mild gastrointestinal symptoms, questioning donors about gastrointestinal disease is not very effective for deferring donors who might have circulating organisms. Other red blood cell suspension contaminants are *Serratia spp. Pseudomonas spp, Enterbacteria spp, Campylobacteria spp,* and *Escherichia coli*, all of which have the potential to cause endotoxic shock in recipients [5, 10, 58].

In Estonia, all platelet concentrates must be microbiologically tested using microbial incubation device and culture media suitable for growth of aerobic and anaerobic bacteria, unless the products are processed with pathogen inactivation system [59]. In NEMC Blood Centre BacT/ALERT is used to culture sample of every produced platelet concentrate (both BC and apheresis) in aerobic and anaerobic culture media bottles suitable for platelet concentrate testing. The sample is usually cultured on the day of production and is incubated for seven days. Platelet concentrates can be issued to hospitals on "negative-to-date" basis. If positive result is obtained after issuing, the hospital is informed. The microbial growth in the culture bottle has to be confirmed and bacteria identified. If platelet concentrate has been transfused, the sensitivity to antibiotics is also determined and results are communicated to the hospital. If the platelet concentrate giving the positive result has not been issued, it will be retested. Also the red blood cell suspensions connected to the positive result (through initial donations) are sampled and cultured [60].

In the period of 2010 – 2016, altogether 27 440 platelet concentrates have been tested in NEMC Blood Centre by BacT/ALERT. 154 of them gave positive result (0.6%) and for 62 cases the microbial growth was confirmed (0.2%). The transfusion of 25 contaminated platelet concentrates was prevented but for 37 cases the positive result was obtained after transfusion. The most prevalent identified contaminant was *Propionibacterium acnes* (32 cases), followed by *Staphylococcus epidermidis* (8 cases) and *Staphylococcus hominis* (4 cases). Other identified bacteria included different *Staphylococcus* strains (*saccharolyticus*, *aureus, haemolyticus, capitis,* etc.), but also *Peptostreptococcus prevotii, Dermacoccus nishinomiyaensis, Peptoniphilus asaccharolyticus, Micrococcus luteus, Porphyromonas gingivalis, Leuconostoc mesenteroides, Sphingomonas paucimobilis, Corynebacterium jeikeium,* etc. [60].

2.5 Pathogen inactivation technology

The concept of pathogen inactivation (PI) technology is to damage pathogen nucleic acids using chemicals and/or treatment with visible or UV light. That would halter the replication and infectivity capability of broad range of viruses, bacteria and protozoa without affecting platelets and red blood cells that do not contain nucleus. Unfortunately, PI technology also has no effect on infectious prion proteins [5].

In literature, pathogen inactivation technologies have been also referred to as pathogen reduction technologies. As this terminology can be confusing, it has been suggested to use rather the term "inactivation" for methods that impair the replication and infectivity of pathogens but do not remove them from product (as using the term reduction would suggest) [61].

Pathogen inactivation technologies for plasma have been in use for more than 20 years. First methods were implemented by plasma fractionation companies for large pools of plasma to ensure the safety of produced plasma products. Later on, methods to pathogen inactivate single units of plasma came into market and were suitable for use in blood centers. Subsequent developments have been the adaption of those methods for usage on cellular blood components [5, 62].

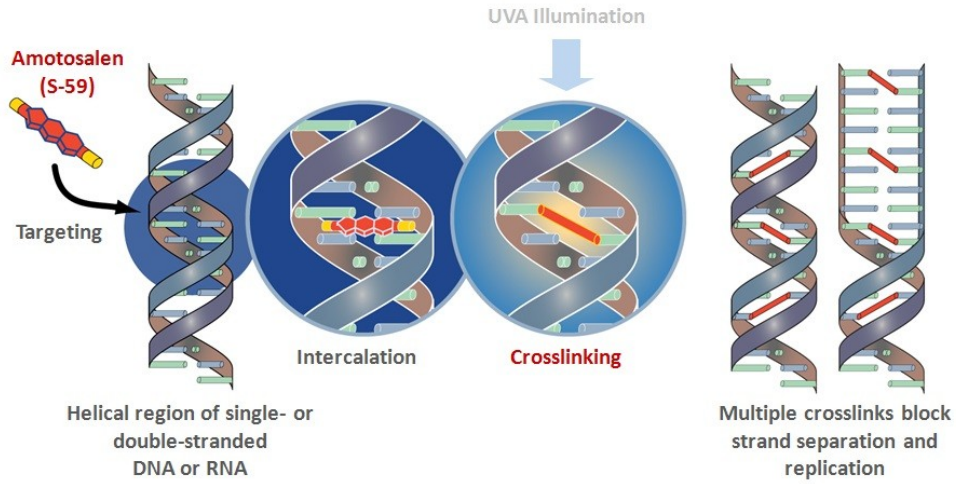
Currently on market there are two methods for pathogen inactivation of plasma only: solvent-detergent method (used at industrial scale) and Theraflex MB. Solvent-detergent method (Octaplas, Octapharma) is used at industrial scale to pathogen inactivate large pools of plasma. Method is based on treatment of plasma with organic solvent in combination with virucidal detergent. These chemicals are extracted later by oil and removed by chromotographic adsorption respectively. Theraflex MB system (MacoPharma) uses methylen blue and is implemented in many European countries for pathogen inactivation of plasma. Method can be applied to a single unit of plasma. Methylen Blue has a natural affinity for nucleic acids and when exposed to visible light (red/white), a photodynamic reaction generates reactive oxygen species, which specifically target the guanine residues of nucleic acids. Residual methylen blue is removed before freezing process [62].

Two methods can be applied for both plasma and platelets: Intercept and Mirasol. These systems are widely used in Europe and Intercept is also approved in the United States. One more system for platelets (Teraflex) is currently on phase III clinical trials stage. Also the

development of one method for red blood cells is under phase III clinical trials (Intercept system for red blood cells that uses amustaline and no UV light activation is required) and Mirasol system is being tested for the adoption to whole blood [62].

2.5.1 Intercept system for platelets

Intercept pathogen inactivation system uses a synthetic photochemical compound amotosalen hydrochloric acid. During illumination with UVA light (320 – 400 nm) this compound reacts with pyrimidine bases of DNA or RNA and forms covalent bond that results in intra- or inter-nucleic acid crosslinks. This kind of crosslinking halts replication of a pathogen. Amotosalen can quickly pass cellular membranes, bacterial walls or viral envelopes and interacts with nucleic acids without interacting with proteins or cellular lipids [62, 63].

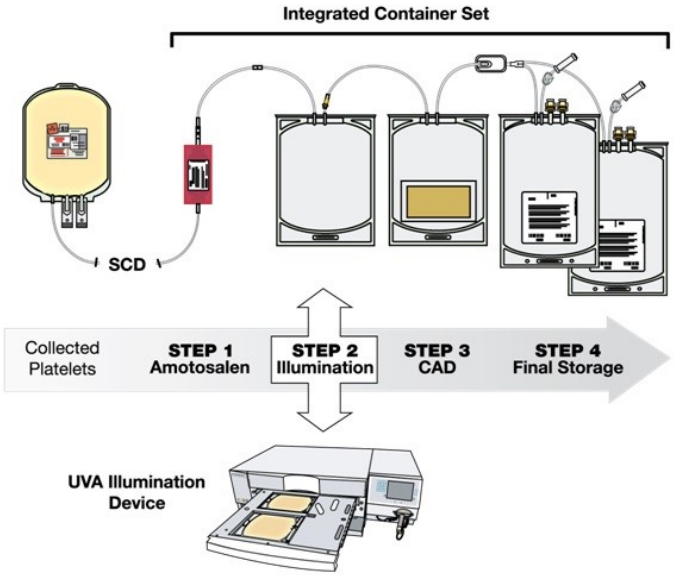


Picture X. Pathogen inactivation mechanism of Intercept system [64].

For treatment with Intercept, platelets have to be suspended with plasma and platelet additive solution. Amotosalen is added and the mixture is exposed to UVA light for 4 – 6 minutes on continuous agitation in Intercept Illuminator [62, 63]. For the removal of most of the remaining amotosalen and the photo by-products of this reaction, the illuminated platelet concentrate has to stay in the container with compound adsorption device (CAD) for 6 – 16 hours at room temperature with agitation. The CAD consists of spherical beads enclosed in a polyester mesh pouch within a plastic container [65]. After CAD phase platelets are transferred and stored in platelet storage bags. All these transfer steps can lead to up to 15% of platelet loss [62, 66].

Platelet concentrates that are to be treated with Intercept double-dose system must meet certain requirements. The plasma content 32 – 47% is required to ensure appropriate UVA

light transmission for maximum inactivation efficacy and minimal impact on platelet viability. Also red blood cells impede UVA light transmission, so the RBC concentration must be lower than 4 x 10^6 in ml. As amotosalen volume in the system is fixed, the volume of platelet concentrate must be between 300 – 420 ml, so that the amotosalen concentration in the illumination container would be 120 – 180 µM. The platelet content before pathogen inactivation must be in range of 250 – 800 x 10^9 as that content of platelets has been validated by the producer during the system development [64].



Picture X. Intercept system for platelets with dual storage container [64].

2.5.2 Mirasol system for platelets

Mirasol pathogen inactivation system uses riboflavin (vitamin B_2), which is a naturally occurring photochemical compound. It binds to nucleic acid (DNA or RNA) bases and upon UV light illumination (at 265 – 370 nm), it oxidizes guanine bases resulting in single strand breaks in nucleic acids. This reaction is a result of direct electron transfer during the oxidation of the guanine bases, which later creates oxygen, peroxide and hydroxyl radicals that breaks DNA and RNA. These breaks are irreversible and cannot be repaired by any endogenous repair mechanism of pathogens. Riboflavin and its byproducts do not need to be removed after UV illumination as they are anyway present in natural foods and also in blood [62].

