

MOTIVATIONS OF VOLUNTARILY WITHDRAWN BLOOD DONORS: A QUALITATIVE ANALYSIS

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BACKGROUND: Retention of blood donors has become one of the main goals of the Dutch blood bank. It is known that many donors withdraw from donating before their fifth donation. In order to retain donors more efficiently, knowledge should be gained about the reasons donors withdraw from donating. Current research on donation intention has mainly been executed in the US and the UK. However, the Dutch blood bank system differs from these systems in that donors are registered at a specific blood bank and can therefore resign as a donor. In order to understand the motivations and beliefs of voluntarily withdrawn donors we conducted an explorative qualitative study to reveal themes that will be further explored in a questionnaire study.

METHODS: Semi-structured interviews were carried out among sixteen ex-donors and sixteen active donors to reveal differences in motivations and beliefs between ex and active donors. The interviews explored start and stop motivations, beliefs on blood donation and experiences at the donor centre. The interviews were transcribed and analysed using thematic analysis (Kwalitan). Themes were identified by defining categories of codes with the same underlying meaning.

RESULTS: The results indicate that active donors are more intrinsically motivated than ex-donors. Also the attribution of negative experiences like being tense, fainting and being deferred from donation is different for each group. In addition, feelings of not being needed as a donor and the need for feelings of commitment exert an important influence on the decision to withdraw from donating.

CONCLUSION: Appreciation of the feelings of being needed and the donation event itself might need more attention. These results will be used to test interventions in blood bank practice. Emergent themes will also be used in a follow-up questionnaire study.

DONOR INSIGHT, A DESCRIPTION OF THE BLOOD DONOR POPULATION IN THE NETHERLANDS: STUDY DESIGN.

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Background Nowadays, over 500.000 donors are responsible for the blood supply in the Netherlands. Unfortunately, little is known about factors that accurately describe the donor population and the considerations underlying the decision to become and stay a donor. Therefore, the Donor InSight study will be conducted. The aims of the study are: 1) to gain insight in factors that characterize the donor population in The Netherlands, 2) to learn more about donor motivation and the donation process.

Methods Donor InSight is designed as a prospective follow-up study. The study will be conducted among whole blood and plasma donors. A random sample of 25.000 donors will comprise the study population. Donors will receive a letter from the principal investigators referring to extensive information on the study and a questionnaire. Donors who are willing to participate are asked to fill in the questionnaire and give informed consent and return these documents to the blood bank. Topics put forward in the questionnaire include: demography, lifestyle, nutrition, medical history, physical activity, donor motivation, and the donation process. The collected data will be merged with data from blood bank records, like factors related to donor screening and donation. With permission of the donor, data will also be connected to medical files and disease registers outside the blood bank. Finally, an extra blood sample will be drawn in a subpopulation for additional tests. The donors included in the Donors InSight study will be followed for several years in order to investigate trends over time.

Expected results A data base with a vast amount of information on donors and donation will be produced. In a pilot study among 600 donors the questionnaire has been tested. A response rate of 70% was achieved. Therefore over 35.000 donors need to be invited in order to achieve a study population of 25.000 donors.

Conclusion By using the collected data, extensive research is possible on many topics, like differences between new and repeat donors, regional differences, and differences between plasma and whole blood donors, as well as trends over time. Most important, more knowledge will be gained about the donation process and donor motivation. In the future, the Donor InSight cohort may also be useful for addressing additional research questions.

QUALITY ASSESSMENT: SANQUIN DONOR SATISFACTION SURVEY

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PURPOSE: To ensure that the interaction between the Sanquin Blood Bank and the donors is positive and that it promotes good customer service, regular inventory of donor opinion through satisfaction surveys is indicated. This study focuses on the overall performance of the Sanquin Blood Banks in the Netherlands assessed by its donors. This study must be regarded as a first measurement, which will have a follow-up after two years to measure improvement of performance. The study will provide the "Sanquin donor service concept" with usable key parameters.

METHOD: questionnaire with structured and open questions regarding crucial aspects of the blood bank service was mailed to a random sample (n=4,000) of all active donors (N=406,403) in the Netherlands (active=visited the blood center or regional site at least once in 24 months preceding the mailing). Items were divided in themes: reception, access, visibility, logistics, image, donor exam, donation, treatment, result donor screening, donor café and catering, information, complaint policy, reliability and miscellaneous.