The treatment consists of adding riboflavin to the illumination bag containing the platelet concentrate unit. The platelet unit containing riboflavin is then subjected to UV illumination

in a controlled temperature and agitation for 10 minutes. About 3% of platelets are lost from bag to bag transfer [62, 67].

2.5.3 Theraflex system for platelets

The Theraflex pathogen inactivation system for platelets does not involve adding a photochemical compound. It uses short wavelength monochromatic UVC light (254 nm) that has a unique ability to interfere with DNA and RNA replication while preserving protein integrity. Short wavelength UVC light leads to the formation of intra-strand or inter-strand cyclobutanepyrimidine and pyrimidine-pyrimidone dimers on DNA and RNA, subsequently halting replication. The Theraflex UVC treatment of PC consists of an illumination device (Macotronic UV illuminator) and processing kit (that contains an illumination bag, a storage bag, and a sampling bag). The platelet concentrate in illumination bag is placed into the UV illuminator under continuous high-speed agitation and exposed to UVC light for one minute. Platelets are then transferred to the storage bag. The whole treatment takes approximately 8 minutes [62, 68].

2.5.4 Concerns regarding pathogen inactivation technology

While the concept of inactivating pathogens in blood components is a promising method to achieve a whole new level in blood transfusion safety, the new treatment of blood also gives rise to multiple concerns. In addition to cost-effectiveness, three major criteria for the pathogen inactivation technology have been defined: it must target a broad spectrum of pathogens, it must not cause damage to the product, and it must be safe for the recipient [62, 69].

To evaluate the efficacy of PI technology, multiple in vitro studies with products spiked with pathogen have been conducted. On those studies different pathogen inactivation systems show different inactivation capability for different pathogens. The problem arises from the fact that it has not been defined how much inactivation is enough, as it is not well known what is the minimal concentration of different pathogens that can cause the infection. Most probably the final evaluation of the efficacy of pathogen inactivation systems is possible only after certain period of routine use, comparing the hemogivilance data before and after the implementation [62, 63, 68 - 70].

For the approval of the pathogen inactivation system, thorough toxicity studies in animal models and clinical trials are required. The chemicals used and created during pathogen

inactivation process may pose a risk to the recipient´s health. In addition, those agents can modify the composition of blood components in a way that could trigger the immune response. As PI chemicals are, by design, capable of interacting and permanently modifying nucleic acids, they have a potential of being genotoxic, carcinogenic, and harmful for the reproductive system. The potential toxicity of PI chemicals to patients needs to be studied for both acute and chronic administrations. No higher frequency of adverse reactions have been shown for PI technologies currently on market [63, 65, 68, 69] but the PI system developed for red blood cells (by Cerus corporation) had a drawback in III phase clinical trials when some of the patients formed antibodies against neoantigens formed on red blood cells during the PI process and the chemical amustaline (S-303) used in the PI system had to be changed in order to prevent such reactions in patients [71, 72].

The chemicals used in PI technology may interact with the cellular and plasma components of the transfusion product. It has been shown in *in vitro* studies that pathogen inactivation process can change the composition and function of the transfusion product. The significance of this effect can be to some extent evaluated by the *in vitro* characteristics and by predicting the *in vivo* importance of these changes. But the studies focusing on *in vivo* effects of PI products and on the final clinical outcome have shown that *in vitro* tests may not always correlate very well with *in vivo* performance as the clinical outcome in some of these studies has not been worse for PI blood components compared to control group. On the other hand, concerns are still existing that PI technology may result in increased demand for blood components due to losses in functionality and more studies are required to assess the effect of PI technologies on blood products [62, 67 - 69].

The whole capacity of PI technology to ensure blood safety cannot be used until systems suitable for red blood cells or whole blood are available for use, as only after implementing PI technology for all blood components, is it possible to get out the maximum of the benefits for the blood transfusion safety that PI technology can offer. Future will show if possibility to pathogen inactivate all blood products will remain as a complementary safety measure or will it also replace at least some of the screening and processing required today to ensure blood transfusion safety [5].

3. Experimental

3.1 Production- and measuring devices

The following devices were used in production of platelet concentrates and Intercept pathogen inactivation process:

- Heraeus Cryofuge 6000i centrifuges with rotor length 29.7 cm (Thermo Electron) for centrifugation of whole blood and pooled buffy coat.
- MacoPress Smart automatic blood components separators (MacoPharma) for the separation of centrifuged whole blood into plasma, red blood cell suspension, and buffy coat.
- Hematron III heat sealers (Fenwal) for sterile sealing of tubing.
- TSCD II sterile connecting devices (Terumo) for sterile welding of tubing.
- Manual expressers (Fenwal) for separation of platelet concentrate from centrifuged pooled buffy coat.
- CompoLift stand (Fresenius Kabi) for hanging blood bags during production processes.
- Intercept Illuminator INT-100-50 (Cerus Corporation) for illuminating platelets with UVA light as part of Intercept pathogen inactivation process.
- Helmer PC1200 platelet incubator and PFS84 platelet agitator (Helmer Labs) for agitating platelet concentrates during the CAD phase of Intercept pathogen inactivation process and for storage of platelet concentrates until expiry.

The following measuring devices were used:

- Sartorius BP8 scales (Sartorius AG) for measuring the weight of tare and of product at different steps of production.
- Ruler for measuring the length of tubing at different steps of production.
- Sysmex KX-21N automated hematology analyzer (Sysmex Corporation) for measuring hematocrit, platelet concentration and white blood cell concentration.
- Inolab pH 730 pH meter with SenTix 81 electrode (WTW) for measuring pH of platelet concentrates
- Intercept Platelets Colour Chart (Cerus Coropration) for assessing the RBC concentration in platelet concentrates.

3.2 Materials and aids in production process

The following materials and aids were used in production:

- Triple blood bag system comprised of 600 ml bag with 63 ml of CPD solution, 500 ml bag with 100 ml of SAGM solution, and 500 ml empty bag (MacoPharma; REF: 1MAMRT6281LU or 1MAMRT6280LU) for collection, centrifugation, and separation of whole blood.
- SSP+ 300 ml storage solution for platelets (MacoPharma; REF: SMASSP2030U) for production of 6BC platelet concentrates.
- Storage container for platelet concentrates (Fenwal; REF: 1FE00R7041) for production of 6BC platelet concentrates.
- Intercept platelet processing set with dual storage containers (Cerus; REF: OCEINT2503) for pathogen inactivation of 6BC platelet concentrates;
- Different pouch bags for sampling.
- Clamps.

3.3 Weight of tare

For the purpose of calculating the volume of the product at different steps of platelet concentrate production and Intercept pathogen inactivation process, all blood bags that were used in the process were weighed with specified tubing length. The tare weights with description and pictures are presented in appendix 1, table 4.

3.4 Buffy coat production

Approximately 450 ml of whole blood collected into 600 ml blood bag with 63 ml of CPD solution (part of triple blood bag system) was centrifuged by the following program:

RPM 3500

RCF 4068

Acceleration 7

Deceleration 5

Time 15 min

Temperature 22° C

Centrifuged whole blood was separated with automatic blood separators into plasma (500 ml empty blood bag), red blood cell suspension (500 ml blood bag with 100 ml of SAGM solution), and buffy coat (remained in the 600 ml blood bag that was used for collecting whole blood). The separation was done by the program validated in 2013.

3.5 Validation of 6BC platelet concentrate production

To assess, whether using 6 buffy coats and 300 ml of SSP+ solution we obtain platelet concentrates that meet the requirements set for Intercept pathogen inactivation process, 12 6BC platelet concentrates were made as described below.

6 BC bags and a bag of SSP+ solution were connected using SCD in a row, keeping between the buffy coat bags the length of plasma tube 15 cm and the length of RBC tube as short as possible, and between the BC bag and SSP+ solution bag the plasma tube of BC bag 20 cm.

The chain of buffy coats and storage solution was hanged onto the stand and blood in all buffy coat bags were let to flow into the lowest buffy coat bag. The buffy coat bags were rinsed by letting the storage solution flow from top into the lowest buffy coat bag (rinsing was done twice, using both times about half of the storage solution volume). The pooled buffy coat bag was sealed off from the chain (leaving the hose length 20 cm), weighed and sampled. The pooled buffy coat bag was connected to the storage container of platelet concentrate using SCD and centrifuged by the following centrifugation program:

RPM 1180
RCF 462
Acceleration 5
Deceleration 3
Time 9 min
Temperature 22° C

The platelet concentrate was separated into the storage container from the centrifuged pooled buffy coat by manual expresser and the pooled BC bag was sealed off. The platelet concentrate in storage container (with hose length 10 cm) was weighed and sampled.

The color of platelet concentrate was assessed by visual inspection (for the estimation of RBC concentration) with the aid of Intercept platelets color chart. Sample of pooled buffy coat and 6BC platelet concentrate were analyzed by hematology analyzer in quality control laboratory of NEMC Blood Centre.

3.6 Validation of 6BC platelet concentrate pathogen inactivation by Intercept platelet processing set with dual storage containers

Validation of pathogen inactivation of 6BC platelet concentrate comprised of producing 12 6BC platelet concentrates, following the treatment with Intercept platelet processing set with dual storage containers. The purpose of the validation was to see the parameters of the final platelet concentrate product.

The 6BC platelet concentrates were made in the same way and the same data was collected as during the validation of 6BC platelet concentrate production (see chapter 3.5). Additionally, also the weight of 6BC PC after sampling was taken.

The platelet concentrate storage container was connected to the amotosalen container of Intercept pathogen inactivation system by using SCD and hanged on the stand. Both the lower and the higher cannula of amotosalen container were broken and the 6BC platelet concentrate was let to flow with 17.5 ml of amotosalen solution into the illumination bag. Platelets in illumination bag were thoroughly mixed by gentle agitation and the air from illumination bag was expressed to amotosalen container. Small amount of platelet and amotosalen mixture was expressed to the tubing connecting illumination bag with amotosalen container and the amotosalen container with empty platelet concentrate storage container was sealed off so that the tubing was no longer than approximately 4 cm from the illumination container inlet port. The discarded amotosalen container with platelet concentrate storage container was weighed.

Platelet concentrate in illumination container was illuminated with UVA light for 4 – 6 minutes by using the Intercept Illuminator. Illuminated platelets with Intercept system were hanged on the stand, the cannula was broken and the platelet concentrate was let to flow into CAD container. Using manual expresser, the air was expressed from CAD container to illumination container and the tubing between CAD and illumination container was sealed close to the inlet port of CAD container. Empty illumination bag was removed and weighed. CAD container with platelets was placed on the flat agitator until early next morning, making sure the CAD agitation duration was between 6 to 16 hours.

After CAD agitation, the platelet concentrate in CAD container was hanged on the stand, air from platelet sampling pouches (one on both storage containers) was expressed, and the sampling pouches were closed with clamps. Cannula was broken and platelets were allowed to flow into both of the two storage containers. Air was expressed from both storage

containers into CAD container, the tubing was clamped and sealed above the Y-fitting, and the empty CAD container was removed and weighed. The platelet concentrate in two connected storage containers was weighed, sample of platelet concentrate was taken into one of the two pouches, and both pouches were sealed off. The platelet concentrate was equally divided between the two storage containers and both containers were sealed off from the Y-fitting (leaving the storage container tubing 10 cm) and weighed.

Sample of pooled buffy coat, platelet concentrate before and after the Intercept treatment were analyzed by hematology analyzer and pH of the Intercept treated platelet concentrate was measured in quality control laboratory of NEMC Blood Centre. From platelet concentrates obtained from 5 Intercept pathogen inactivation processes (all together 10 PC products) the sample was taken on expiry (7^{th} day of storage) to be analyzed by hematology analyzer and pH meter.

3.7 Production after validations

After validation, 47 6BC platelet concentrates were processed with Intercept pathogen inactivation system (from January to March 2015), producing all together 94 pathogen inactivated platelet concentrates. For 26 Intercept processes, the weight of the 6BC PC after sampling and Intercept treated platelet concentrates after sampling and splitting were taken and the samples of PC before and after the Intercept treatment were analyzed by hematology analyzer.

3.8 Collected data and calculations

3.8.1 Collected data

The following data was collected during validation of 6BC PC production:

- For BC pool: weight including tare before sampling, PLT concentration, hematocrit.
- For 6BC PC: weight including tare before sampling, PLT concentration, RBC concentration (visual assessment of acceptability).

The following data was collected during validation of 6BC PC pathogen inactivation with Intercept platelet processing set with dual storage containers:

- For BC pool: weight including tare before sampling, PLT concentration, hematocrit.
- For 6BC PC: weight including tare before and after sampling, PLT concentration, RBC concentration (visual assessment of acceptability).

- During the Intercept treatment, the weights of the following discarded containers were taken: PC storage container connected to amotosalen container, illumination bag, CAD container. Also the weight of Intercept treated PC before sampling and split was taken.
- For Intercept treated PC: weight including tare after sampling and split, PLT concentration, WBC concentration, pH.
- For 10 Intercept treated PC (from 5 Intercept treatment processes) on 7th storage day: PLT concentration and pH.

After validation, the following data was collected for 26 Intercept treatment processes:

- For 6BC PC: weight including tare after sampling, PLT concentration.
- For Intercept treated PC: weight including tare after sampling and split, PLT concentration and WBC concentration.

The measured data is presented in appendices 3 and 4 (marked with light pink background color).

3.8.2 Calculations

From the measured data, following quantities were calculated:

- Volume of BC pool before sampling, 6BC PC before and after sampling, Intercept treated PC after sampling and split were calculated by the formula 1. The density of BC pool was estimated to be 1.04 g/ml [73] and the density of PC composed of approximately 30% of plasma and 70% of SSP+ storage solution to be 1.01 g/ml [74].

$$\boldsymbol{volume\ of\ product\ (ml)} = \frac{\boldsymbol{weight\ of product\ including\ tare\ (g) - weight\ of\ tare\ (g)}}{\boldsymbol{density\ of\ product\ (\frac{g}{ml})}} \quad (1)$$

- PLT content of BC pool before sampling, 6 BC PC before and after sampling, Intercept treated PC after sampling and split (including 7th storage day analysis) were calculated by the formula 2. Also the WBC content of Intercept treated PC after sampling and split was calculated by the formula 2.

$$\boldsymbol{cell\ content\ in\ product\ (\frac{10^9}{unit})} = \frac{\boldsymbol{cell\ concentration\left(\frac{10^3}{\mu l}\right) \times volume\ of\ product\ (ml)}}{\boldsymbol{1000}} \quad (2)$$

- Plasma content of 6BC PC was calculated by the formula 3. The corrected volume 290 ml of SSP+ storage solution was used for calculations as approximately 10 ml was estimated to be lost in containers during production.

$$\textbf{\textit{plasma content}}\ (\%) = \left(1 - \frac{\textbf{\textit{volume of SSP+solution in BC pool (ml)}}}{(1-\textbf{\textit{hematocrit of BC pool}}) \times \textbf{\textit{volume of BC pool (ml)}}}\right) \times 100\% \quad (3)$$

- Platelet recovery for 6BC PC production process, Intercept treatment process and storage were calculated by the formula 4. For 6BC PC production process the PLT content of BC pool before sampling and of 6BC PC before sampling were used. For Intercept treatment process the PLT content of 6BC PC after sampling and the PLT content together in both final PC doses after sampling and split were used. For platelet recovery of storage, the PLT content of the final product (Intercept treated PC after sampling and split) after production and on the last day of storage (7^{th} day) were used.

$$\textbf{\textit{platelet recovery}}\ (\%) = \frac{\textbf{\textit{platelet content at the end of the process}}\left(\frac{10^9}{unit}\right) \times 100\%}{\textbf{\textit{platelet content in the beginning of the process}}\left(\frac{10^9}{unit}\right)} \quad (4)$$

- For the estimation of volume losses during Intercept treatment, the residual volume of PC left in PC storage container connected to amotosalen container, illumination bag, and CAD container, also the volume of Intercept treated PC before sampling and split were calculated using formula 1. The volume lost during sampling and splitting was calculated by the formula 5.

$$\textbf{\textit{PC vol lost}}\ (\textbf{\textit{ml}}) = \textbf{\textit{PC vol before split}}\ (\textbf{\textit{ml}}) - \textbf{\textit{vol of both PC doses after split}}\ (\textbf{\textit{ml}}) \quad (5)$$

The calculated values are presented in appendices 3 and 4 (marked with light green background color).

3.8.3 Estimation of measurement uncertainties

To assess the reliability of results obtained during the experimental work, the measurement uncertainty estimations are given both for directly measured and calculated quantities, as well as for other quantities used in calculations (SSP+ volume in BC pool, density of BC pool and PC). The explanations of obtaining the uncertainty estimates are given below and the numerical values of combined standard uncertainties (u_c) and expanded uncertainties (U, k = 2) are presented in appendix 5, table 11.

The standard uncertainty of weight was taken from the calibration certificate (ATLM-14/0303) of Sartorius BP8 scales issued by AS Metrosert in April 2014. For the weight of tare, in addition to uncertainty from scales (1 g), also the difference between containers used in production and container used for measuring the weight of tare (resulting from variation in

container production, measuring the length of tubing, and sealing) were taken into account and estimated to be up to 2 g. The combined standard uncertainty that takes into account both uncertainty sources was calculated for the weight of tare by the formula 6.

$$\boldsymbol{u_c = \sqrt{u_1{}^2 + u_2{}^2}} \quad (6)$$

The uncertainty of measurements obtained by hematology analyzer Sysmex (hematocrit, PLT concentration, WBC concentration) comprised of two sources: the uncertainty component accounting for random effects was calculated using routine laboratory quality control results for 2014 and the component accounting for systematic effects was calculated using inter-laboratory comparison results for 2014. The calculations were done using the Nordtest approach [75]. The combined standard uncertainty was calculated using formula 6.

For pH measurements, the uncertainty was calculated by the web application provided by Testing Centre of University of Tartu [76]. The highest uncertainty of individual results is presented and generalized to all pH measurements.

For the SSP+ solution volume in BC pool two uncertainty sources were taken into account. The possible deviation of the initial volume in storage solution container from the indicated 300 ml was estimated to be up to 3 ml. The maximum differences in SSP+ volume lost during production (residues left in SSP+ container, connected BC bags, and tubing), from the 10 ml that was taken into account in calculations, was also evaluated to be up to 3 ml. The combined standard uncertainty was calculated according to formula 6.

To obtain uncertainty estimates for calculated results: volume, plasma content, PLT and WBC content in product, the ISO GUM modeling approach was used [77] and the uncertainty calculations were done by the Kragten spreadsheet method [78]. The formulas 1 - 3 were used as measurement models and the uncertainties of input quantities are presented in appendix 5, table 11. The uncertainty calculations were done for individual results and as the calculated uncertainty values differed to a small degree, the highest uncertainty is presented and generalized to all corresponding results.