RESULTS: Response 2245/4000 (62%). Majority is (very) satisfied with the overall performance of the Blood Bank (mark 8) and there are no major differences between the performance of the divisions. Some important points of dissatisfaction: parking problems, waiting time (objective and perceived), personal attention while, before, and after donating, information materials and media, image Sanquin organization, complaint procedure, communication with the donor and call up system.

CONCLUSION: Although there are no extreme points of dissatisfaction found that require immediate action – at least on the level of Blood Bank performance that can be influenced by the blood bank personnel – the results reveal attention points which deserve improvement. The Sanquin donor service concept will be used to emphasize the points and take action on management level to improve on the defined items. After two year the results of these efforts will be measured through a repeat survey.

CAN WE EXPECT MORE OF OUR DONORS THAN JUST GIVING BLOOD?

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OBJECTIVES. Blood donors are role models for their family and friends. Previous research showed that most non-donors hardly ever considered blood donation and that they do not know any blood donors. It is also known that most donors started donating blood because they were stimulated by others. This study investigated psychosocial correlates of our donor's willingness to actively recruit new donors among their family/friends. This can be especially helpful in recruiting AB0-specific donors.

METHODS. Questionnaires were sent to the donor's home address (n = 400) and returned by 224 (54% respons) or filled out at the blood centre (n = 191).

RESULTS. Donors are willing to recruit new donors among their family/friends. Self-efficacy ($\Delta R^2 = .27$), personal objections ($\Delta R^2 = .10$), pleasant experience at blood centre ($\Delta R^2 = .06$), and feelings of responsibility ($\Delta R^2 = .03$) accounted for 46% of variation in intention. Donors at the blood centre were overall more positive about recruiting new donors than donors at home. Donors indicated that they would like to receive support in the recruitment of new donors, e.g. a leaflet with tips to start a conversation. Financial rewards for successful recruitment seemed unrelated to recruitment motivation.

CONCLUSIONS. Blood donors can help in recruiting new donors. To facilitate this, we need to develop new strategies for donor recruitment.

BLOOD DONORS, HOW WELL DO THEY REPRESENT AN OPEN POPULATION; HERITABILITY AS AN EXAMPLE

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BACKGROUND: Voluntary blood donors are often considered to represent the rather healthy/disease free fraction of the open population and for that reason have been used to provide reference groups in comparative studies, or to define normal lab values. Results from an epidemiologic oriented “Donors-In-Sight” project allowed to explore the validity of above assumptions. We looked at Hb and blood pressure as traits claimed to be co-determined by genetic factors, and compare their heritability (h^2) among donors with h^2 values from studies in open populations. Apart from pure additive genetic aspects heritability may also reflect shared “cultural” transmitted aspects like diet or in our case blood donations.

A previous study already had shown that inter- and intra- individual variation in Hb levels among donors are rather similar, and thus cannot have discriminative value.

MATERIALS AND METHODS: Among the $n= 128.685$ donors in the ZON region, heritability estimates were calculated based on the correlations in donation based data from 2004 in over 400 twin donors. Twins were traced by probabilistic linkage on identical birthdates, gender, ABO-Rhesus blood groups, and Zip-code.

RESULTS: Heritability estimated from mono- and di-zygote same gender twins.

Antropometric trait compared	In donor twins Heretability h^2	Based on published data from open population studies Heritability h^2
Haemoglobin	0.20	0.84
Systolic blood pressure	0.61	0.44 – 0.57
Diastolic blood pressure	0.74	0.32 – 0.50

DISCUSSION: Compared to heritability estimates from non blood donor based studies, our donor-twins based estimates for Hb levels were significant lower than those published in the literature, while heritability estimates for both systolic and diastolic blood pressure tended to be even higher than h^2 values reported in the literature.

The latter we interpret as reflecting the fact that most studies in the literature will have more misclassified twins than in our donor-twins that include blood group identity.

The lower heritability for Hb among donors on the other hand reflects that in contrast to non-donors, donor Hb levels include the additional variation induced by the Hb lowering effects of regularly blood donations.

Heritability estimates reflect explained variance, which for a pure genetic trait would in identical twins result in a $h^2 = 1$. Where blood donations adds an extra source of variability to what is to be explained, blood donation effects on haemoglobin levels can provide a valid explanation for the observed lower heritability estimate h^2 .

CONCLUSION: A population of regular blood donors may provide not automatically a good representation for reference Hb and/or related blood parameters in a healthy population.