The expanded uncertainties were calculated by formula 7. The coverage factor k = 2 was used, giving the confidence level about 95%.

$$\boldsymbol{U = u_c \times k} \quad (7)$$

4. Results and Discussion

4.1 Buffy coat adjustment

During the validation of separation process from July to November in 2013 the separation program settings for four MacoPress Smart separators for use with MacoPharma triple blood bag systems were adjusted. The goal was to optimize the separation process in order to achieve buffy coasts with as low volume and hematocrit as possible without hindering the quality of plasma and red blood cell suspension. The purpose for low volume and hematocrit was to obtain buffy coats suitable for the production of platelet concentrates that meet the requirements set for Intercept treatment.

Prior to the changeover to MacoPress Smart separators, Dualpress separators (LMB Technologie GmbH) were used to separate the whole blood (collected into MacoPharma triple blood bag systems) into plasma, red blood cell suspension and buffy coat. In June 2013 the buffy coat volume was in range of 51 – 60 ml, with an average 56 ml and BC hematocrit was in range of 0.42 – 0.54, with an average 0.50 (the results of the regular quality control tests in June 2013 are presented in appendix 2, table 5).

With that large volume and high hematocrit it was not possible to produce platelet concentrates that would meet the requirements set for Intercept treatment. The desired number of buffy coats used for production of double-dose platelet concentrate would be 7 or 8, but at least minimum 6 would be required. Therefore the maximum capacity of buffy coat bag (600 ml) that would be used for pooling the buffy coats, set the restrictions for buffy coat volume (considering that 300 ml of SSP+ would be used). On the other hand, by diminishing the volume also lower hematocrit was required to meet the norm of plasma content. Hence, the reduction of buffy coat volume had to come from reduction of red blood cells while keeping the appropriate amount of plasma in buffy coat.

In August 2014 the volume range of buffy coat was 47 – 52 ml, with an average 49 ml. The range for hematocrit was 0.35 – 0.51, with an average 0.41 (the results of the regular quality control tests in August 2014 are presented in appendix 2, table 6). These indicators were assessed to be suitable for production of platelet concentrates from 6 buffy coats, as the volume did not allow addition of no more. Further reduction of buffy coat volume was not possible without seriously compromising the quality of red blood cell suspension, as

minimizing the amount of red blood cells in buffy coat led to excess amount of white blood cells in red blood cell suspension.

4.2 6BC platelet concentrate production

The results for 6BC PC production are presented in appendix 3, table 7. The average, standard deviation, minimum, and maximum values are brought out separately for 6BC PC production validation, Intercept pathogen inactivation process validation, and for all 24 units together.

The requirements for platelet concentrates acceptable for use with Intercept platelet processing set with dual storage containers are given in table 2.

Table 2. PC acceptance criteria for Intercept treatment [64].

Platelet content	Volume	Plasma content	RBC concentration
$2.5 – 7.0 \times 10^{11}$	300 – 420 ml	32 – 47 %	$< 4 \times 10^{6}$/ml
$7.1 – 8.0 \times 10^{11}$	375 – 420 ml		

The platelet content of 6BC PC was in range of $469 – 714 \times 10^{9}$, with an average 555×10^{9}. The volume was in range of 362.4 – 394.1 ml, with an average 379.1 ml. All except one 6BC PC fell in platelet content range of $2.5 – 7.0 \times 10^{11}$ and the corresponding volumes were in acceptable range 300 – 420 ml. The conformity to criteria held also when considering the expanded uncertainty for 6BC PC platelet content $\pm 14 \times 10^{9}$ and for volume ± 6.2 ml. Only one 6BC PC, with the highest platelet content 714×10^{9}, was in range of $7.1 – 8.0 \times 10^{11}$ and the corresponding volume was 375.2 ml. Both the platelet content and the volume fell in-between the ranges as, when taking into account the uncertainty, the conformity to either one of the platelet content – volume ranges was possible. The possibility that platelet content and volume would fall in different ranges was considered relatively unlikely and not too relevant if both quantities would be close to changeover from one acceptability range to another.

The plasma content of 6BC PC was in range of 34.6 – 37.6 %, with an average 36.2 %. All 6BC PC had acceptable plasma content also considering the expanded uncertainty ± 2.4 %.

For 6BC PC products the conformity to the RBC concentration criteria was assessed by the visual inspection with the aid of colour chart provided for use with Intercept system. All 24 6BC PC were with yellow or light orange color and considered to meet the criteria.

In addition, to evaluate the efficacy of the 6BC PC production process, the recovery of platelets was calculated. The results were in range of 71 – 92%, with an average 82%. While for other measured and calculated quantities, the average, standard deviation and range were similar for validation of 6BC PC production and validation of Intercept treatment process, so that the differences could be attributed to the normal variability of donor blood and production process, the difference for platelet recovery was outstandingly bigger. Though the average was quite alike (83% compared to 81%), the standard deviation and range were considerably bigger for validation of Intercept treatment (6.4% and 71 – 92% compared to 2.3% and 77 – 87% for 6BC PC production validation).

As the obtained platelet recovery values were not found to correlate with none of the characteristics of BC pool, the author of the thesis believes that the high variability of platelet recovery can be ascribed to the inconstancy of centrifuged BC pool separation process with manual presses, caused by the not well controlled duration of separation time, change in speed of flow during separation, and endpoint of separation. Whether the separation process of centrifuged pooled buffy coat could be more standardized using automatic blood separators and would it result in overall better recovery or not would need a further investigation. But despite of the high variability of platelet recovery, the validated 6BC PC production process was assessed to be overall performing well and fit for manufacturing platelet concentrates suitable for Intercept treatment.

4.3 Intercept treatment process

The data for 6BC PC treatment with Intercept double-dose system are presented in appendix 4. In table 8 the results for the final platelet concentrate product (Intercept treated PC after sampling and split) are presented. The average, standard deviation, minimum, and maximum values are brought out separately for validation of Intercept treatment process, production after validation, and for all 38 processes. The results were assessed to be similar in two groups and the minor differences could be attributed to the normal variability of donor blood and production processes. The storage analysis are presented in table 9 and the volume losses during Intercept treatment process in table 10.

The requirements from the legislation and the recommended quality parameters by the “Guide to the preparation, use and quality assurance of blood components” for pooled BC platelets in additive solution are given in table 3.

Table 3. Requirements and recommendations for pooled BC platelets in additive solution.

Source of requirement/ recommendation	Platelet content	Volume	WBC content	pH (at the end of shelf-life)
Quality requirements by legislation [59]	As validated by the blood establishment	Must assure the compliance with the pH requirement	$< 0.15 \times 10^9$ for 3BC PC ($< 0.05 \times 10^9$ per BC)	> 6.4
Guide recommendations [12]	$> 200 \times 10^9$	> 40 ml per 60 $\times 10^9$ platelets	$< 0.3 \times 10^9$	> 6.4
	75% of conformity required	-	90% of conformity required	-

The platelet content of the final platelet concentrate at the end of production was in range of 205 – 319 x 10^9, with an average 256 x 10^9. When taking into account the expanded uncertainty ± 10 x 10^9, 4 products could be below the limit and the conformity to platelet content requirement would be 95%.

The 75% conformity to platelet content should be met also at the end of shelf life. The platelet recovery at storage was in range of 93 – 97% , with an average 95%. Applying the lowest (93%) recovery on all results and also considering the expanded uncertainty of the final product platelet content (± 10 x 10^9), 11 products were found to be at risk of falling below the limit by the time of expiry, making the conformity to platelet content requirement 86%.

The volume of the final PC was in range of 163.4 – 189.1 ml, with an average 175.2 ml. The proper platelet content – volume ratio is needed to keep the optimal storage conditions for platelets. For 32 products the volume per platelet content was smaller than recommended, 6 more could fall below the volume limit if the expanded uncertainty ± 5.2 ml was taken into account. As those were products with higher platelet content (majority of them had platelet content above the average, only three had slightly below), there was not much concern, even if the platelet loss during storage would be somewhat higher than on average. Out of 10 platelet concentrates used for storage analysis, 4 had lower than recommended volume, but all of them had average or higher platelet recovery. Though storage analysis did not show lower platelet recovery for products that did not meet the volume criteria, the number of products analyzed was too small to make any firm assumptions whether the deviation from the recommended platelet content – volume ratio would result in somewhat higher platelet losses during storage.

To assess where and how much volume was lost during Intercept treatment process, the volume losses at different steps of the process were calculated. As expected, the amount and variability in losses was in accordance with the complexity of the container. Highest volume was lost in CAD container where platelet concentrate can stay in CAD or filter pouch (attached to the tubing for catching the smaller pieces in case of breakdown of CAD). Much less volume was lost in connected platelet concentrate storage container and amotosalen container, as well as during the sampling and splitting of Intercept treated PC. In routine work only a portion of products would be sampled, saving a few ml in most of the products in this step. As could be assumed, the volume that stayed in illumination container was relatively small compared to other processing steps.

Some residues in containers are inevitable and the volume losses were quite constant in all the other steps except for CAD, for which not much can be done to minimize the volume lost, as the CAD container needs to be handled with extra care not to cause breakage. Looking at the calculated volume losses and based on visual assessment of residues left in discarded containers during processing, the handling of the system was evaluated to be sparing enough to avoid unnecessary volume losses.