BLOOD-VOLUME; A CHEAP AND SIMPLE ADDITIONAL PARAMETER TO HELP INCREASE THE EFFICIENCY OF BLOOD COLLECTION AT THE LEVEL OF INDIVIDUAL DONORS.

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BACKGROUND: In transfusion medicine Hb as mmol/l is a *relative* measure of the fraction haemoglobin in the 10cc whole blood sampled. This relative measure plays a pivotal role in deciding which individual can give blood. The clinical relevant issue however is the total *absolute* amount of Haem and/or number of erythrocytes, representing the total oxygen transport capacity of these individuals which depends on total blood-volume.

We defend the thesis that this cheap and simple parameter should explicitly be included in evidence based rational decision making in transfusion medicine.

MATERIALS: As part of a project "Donors-In-Sight" info on height was collected from regular blood Dutch donors ages 18 – 70, 40% female 60% male to estimate body surface and gender specific blood-volumes based on published formulas (1).

RESULTS: Male mean blood volumes 5,01 l (range 3,3 - 7,5) Hb 9,46 (range 6,4 - 12,4).

Female mean blood volumes 4,09 l (range 3,0 - 6,1) Hb mean 8,54 (range 5,7 - 12,1).

Blood volumes varied by a factor 2,1 which is larger than both the observed ranges as well as the Hb levels set for safe blood donations (7,8 to 12 in females and 8,5 to 12 in males).

Within these regular donors, regression analysis to explain last measured Hb depended largely (R^2 35%) on gender specific estimated blood volume (r 0.44 $p=0.000$), gender itself ($r=0.57$ $p=0.000$) and age ($r=0.13$ $p=0.000$), with the highest Hb levels in donors with the largest estimated blood volumes.

DISCUSSION: This would imply that donors with e.g. an Hb 10 may vary a factor 2 in frequencies with which they could safely donate regularly blood without jeopardizing over time their own total amounts of haemoglobin = oxygen transport capacity.

Blood volume is a cheap, simple to estimate and rather fixed patient/donor trait. Given its greater variability than Hb, blood-volume thereby determines a larger percentage of variance explained in how many erythrocytes can successfully be taken from a donor than Hb itself.

CONCLUSION: Including estimated blood-volume to each individual decision to take or donate blood seems both a simple and cost-effective way to optimize the practice of blood-banking, while making blood collection a more rational evidence based practice.

It would result in measurable lower deferral and complication rates in donors while improving effectiveness of transfusions in patients.

1.Keszthelyi B, Lakatos-Novotny S, Toth A. Measurement of blood loss by total-body counting on the basis of calculated and predicted blood volumes. Acta Medica Academiae Scientiarum Hungaricae 1979;36:71-78.

STORED RED CELL CONCENTRATES OF HEMOCHROMATOSIS PATIENTS: ARE THEY DIFFERENT?

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BACKGROUND: The preferred treatment for iron overload associated with hemochromatosis is therapeutic phlebotomy. This can be done by either whole blood collection (WB) or by erythrocytapheresis (EA). In the Netherlands, red cell concentrates (RCC) from hemochromatosis (HH) patients are discarded or used for research or quality testing purposes only. In other countries, these RCC are used for transfusion purposes. There is an ongoing ethical discussion about the use of blood from HH patients for transfusion into patients.

AIM: To establish whether RCC originating from HH patients can be stored for transfusion purposes and whether they comply with the European and Dutch guidelines during storage. To evaluate whether there are any differences between RCC obtained by apheresis and whole blood collection.

METHODS: RCC of HH patients and regular donors were obtained by whole blood collection or apheresis. All RCC were leukocyte depleted and stored for a maximum of 7 weeks in SAGM under blood bank conditions. Starting in the first week of storage a set of hematologic, biophysical and biochemical measurements was performed on all units and weekly thereafter.

RESULTS: Whole blood derived RCC were collected from four HH patients and eleven regular donors. From four HH patients and four regular donors, EA-derived RCC were collected. A short overview of the results is presented in the table (mean \pm SD).