In addition to volume losses, the recovery of platelets during the Intercept treatment was calculated. The results were in range of 88 – 96 %, with an average 92%. The standard deviation over all results was 1.8% and similar for validation of Intercept treatment (1.5%) and production after validation (2.0%).

All final PC products had less white blood cells than allowed ($< 0.15 \times 10^9$). The results were in range of $0.016 – 0.054 \times 10^9$, with an average 0.027×10^9 in product. Even though the expanded uncertainty $\pm 0.086 \times 10^9$ was high compared to the results, it was acceptable to assess the conformity to criteria as all results were below the limit also when uncertainty was taken into account.

The pH measurements on the day of production were in range of 6.88 – 7.09, with an average 6.96. On the last day of storage the pH of the product was in range of 6.99 – 7.05, with an average 7.01, being noticeably above the limit also when the expanded uncertainty ± 0.062 was considered.

4.4 Production planning

As the pathogen inactivation of platelet concentrates by Intercept system is a time-consuming multi-step process, requiring quite a lot of hands-on work, many aspects in addition to conformity to quality requirements need to be considered.

First of all, the implementation of the process into routine production needs a thorough analysis in terms of staff planning and working hours. In NEMC Blood Centre the production of platelet concentrates starts in the afternoon (around 2 pm), after the initial processing (centrifugation, separation) of blood, collected the day before, has finished and the laboratory has conducted the mandatory testing. Due to the time limit of 6 – 16 hours for the CAD phase, the production of pathogen inactivated platelet concentrates could not be finished on the same day but was required to continue early next morning. Working in routine would require some of the staff to stay a bit longer and some of the staff to come earlier in the morning. The impact on the smooth operation of other production processes would need to be analyzed carefully, but most likely, despite of extra work, no additional staff would be needed and a carefully planned change of production arrangement would be enough.

After proper validation and implementation of the Intercept process into routine production, the stability of production has to be assured as the changes in the first steps of processing can result in changes in BC composition, which can lead to nonconformity to requirements set for platelet concentrates to be treated with Intercept, influencing the pathogen inactivation capability of the system. Proper quality control program for routine production should include the assessment of stability of BC characteristics (volume, hematocrit) in addition to regular checks of BC pool as well as PC before and after pathogen inactivation, to assure the conformity to criteria, ensuring the process effectiveness.

The most problematic aspect about implementing 100% pathogen inactivated platelet concentrate production would be the difficulty to plan the PC stock due to the prolonged production. Also currently, the maintenance of optimal PC stock (per blood group) is not an easy task due to the short shelf-life, relatively low issuing numbers (compared to red blood cell suspensions and plasma), and quite unstable demand (per blood group), resulting in relatively high outdating (compared to other blood products). But in the even more difficult planning situation, the added cost would put even more pressure to minimize the expiry. So the target outdating and measures to achieve it need to be put in place to avoid unnecessary waste of resources.

5. Summary

The aim of this thesis was to develop a method for production of buffy coat platelet concentrates suitable for use with Intercept platelet processing set with dual-storage containers and to assess the conformity of pathogen inactivated platelet products with the required and recommended quality parameters. During the six month testing period, validations of 6BC PC production and subsequent processing with Intercept system were carried out, followed by the production and processing of limited number of platelet products after validations.

In order to produce suitable platelet concentrates, the adjustment of whole blood separation process was necessary to find the compromise between the desired buffy coat parameters and the quality of plasma and red blood cell suspension. The succeeding production of platelet concentrates using 6 buffy coats proved the conformity to requirements for platelet content, volume, plasma content, and red blood cell concentration set for platelet concentrates suitable to be processed with Intercept system.

After processing with Intercept system, the platelet and white blood cell content were assessed to be appropriate. A little concern was the lower than recommended amount of volume per platelet content in about half of the products, but as those were the units containing about average or higher platelet content and storage analysis showed good platelet recovery and normal pH values at the end of shelf life, the overall results for volume were also considered to be acceptable.

Both the 6BC PC production and Intercept treatment process, as well as the storage of pathogen inactivated platelet products were evaluated to be efficient in terms of platelet recovery. Both the recovery results as well as the analysis of volume losses showed stability of handling the Intercept platelet processing system. Though the overall performance of 6BC PC production was showing adequate results, the high variability in platelet recovery shows an opportunity for further improvement and standardization of the platelet concentrate separation process.

In conclusion, the testing of buffy coat platelet concentrate pathogen inactivation in NEMC Blood Centre can be considered successful, as the results show the readiness for routine production of pathogen inactivated buffy coat platelet concentrates and the experience gained gives confidence to overcome any obstructions either in processing or production planning.

6. Kokkuvõte

Antud magistritöö eesmärgiks oli välja töötada tootmismeetod, mis võimaldaks valmistada buffy coat (BC) trombotsüütide kontsentraate, mis sobiksid töötlemiseks kahe säilituskotiga Intercept trombotsüütide patogeeninaktiveerimise süsteemiga ning vastaksid töötlemise järgselt kohustuslikele ning soovituslikele kvaliteedinõuetele. Kuue kuu pikkuse testperioodi jooksul teostati 6BC trombotsüütide kontsentraadi valmistamise valideerimine ja sellele järgnev Interceptiga töötlemise valideerimine ning valideerimisjärgselt toodeti limiteeritud arv patogeeninaktiveeritud trombotsüütide kontsentraate.

Selleks, et sobivaid trombokontsentraate valmistada, oli eelnevalt vajalik modifitseerida täisvere separeerimisprotsessi, et leida kompromiss soovitud BC parameetrite ning plasma ja erütrotsüütide suspensiooni kvaliteedinäitajate vahel. Järgnev trombotsüütide kontsentraadi valmistamine kuuest BC-st tõestas, et saadud trombokontsentraadid vastavad tootja poolt kehtestatud trombotsüütide sisalduse, mahu, plasma sisalduse ja erütrotsüütide kontsentratsiooni kriteeriumitele ning sobivad seega töötlemiseks Intercept süsteemiga.

Intercept patogeeninaktiveerimise süsteemiga töötlemise järgselt hinnati toote trombotsüütide ja leukotsüütide sisaldus sobivaks. Kuigi kõigis toodetes maht ei vastanud soovitatud nõuetele, hinnati see siiski sobivaks kuna antud toodetes oli trombotsüütide sisaldus pigem kõrgem kui keskmiselt ning säilivusanalüüsid näitasid häid tulemusi nii trombotsüütide sisalduse kui ka pH suhtes.

Nii 6BC trombotsüütide kontsentraadi tootmise kui ka Intercept süsteemiga töötlemise protsess hinnati efektiivseks trombotsüütide saagise koha pealt. Võttes arvesse ka mahu kadu töötlemise erinevates etappides, hinnati Intercept süsteemi käsitlemine stabiilseks. Kuigi üleüldiselt hinnati 6BC trombotsüütide kontsentraadi tootmise protsessi käigus saadud tulemused heaks, leiti töö käigus saadud trombotsüütide saagise suurt varieeruvust analüüsides, et käsipressidega trombotsüütide kontsentraadi separeerimisprotsess ei ole hästi kontrollitav. Kas automaatsete separaatoritega oleks võimalik antud protsessi standardiseerida ning kas selle käigus oleks võimalik saavutada ka keskmiselt kõrgem trombotsüütide saagis, vajaks eraldi katsetamist.

Kokkuvõttes võib hinnata BC trombotsüütide kontsentraadi patogeeninaktiveerimise testimist Intercept süsteemiga Põhja-Eesti Regionaalhaigla Verekeskuses edukaks kuna saadud tulemused näitavad valmisolekut antud protsessi juurutamiseks rutiintöösse.

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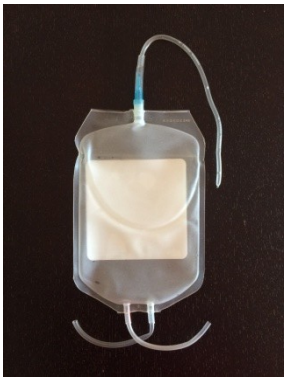
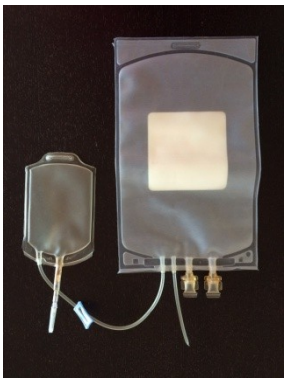
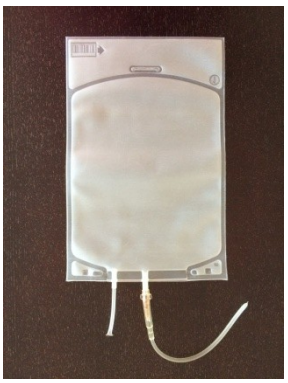
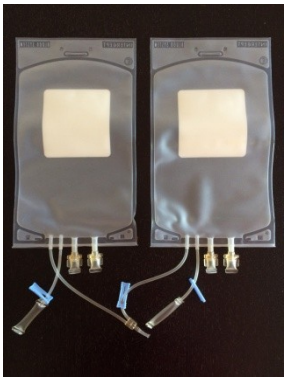
Appendix 1

Table 4. Weight of tare.

Description of tare	Weight of tare	Picture of tare
Tare BC pool before sampling BC (pool) bag including tube length 10 cm + 10 cm + 20 cm. (MacoPharma, REF: 1MAMRT6281LU or 1MAMRT6280LU)	28 g	
Tare 6BC PC before sampling PC storage container including sampling pouch and tube length 10 cm. (Fenwal, REF: 1FE00R7041)	56 g	
Tare 6BC PC after sampling PC storage container including tube length 10 cm. (Fenwal, REF: 1FE00R7041)	39 g	
Tare PC storage container with amotosalen container PC storage container + amotosalen container (the weight of amotosalen solution subtracted) including tube length 12 cm (between containers) + 46 cm (Fenwal, REF: 1FE00R7041; Cerus, REF: OCEINT2503)	52 g	

Description of tare	Weight of tare	Picture of tare
Tare illumination bag Illumination bag with tube length 4 cm + 14 cm. (Cerus, REF: OCEINT2503)	32 g	
Tare CAD container CAD bag and filter pouch, with tube length 4 cm. (Cerus, REF: OCEINT2503)	52 g	
Tare Intercept treated PC before sampling and split Two connected PC storage containers including two sampling pouches. (Cerus, REF: OCEINT2503)	93 g	
Tare of final product: Intercept treated PC after sampling and split PC storage container including tube length 10 cm. (Cerus, REF: OCEINT2503)	42 g	

Appendix 2

Table 5. Quality control results for buffy coats in June 2013.

Analysis number	Date	Blood nr H050013	Volume of BC (ml)	Hct of BC	Platelet content (10^9/1BC)
1	4.06.2013	009927	56	0.51	71
2	4.06.2013	010906	59	0.50	117
3	4.06.2013	013719	58	0.49	122
4	5.06.2013	010100	55	0.48	106
5	5.06.2013	010891	55	0.49	128
6	5.06.2013	013656	59	0.54	91
7	6.06.2013	009996	57	0.50	126
8	6.06.2013	010879	57	0.50	123
9	6.06.2013	013686	57	0.52	77
10	7.06.2013	010392	51	0.50	107
11	7.06.2013	010958	53	0.49	103
12	7.06.2013	011725	58	0.50	119
13	11.06.2013	010600	55	0.42	118
14	11.06.2013	011020	55	0.49	107
15	11.06.2013	011704	58	0.52	93
16	12.06.2013	010362	55	0.50	77
17	12.06.2013	011032	56	0.50	101
18	12.06.2013	011677	55	0.48	67
19	13.06.2013	010344	53	0.49	94
20	13.06.2013	010833	51	0.48	99
21	13.06.2013	011746	55	0.53	96
22	14.06.2013	011270	55	0.48	119
23	14.06.2013	013812	55	0.49	96
24	14.06.2013	014527	56	0.54	133
25	18.06.2013	010544	53	0.51	72
26	18.06.2013	011124	60	0.51	97
27	18.06.2013	013937	57	0.51	48
28	19.06.2013	010273	58	0.48	121
29	19.06.2013	011128	56	0.49	126
30	19.06.2013	013859	59	0.51	84
31	20.06.2013	011200	57	0.50	158
32	20.06.2013	011271	59	0.51	99
33	20.06.2013	013990	58	0.51	92
34	21.06.2013	010323	59	0.50	93
35	21.06.2013	011145	51	0.51	97
36	21.06.2013	014001	60	0.50	115
37	26.06.2013	014199	52	0.51	138
38	26.06.2013	014614	58	0.49	88
39	27.06.2013	014108	51	0.53	98
40	27.06.2013	014713	60	0.51	127
41	28.06.2013	014893	57	0.50	102
42	28.06.2013	015219	57	0.43	110
Average			**56**	**0.50**	**104**
Minimum			**51**	**0.42**	**48**
Maximum			**60**	**0.54**	**158**
Standard deviation			**2.6**	**0.023**	**21**

Table 6. Quality control results for buffy coats in August 2014.

Analysis number	Date	Blood nr H050014	Volume of BC (ml)	Hct of BC	Platelet content (10^9/1BC)
1	1.08.2014	019143	49	0.45	100
2	1.08.2014	020030	50	0.40	125
3	5.08.2014	019558	49	0.35	130
4	5.08.2014	021137	51	0.43	147
5	6.08.2014	019036	48	0.38	122
6	6.08.2014	021172	51	0.51	75
7	7.08.2014	019051	48	0.43	71
8	7.08.2014	020105	48	0.37	105
9	8.08.2014	019631	50	0.37	116
10	8.08.2014	020150	49	0.43	146
11	12.08.2014	017969	48	0.41	94
12	12.08.2014	019080	50	0.40	109
13	12.08.2014	020246	50	0.41	150
14	13.08.2014	019097	50	0.38	124
15	13.08.2014	020327	49	0.46	116
16	13.08.2014	022016	51	0.38	100
17	14.08.2014	018435	48	0.42	145
18	14.08.2014	019339	52	0.48	116
19	14.08.2014	020348	48	0.48	75
20	15.08.2014	019360	52	0.40	100
21	15.08.2014	020299	52	0.48	102
22	15.08.2014	021501	51	0.42	93
23	19.08.2014	019801	50	0.40	95
24	19.08.2014	020419	47	0.42	110
25	19.08.2014	021547	48	0.39	144
26	22.08.2014	019440	47	0.39	112
27	22.08.2014	021255	47	0.41	113
28	22.08.2014	021677	51	0.39	108
29	26.08.2014	019791	49	0.39	118
30	26.08.2014	020499	49	0.43	75
31	26.08.2014	021706	49	0.38	87
32	27.08.2014	019836	48	0.37	112
33	27.08.2014	021296	51	0.41	96
34	27.08.2014	021755	51	0.45	125
35	28.08.2014	019932	49	0.38	121
36	28.08.2014	020539	51	0.46	138
37	28.08.2014	021784	49	0.38	151
38	29.08.2014	019858	49	0.45	127
39	29.08.2014	020626	48	0.40	113
40	29.08.2014	022128	49	0.43	163
Average			**49**	**0.41**	**114**
Minimum			**47**	**0.35**	**71**
Maximum			**52**	**0.51**	**163**
Standard deviation			**1.4**	**0.037**	**23**

Appendix 3

Table 7. Data of 6BC PC production (d – density, rec – recovery, u – unit, W - weight).

Validation of 6BC PC production													
		BC pool before sampling (tare 28 g, d = 1.04 g/ml)					6BC PC before sampling (tare 56 g, d =1.01 g/ml)						
Product	Date of production	W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	Hct	Plasma (%)	W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT rec (%)	RBC (visual)
6BC-PC-1	24.09.14	614	563.5	1096	618	0.184	36.9	454	394.1	1260	497	80	ok
6BC-PC-2	24.09.14	628	576.9	1013	584	0.202	37.0	438	378.2	1243	470	80	ok
6BC-PC-3	25.09.14	610	559.6	1174	657	0.182	36.6	444	384.2	1426	548	83	ok
6BC-PC-4	25.09.14	617	566.3	1047	593	0.203	35.8	434	374.3	1279	479	81	ok
6BC-PC-5	30.09.14	603	552.9	1051	581	0.174	36.5	445	385.1	1247	480	83	ok
6BC-PC-6	30.09.14	608	557.7	1237	690	0.189	35.9	433	373.3	1500	560	81	ok
6BC-PC-7	1.10.14	626	575.0	1015	584	0.192	37.6	452	392.1	1197	469	80	ok
6BC-PC-8	1.10.14	618	567.3	1188	674	0.189	37.0	448	388.1	1421	552	82	ok
6BC-PC-9	2.10.14	609	558.7	1319	737	0.200	35.1	429	369.3	1580	584	79	ok
6BC-PC-10	7.10.14	612	561.5	1341	753	0.185	36.6	441	381.2	1627	620	82	ok
6BC-PC-11	9.10.14	607	556.7	1657	923	0.190	35.7	435	375.2	1904	714	77	ok
6BC-PC-12	9.10.14	615	564.4	1374	776	0.201	35.7	437	377.2	1782	672	87	ok
Average		**614**	**563.4**	**1209**	**681**	**0.191**	**36.4**	**441**	**381.0**	**1456**	**554**	**81**	-
Minimum		**603**	**552.9**	**1013**	**581**	**0.174**	**35.1**	**429**	**369.3**	**1197**	**469**	**77**	-
Maximum		**628**	**576.9**	**1657**	**923**	**0.203**	**37.6**	**454**	**394.1**	**1904**	**714**	**87**	-
Standard deviation		**7.5**	**7.2**	**191**	**103**	**0.0091**	**0.73**	**7.9**	**7.8**	**230**	**82**	**2.3**	-
Validation of 6BC PC pathogen inactivation with Intercept double-dose system													
6BC-PC-INT-1	22.10.14	622	571.2	1176	672	0.197	36.8	446	386.1	1355	523	78	ok
6BC-PC-INT-2	22.10.14	617	566.3	1002	567	0.201	35.9	441	381.2	1280	488	86	ok
6BC-PC-INT-3	29.10.14	610	559.6	1235	691	0.190	36.0	429	369.3	1523	562	81	ok
6BC-PC-INT-4	29.10.14	609	558.7	1105	617	0.197	35.4	439	379.2	1465	556	90	ok