Parameter	Shelf life (days)	HH patients		Regular donors	
		WB	EA	WB	EA
MCV (fL)	1	99.6 \pm 8.3	99.3 \pm 5.6	92.9 \pm 3.4	90.6 \pm 1.6
	36	102.5 \pm 9.5	109.4 \pm 4.7	96.2 \pm 3.8	97.5 \pm 4.8
	50	105.9 \pm 8.6	111.6 \pm 3.9	98.5 \pm 4.2	102.3 \pm 4.3
Glucose (mM)	1	28.4 \pm 1.7	24.3 \pm 1.7	26.8 \pm 2.0	24.4 \pm 2.1
	36	12.3 \pm 2.4	6.8 \pm 0.7	13.3 \pm 2.8	6.6 \pm 0.5
	50	9.7 \pm 2.3	4.1 \pm 0.6	10.3 \pm 3.3	3.8 \pm 0.8
Hemolysis (%)	1	0.06 \pm 0.01	0.08 \pm 0.02	0.08 \pm 0.03	0.16 \pm 0.09
	36	0.28 \pm 0.04	0.16 \pm 0.06	0.34 \pm 0.19	0.25 \pm 0.11
	50	0.85 \pm 0.27	0.48 \pm 0.09	0.76 \pm 0.33	0.49 \pm 0.10
ATP (μ mol/g Hb)	1	3.47 \pm 0.32	3.58 \pm 0.33	3.76 \pm 0.49	3.82 \pm 0.52
	36	1.73 \pm 0.43	1.58 \pm 0.55	2.21 \pm 0.75	2.16 \pm 0.33
	50	0.80 \pm 0.34	0.67 \pm 0.45	1.12 \pm 0.52	1.10 \pm 0.15

CONCLUSIONS: Independent of their collection method, RCC of HH patients comply with the European guidelines for stored RCC during a shelf life of 7 weeks. There are no significant differences between RCC from HH patients and RCC from regular donors. However, there are significant differences in some storage-related changes between the RCC that were collected by erythrocytapheresis and the RCC that had been obtained by whole blood collection.

THE EFFECTS OF TRANSFUSIONS ON CYTOKINE GENE EXPRESSION MODIFICATION AFTER CARDIAC SURGERY

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The systemic inflammatory response syndrome (SIRS) has been recognized as a precursor of multi organ failure (MOF), contributing to mortality risk of cardiac surgery patients. No direct marker identifying patients at high risk for developing MOF nor the mechanism by which MOF evolves from SIRS has been discerned. Gene expression (GE) arrays may provide a promising way of exploring which genes are involved. Aim of the study is to define the effect of blood transfusions (BT) in cytokine GE in whole blood from cardiothoracic surgery patients. RNA was extracted from pre- and 24-h postoperative blood samples from 10 patients, matched according to gender, age, CPB time and BT products. The oligo GEArray for 114 human common cytokines was used to identify changes in GE profiles of 4 patients developing MOF and six patients with SIRS.

Table Δ expression*	10 cardiac surgery patients			
	5 non-transfused patients		5 transfused patients	
	SIRS (N=3)	MOF (N=2)	SIRS (N=3)	MOF (N=2)
BMP	0.03	-0.03	0.06	0.31
CSF	0.01	-0.21	-0.16	0.22
IFN-α	0.02	-0.05	0.05	0.35
IL-17	-0.04	-0.04	-0.10	0.19
IL-8	-0.17	-0.72	-0.26	-0.43
PDGF-α	0.01	0.03	0.09	0.29
TGF-α	0.26	0.11	0.32	0.61
VEGF-β	-0.23	-0.40	0.02	-0.14

* Surgery effect in different groups (24-h postoperative – preoperative GE levels)
- = downregulation, + = upregulation.

Preliminary results (table) indicate two GE profiles in MOF developing patients as compared to SIRS: those without BT show down-regulation of wound/vascular repair GE (VEGF-β), whilst patients with BT reveal haematopoietic and Th1 GE upregulation. Downregulation of IL-8 GE in both MOF patient groups may affect chemotaxis. In conclusion, MOF in transfused patients appears to be accompanied by GE-upregulation for Th1 cytokines and haematopoietic mediators.

SHORT-TERM AND LONG-TERM SURVIVAL OF TRANSFUSED RBCS: A COMPARISON BETWEEN STORED RCC OF SHORT AND LONG SHELF LIFE

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BACKGROUND: According to the European guidelines at least 75% of the transfused RBCs should survive in the patient's circulation during the first 24 hours after transfusion. Measuring this 24-hour *in vivo* survival by ⁵¹Cr-labeled RBCs is an established and generally accepted technique. In the Netherlands, however, it is not allowed to use this technique. We therefore developed a method for flow cytometric determination of the survival of transfused RBCs using differences in erythrocyte antigenic phenotype between donor and patient. This approach has also potential to determine the long-term survival of transfused donor RBCs, and to compare the survival of different RBC populations in one patient.