Product	Date of production and analysis	BC pool before sampling (tare 28 g, d = 1.04 g/ml)					6BC PC before sampling (tare 56 g, d =1.01 g/ml)						
		W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	Hct	Plasma (%)	W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT rec (%)	RBC (visual)
6BC-PC-INT-5	5.11.14	621	570.2	1422	811	0.194	36.9	437	377.2	1520	573	71	ok
6BC-PC-INT-6	5.11.14	606	555.8	1365	759	0.190	35.6	441	381.2	1630	621	82	ok
6BC-PC-INT-7	6.11.14	622	571.2	1096	626	0.205	36.1	432	372.3	1283	478	76	ok
6BC-PC-INT-8	6.11.14	614	563.5	1045	589	0.199	35.7	442	382.2	1246	476	81	ok
6BC-PC-INT-9	12.11.14	624	573.1	1323	758	0.209	36.0	438	378.2	1800	681	90	ok
6BC-PC-INT-10	12.11.14	619	568.3	1162	660	0.220	34.6	422	362.4	1609	583	88	ok
6BC-PC-INT-11	13.11.14	617	566.3	1021	578	0.189	36.9	437	377.2	1411	532	92	ok
6BC-PC-INT-12	13.11.14	617	566.3	1249	707	0.185	37.2	440	380.2	1568	596	84	ok
Average		**617**	**565.9**	**1183**	**670**	**0.198**	**36.1**	**437**	**377.2**	**1474**	**556**	**83**	-
Minimum		**606**	**555.8**	**1002**	**567**	**0.185**	**34.6**	**422**	**362.4**	**1246**	**476**	**71**	-
Maximum		**624**	**573.1**	**1422**	**811**	**0.220**	**37.2**	**446**	**386.1**	**1800**	**681**	**92**	-
Standard deviation		**5.7**	**5.5**	**138**	**78**	**0.0098**	**0.75**	**6.5**	**6.4**	**167**	**61**	**6.4**	-
Results of all 24 units of 6BC PC													
Average		**615**	**564.6**	**1196**	**675**	**0.195**	**36.2**	**439**	**379.1**	**1465**	**555**	**82**	**-**
Minimum		**603**	**552.9**	**1002**	**567**	**0.174**	**34.6**	**422**	**362.4**	**1197**	**469**	**71**	**-**
Maximum		**628**	**576.9**	**1657**	**923**	**0.220**	**37.6**	**454**	**394.1**	**1904**	**714**	**92**	**-**
Standard deviation		**6.6**	**6.4**	**163**	**90**	**0.0099**	**0.74**	**7.3**	**7.3**	**197**	**71**	**4.8**	**-**

Appendix 4

Table 8. Data of 6BC PC Intercept treatment process (d – density, rec – recovery, u – unit, W - weight).

Validation of 6BC PC pathogen inactivation with Intercept double-dose system														
		6BC PC after sampling (tare 39 g, d = 1.01 g/ml)				Intercept treated PC after sampling and split (tare 42 g, d = 1.01 g/ml)								
Product	Date of production	W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT (10^3/µl)	WBC (10^3/µl)	Dose	W incl tare (g)	Volume (ml)	PLT (10^9/u)	WBC (10^9/u)	PLT rec (%)	pH
6BC-PC-INT-1	22. - 23.10.14	426	383.2	1355	519	1365	0.1	1	211	167.3	228	0.017	91	6.90
								2	223	179.2	245	0.018		
6BC-PC-INT-2	22. - 23.10.14	421	378.2	1280	484	1258	0.2	1	220	176.2	222	0.035	91	6.90
								2	218	174.3	219	0.035		
6BC-PC-INT-3	29. - 30.10.14	409	366.3	1523	558	1503	0.1	1	207	163.4	246	0.016	90	6.88
								2	216	172.3	259	0.017		
6BC-PC-INT-4	29. - 30.10.14	419	376.2	1465	551	1457	0.2	1	218	174.3	254	0.035	92	6.94
								2	218	174.3	254	0.035		
6BC-PC-INT-5	05. - 06.11.14	417	374.3	1520	569	1552	0.2	1	218	174.3	270	0.035	93	6.94
								2	210	166.3	258	0.033		
6BC-PC-INT-6	05. - 06.11.14	421	378.2	1630	616	1650	0.2	1	215	171.3	283	0.034	93	6.90
								2	221	177.2	292	0.035		
6BC-PC-INT-7	06. - 07.11.14	412	369.3	1283	474	1257	0.1	1	216	172.3	217	0.017	90	7.05
								2	211	167.3	210	0.017		
6BC-PC-INT-8	06. - 07.11.14	423	380.2	1246	474	1196	0.1	1	215	171.3	205	0.017	88	7.09
								2	222	178.2	213	0.018		
6BC-PC-INT-9	12. - 13.11.14	418	375.2	1800	675	1801	0.1	1	221	177.2	319	0.018	93	6.91
								2	215	171.3	308	0.017		
6BC-PC-INT-10	12. - 13.11.14	402	359.4	1609	578	1602	0.2	1	210	166.3	266	0.033	92	6.95
								2	211	167.3	268	0.033		
6BC-PC-INT-11	13. - 14.11.14	417	374.3	1411	528	1364	0.1	1	216	172.3	235	0.017	91	7.09
								2	222	178.2	243	0.018		

Product	Date of production	6BC PC after sampling (tare 39 g, d = 1,01)				Intercept treated PC after sampling and split (tare 42 g, d = 1,01 g/ml)								
		W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT (10^3/µl)	WBC (10^3/µl)	Dose	W incl tare (g)	Volume (ml)	PLT (10^9/u)	WBC (10^9/u)	PLT rec (%)	pH
6BC-PC-INT-12	13. - 14.11.14	422	379.2	1568	595	1568	0.1	1	223	179.2	281	0.018	91	6.91
								2	209	165.3	259	0.017		
Average		**417**	**374.5**	**1474**	**552**	**1464**	**0.14**	**-**	**216**	**172.4**	**252**	**0.024**	**91**	**6.96**
Minimum		**402**	**359.4**	**1246**	**474**	**1196**	**0.1**	**-**	**207**	**163.4**	**205**	**0.016**	**88**	**6.88**
Maximum		**426**	**383.2**	**1800**	**675**	**1801**	**0.2**	**-**	**223**	**179.2**	**319**	**0.035**	**93**	**7.09**
Standard deviation		**6.7**	**6.6**	**167**	**61**	**182**	**0.051**	**-**	**4.8**	**4.8**	**31**	**0.0087**	**1.5**	**0.077**
Production after validation														
H050014227109	14. - 15.01.15	420	377.2	1484	560	1495	0.1	1	216	172.3	258	0.017	93	-
								2	218	174.3	261	0.017		
H050014227110	14. - 15.01.15	422	379.2	1476	560	1432	0.1	1	220	176.2	252	0.018	91	-
								2	224	180.2	258	0.018		
H050014227111	15. - 16.01.15	414	371.3	1369	508	1359	0.1	1	224	180.2	245	0.018	93	-
								2	210	166.3	226	0.017		
H050014227112	15. - 16.01.15	421	378.2	1655	626	1649	0.2	1	219	175.2	289	0.035	92	-
								2	218	174.3	287	0.035		
H050014227191	22. - 23.01.15	415	372.3	1703	634	1611	0.1	1	212	168.3	271	0.017	88	-
								2	223	179.2	289	0.018		
H050014227192	22. - 23.01.15	438	395.0	1722	680	1721	0.2	1	226	182.2	314	0.036	92	-
								2	227	183.2	315	0.037		
H050015200031	27. - 28.01.15	423	380.2	1652	628	1603	0.3	1	225	181.2	290	0.054	91	-
								2	221	177.2	284	0.053		
H050015200032	27. - 28.01.15	421	378.2	1375	520	1350	0.2	1	224	180.2	243	0.036	94	-
								2	226	182.2	246	0.036		
H050015200048	28. - 29.01.15	429	386.1	1151	444	1135	0.2	1	225	181.2	206	0.036	92	-
								2	224	180.2	205	0.036		

Product	Date of production	6BC PC after sampling (tare 39 g, d = 1.01 g/ml)				Intercept treated PC after sampling and split (tare 42 g, d = 1.01 g/ml)								
		W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT (10^3/µl)	WBC (10^3/µl)	Dose	W incl tare (g)	Volume (ml)	PLT (10^9/u)	WBC (10^9/u)	PLT rec (%)	pH
H050015200049	28. - 29.01.15	419	376.2	1379	519	1318	0.2	1	219	175.2	231	0.035	88	-
								2	216	172.3	227	0.034		
H050015200114	03. - 04.02.15	418	375.2	1654	621	1595	0.3	1	215	171.3	273	0.051	89	-
								2	219	175.2	280	0.053		
H050015200122	04. - 05.02.15	433	390.1	1511	589	1487	0.1	1	223	179.2	266	0.018	91	-
								2	225	181.2	269	0.018		
H050015200123	04. - 05.02.15	423	380.2	1381	525	1345	0.1	1	222	178.2	240	0.018	90	-
								2	218	174.3	234	0.017		
H050015200134	05. - 06.02.15	424	381.2	1702	649	1622	0.1	1	223	179.2	291	0.018	89	-
								2	221	177.2	287	0.018		
H050015200135	05. - 06.02.15	418	375.2	1373	515	1329	0.1	1	218	174.3	232	0.017	91	-
								2	221	177.2	236	0.018		
H050015200166	10. - 11.02.15	418	375.2	1504	564	1502	0.1	1	225	181.2	272	0.018	96	-
								2	223	179.2	269	0.018		
H050015200169	11. - 12.02.15	412	369.3	1382	510	1318	0.2	1	212	168.3	222	0.034	88	-
								2	216	172.3	227	0.034		
H050015200170	11. - 12.02.15	423	380.2	1464	557	1442	0.2	1	228	184.2	266	0.037	94	-
								2	223	179.2	258	0.036		
H050015200181	12. - 13.02.15	405	362.4	1431	519	1363	0.2	1	218	174.3	238	0.035	92	-
								2	220	176.2	240	0.035		
H050015200182	12. - 13.02.15	409	366.3	1362	499	1373	0.2	1	213	169.3	232	0.034	93	-
								2	214	170.3	234	0.034		
H050015200223	17. - 18.02.15	427	384.2	1635	628	1633	0.2	1	225	181.2	296	0.036	93	-
								2	221	177.2	289	0.035		
H050015200224	17. - 18.02.15	418	375.2	1647	618	1589	0.2	1	217	173.3	275	0.035	90	-
								2	221	177.2	282	0.035		