AIM: To determine the survival of RBCs from the currently used red cell concentrate (RCC) in the Netherlands. To establish whether there are differences in survival between short and longer stored RBCs. To evaluate whether the common parameters to assess stored erythrocyte quality can be used as a predictor for the *in vivo* survival.

METHODS: Immuno-compromised patients received two RCC with different shelf lives, one of 0-10 days (short) and the other of 25-35 days (long). The donor RBCs of both products and of the patient had different erythrocyte phenotypes. After irradiation, 30 mL of RBCs was collected from each RCC and used for quality control. At several time points after transfusion, blood samples were taken from the patient for the determination of *in vivo* survival of the transfused RBCs. The RBCs of the collected samples were first incubated with an Ab against one of the selected Ag (anti-E, -c, -C, -K, -Fya, -Jka, -Jkb, -s), and subsequently incubated with a FITC-conjugated goat anti-human IgG antibody. The percentages of donor RBCs could then be determined by flow cytometry with a sensitivity of 0.1%.

RESULTS: Ten patients received a transfusion according to our protocol. The mean age of the transfused patients was 48 years (range 25-59 years), and most of these patients were treated with an autologous stem cell transplantation for Multiple Myeloma. A short overview of the results is presented in the table (mean \pm SD).

	Short	Long
Storage time (days)	5 \pm 2	30 \pm 3
24hr recovery (%)	86.4 \pm 17.8	73.5 \pm 13.7
28 days recovery (%)	47.6 \pm 22.3	42.1 \pm 17.1
84 days recovery (%)	11.2 \pm 10.0	9.5 \pm 6.7
126 days recovery (%)	1.6 \pm 1.8	0.4 \pm 0.5

CONCLUSIONS: The 24 hr recoveries of short and longer stored RBCs are significantly different from each other. This could be caused by the rapid removal of the fraction with the oldest and most damaged RBCs in the first 24 hours after transfusion, which increases in RCC with the shelf life. Statistically, the 24hr posttransfusion survival of RCC with a short and a long shelf life comply with the European guidelines. However, only 4 out of 10 of the RCC with a long shelf life versus 7 out of 10 of the RCC with a short shelf life have a 24hr posttransfusion survival which is 75% or more. After the first 24 hours of transfusion, the RBCs with a short and a long shelf life have an identical survival time.

STORAGE OF WHOLE BLOOD INFLUENCES THE RESPONSE OF RED CELLS TO AN ALKALINE STORAGE MEDIUM

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Introduction. Recently, we developed an additive solution allowing maintenance of high 2,3-DPG levels without concurrent ATP decline. Our new solution is based on the "chloride-shift" principle demonstrated 10 years ago, resulting in a more alkaline cytosol favouring 2,3-DPG formation. Early results indicated large variation in the effects on 2,3-DPG when the new solution was used to replace the standard SAGM solution. This could be caused by donor variation or by variation in time between collection and RCC preparation.

Material and methods. Whole blood (WB) collections in CPD (13 ml) from various donors were stored at 20 °C for 4 or 24h, subsequently centrifuged and separated in plasma, buffy coat and packed cells. From each donation, part of the packed cells was resuspended in our experimental solution (PAGGG-M containing gluconate instead of saline, pH 8.2) and the other part in PAGGS-M (pH 6.2). Samples were taken from WB at 4 and 24h and from the RCC immediately after preparation and after 14 days of storage at 4°C.

Results. When prepared after 4 h storage as WB, the initial value of 2,3-DPG was about 15 µmol/g Hb in WB and in both types of RCC, whereas after 24 h storage this value had declined to 7 µmol/g Hb. During storage for 14 days in PAGGG-M, the RCC prepared after 4 h showed an increase in 2,3-DPG from 15 to 26 µmol/g Hb, whereas in the RCC prepared after 24 h this only increased from 7 to 11 µmol/g Hb. Upon storage in PAGGS-M, units prepared after 4 h showed a decrease to 10 µmol/g Hb and units prepared after 24 h to 2 µmol/g Hb. For all RCC, the ATP level remained unchanged during the first two weeks. During storage as whole blood, the intracellular pH was 7.17 after 4 h and 7.03 after 24 h. This initial difference in whole blood was also observed in the RCC after preparation.