Product	Date of production	6BC PC after sampling (tare 39 g, d = 1.01)				Intercept treated PC after sampling and split (tare 42 g, d = 1.01 g/ml)								
		W incl tare (g)	Volume (ml)	PLT (10^3/µl)	PLT (10^9/u)	PLT (10^3/µl)	WBC (10^3/µl)	Dose	W incl tare (g)	Volume (ml)	PLT (10^9/u)	WBC (10^9/u)	PLT rec (%)	pH
H050015200231	18. - 19.02.15	430	387.1	1569	607	1512	0.1	1	227	183.2	277	0.018	93	-
								2	233	189.1	286	0.019		
H050015200232	18. - 19.02.15	403	360.4	1504	542	1453	0.2	1	215	171.3	249	0.034	92	-
								2	215	171.3	249	0.034		
H050015200240	19. - 20.02.15	412	369.3	1493	551	1446	0.1	1	213	169.3	245	0.017	90	-
								2	216	172.3	249	0.017		
H050015200281	26. - 27.02.15	419	376.2	1293	486	1281	0.1	1	221	177.2	227	0.018	93	-
								2	219	175.2	224	0.018		
Average		**420**	**377.0**	**1495**	**564**	**1460**	**0.16**	**-**	**220**	**176.6**	**258**	**0.029**	**92**	**-**
Minimum		**403**	**360.4**	**1151**	**444**	**1135**	**0.1**	**-**	**210**	**166.3**	**205**	**0.017**	**88**	**-**
Maximum		**438**	**395.0**	**1722**	**680**	**1721**	**0.3**	**-**	**233**	**189.1**	**315**	**0.054**	**96**	**-**
Standard deviation		**8.0**	**8.0**	**145**	**59**	**141**	**0.064**	**-**	**4.8**	**4.8**	**27**	**0.011**	**2.0**	**-**
All units together														
Average		**419**	**376.2**	**1488**	**560**	**1461**	**0.16**	**-**	**219**	**175.2**	**256**	**0.027**	**92**	**-**
Minimum		**402**	**359.4**	**1151**	**444**	**1135**	**0.1**	**-**	**207**	**163.4**	**205**	**0.016**	**88**	**-**
Maximum		**438**	**395.0**	**1800**	**680**	**1801**	**0.3**	**-**	**233**	**189.1**	**319**	**0.054**	**96**	**-**
Standard deviation		**7.6**	**7.6**	**151**	**59**	**152**	**0.060**	**-**	**5.2**	**5.1**	**28**	**0.011**	**1.8**	**-**

Table 9. Data of storage analysis (d – density, rec – recovery, u - unit).

Product	Dose	Volume (ml)	After production, 1st storage day (d = 1.01 g/ml)				7th storage day (d = 1.01 g/ml)				
			Date	PLT (10^3/µl)	PLT (10^9/u)	pH	Date	PLT (10^3/µl)	PLT (10^9/u)	pH	PLT rec (%)
6BC-PC-INT-1	1	167.3	23.10.14	1365	228	6.90	29.10.14	1288	215	7.00	94
	2	179.2			245			1271	228	7.00	93
6BC-PC-INT-2	1	176.2	23.10.14	1258	222	6.90	29.10.14	1183	208	7.00	94
	2	174.3			219			1214	212	7.00	97
6BC-PC-INT-3	1	163.4	30.10.14	1503	246	6.88	05.11.14	1427	233	7.00	95
	2	172.3			259			1432	247	7.02	95
6BC-PC-INT-4	1	174.3	30.10.14	1457	254	6.94	05.11.14	1387	242	6.99	95
	2	174.3			254			1407	245	7.00	97
6BC-PC-INT-5	1	174.3	06.11.14	1552	270	6.94	12.11.14	1504	262	7.05	97
	2	166.3			258			1478	246	7.05	95
Average		**172.2**	-	**1427**	**245**	**6.91**	-	**1359**	**234**	**7.01**	**95**
Minimum		**163.4**	-	**1258**	**219**	**6.88**	-	**1183**	**208**	**6.99**	**93**
Maximum		**179.2**	-	**1552**	**270**	**6.94**	-	**1504**	**262**	**7.05**	**97**
Standard deviation		**4.9**	-	**117**	**17**	**0.027**	-	**112**	**18**	**0.022**	**1.2**

Table 10. Data of volume losses during Intercept treatment (d – density, W – weight).

Product	PC storage container + amotosalen bag (tare 52 g, d = 1.01 g/ml)		Illumination bag (tare 32 g, d = 1.01 g/ml)		CAD container (tare 52 g, d = 1.01 g/ml)		Final product before sampling and split (tare 93 g, d = 1.01 g/ml)		Final product after split		Lost at split
									Dose 1	Dose 2	
	W incl tare (g)	Volume (ml)	W incl tare (g)	Volume (ml)	W incl tare (g)	Volume (ml)	W incl tare (g)	Volume (ml)	Volume (ml)	Volume (ml)	Volume (ml)
6BC-PC-INT-1	59	6.9	36	4.0	84	31.7	451	354.5	167.3	179.2	8.0
6BC-PC-INT-2	60	7.9	35	3.0	85	32.7	455	358.4	176.2	174.3	7.9
6BC-PC-INT-3	60	7.9	35	3.0	82	29.7	441	344.6	163.4	172.3	8.9
6BC-PC-INT-4	60	7.9	35	3.0	79	26.7	454	357.4	174.3	174.3	8.8
6BC-PC-INT-5	60	7.9	35	3.0	85	32.7	446	349.5	174.3	166.3	8.9
6BC-PC-INT-6	60	7.9	34	2.0	82	29.7	455	358.4	171.3	177.2	9.9
6BC-PC-INT-7	60	7.9	35	3.0	81	28.7	445	348.5	172.3	167.3	8.9
6BC-PC-INT-8	62	9.9	35	3.0	81	28.7	454	357.4	171.3	178.2	7.9
6BC-PC-INT-9	59	6.9	34	2.0	80	27.7	453	356.4	177.2	171.3	7.9
6BC-PC-INT-10	60	7.9	36	4.0	77	24.8	436	339.6	166.3	167.3	6.0
6BC-PC-INT-11	59	6.9	35	3.0	76	23.8	455	358.4	172.3	178.2	7.9
6BC-PC-INT-12	59	6.9	35	3.0	85	32.7	450	353.5	179.2	165.3	9.0
Average	**60**	**7.8**	**35**	**3.0**	**81**	**29.1**	**450**	**353.1**	**172.1**	**172.6**	**8.3**
Minimum	**59**	**6.9**	**34**	**2.0**	**76**	**23.8**	**436**	**339.6**	**163.4**	**165.3**	**6.0**
Maximum	**62**	**9.9**	**36**	**4.0**	**85**	**32.7**	**455**	**358.4**	**179.2**	**179.2**	**9.9**
Standard deviation	**0.83**	**0.83**	**0.60**	**0.60**	**3.1**	**3.0**	**6.3**	**6.2**	**4.6**	**5.1**	**1.0**

Appendix 5

Table 11. Uncertainty estimations.

	Combined standard uncertainty u_c	**Expanded uncertainty U (k = 2)**	**Uncertainty components used for calculating u_c**
Measurements			
Weight	1.0 g	-	u(scales) = 1 g
Weight of tare	2.2 g	-	u(scales) = 1 g u(variation between containers) = 2 g
Hematocrit	0.0060	-	u(routine quality control) = 0.0024 u(interlaboratory comparison) = 0.0055
PLT concentration	9.5 x 10^3/µl	-	u(routine quality control) = 5.8 x 10^3/µl u(interlaboratory comparison) = 7.5 x 10^3/µl
WBC concentration	0.24 x 10^3/µl	-	u(routine quality control) = 0.087 x 10^3/µl u(interlaboratory comparison) = 0.22 x 10^3/µl
pH	0.031	0.062	(calculations done by web application)
Quantities used for calculation			
Density of BC pool	0.005 g/ml	-	u(variation of composition) = 0.005 g/ml
Density of PC	0.005 g/ml	-	u(variation of composition) = 0.005 g/ml
Volume of SSP+ solution in BC pool	4.2 ml	-	u(variation of initial volume) = 3 ml u(variation of lost volume) = 3 ml
Calculated results			
Volume of BC pool (before sampling)	3.6 ml	-	u_c(weight) u_c(weight of tare) u_c(density of BC pool)
Volume of 6BC PC (before sampling)	3.1 ml	6.2 ml	u_c(weight) u_c(weight of tare) u_c(density of PC)
Volume of Intercept treated PC (after sampling and split)	2.6 ml	5.2 ml	u_c(weight) u_c(weight of tare) u_c(density of PC)
Plasma content of 6BC PC	1.2 %	2.4 %	u_c(hematocrit) u_c(volume of BC pool) u_c(volume of SSP+ solution in BC pool)
PLT content of 6BC PC (before sampling)	6.9 x 10^9/unit	14 x 10^9/unit	u_c(PLT concentration) u_c(volume of 6BC PC)
PLT content of Intercept treated PC (after sampling and split)	5.0 x 10^9/unit	10 x 10^9/unit	u_c(PLT concentration) u_c(volume of Intercept treated PC)
WBC content of Intercept treated PC (after sampling and split)	0.043 x 10^9/unit	0.086 x 10^9/unit	u_c(WBC concentration) u_c(volume of Intercept treated PC)

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