Conclusions. The variable response with respect to 2,3-DPG levels with our experimental additive solution was not caused by donor variation. The effect was dependent on the time between collection and component preparation. Apparently, the decrease in intracellular pH during WB storage has a long-lasting effect on the response to an alkaline storage medium.

TEN YEARS OF BLOOD COMPONENT TRANSFUSIONS: USAGE, DISEASE AND SURVIVAL¹M.P. Janssen, ²C.L. van der Poel, ³W.P. Schaasberg, ⁴G.J. Bonsel¹Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, ²Sanquin Blood Supply Foundation, ³Statistics Netherlands, ⁴Amsterdam Medical Center

In the Netherlands around a 750,000 blood components are being transfused annually. As the blood establishment and hospital organizations are separate entities, no information on the end user (the patient) is known to the Dutch Blood Supply Foundation. With the intention to aim for "optimal" instead of "maximal" blood safety, this information is required to enable evidence based decision making concerning blood safety interventions. Both underlying disease of the blood recipient, the amount and type(s) of components obtained, and the survival of the recipient are parameters required for evaluating the (cost-) effectiveness of blood safety interventions.

The aim of the study is to obtain insight in the "recipient profile", which refers to type of disease, type(s) and amount of blood products, age and life expectancy of the transfusion recipient.

All recorded transfusions in the University Medical Hospital Utrecht (UMCU) from 1993 to 2003 were collected. Transfusion records were linked to other hospital patient records, which were subsequently (anonymously) linked to the national decedents register at the National Bureau of Statistics Netherlands. This enabled analysis of patient survival in terms of blood usage and patient disease. Products are grouped by erythrocyte concentrates, trombocyte concentrates and fresh frozen plasma (FFP) units. Patient survival was calculated from first recorded transfusion and compared to the survival of the general Dutch population. In the period 1993 to 2003 33,473 UMCU patients received 414,978 blood component transfusions. Of these, 55% were erythrocytes, 25% trombocytes and 21% FFP units. Of all patients 96% received erythrocytes, 23% trombocytes and 34% plasma. The median number of units per type of product treated patient was 4 erythrocytes (mean of 7), 3 trombocytes (mean of 13) and 4 FFP units (mean of 8). The median total number of products per patient was 4. The distribution of number of products used per patient is highly skewed. The recipient mean age is 50 years, but most transfusions took place around age 68 and 73 for females and males. Neonates have the highest transfusion intensity however. Patients with neoplasms and patients with disease of the circulatory system receive about 25% of all blood products each. About 13% of the components are used for trauma treatment and 6% for patients with disease of the digestive system. Of 11% of all components the associated illness is unknown. The one, five and ten year patient survival after erythrocyte, trombocyte and plasma transfusion is respectively 78%-65%-52%, 73%-60%-48%, and 77%-66%-54%. There is a highly significant increase in mortality rate with an increase of the number of products transfused. After 10 years survival there is still a significant increase in mortality rate of transfusion recipients as compared to the general Dutch population. The number of transfusions per patient is high, but patient survival for UMCU patients is comparable to data published in literature. The high number of transfusions is caused by a limited number of patients receiving high amounts of components. This can be explained by the fact that the UMCU is a highly specialized academic hospital treating patients with complex disease specifics.

PROTOCOLS AND BLOOD SAVING TECHNIQUES REDUCED RED CELL TRANSFUSIONS BY 45%

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Background: Until 1999 no special blood saving techniques were used in our 300 bed general hospital, apart from loco-regional anaesthesia techniques. Realising the risks of donor blood transfusions, a blood transfusion reduction programme was started.

Methods: Introduction of the following blood saving measures and techniques:

- 2000: The so-called 4-5-6 transfusion protocol [1];
- 2000: Active patient temperature control, aiming at a perioperative temperature loss less than 1 °C;
- 2000: Surgical damage-control strategy;
- 2000: Perioperative and postoperative cell saving in all major surgery (hip/knee/vascular/trauma);
- 2001: Introduction symposium on the subject of blood saving techniques to increase awareness;
- 2003: 4-week preoperative epoetin alfa treatment in total hip/knee surgery for patients with Hb 10-13 g/dl at preoperative screening visit [2].

Results: Both in overall hospital and in total hip and knee replacement surgery allogenic transfusion needs decreased considerably from the introduction of the first measures onward, despite increasing operation production figures. RBC use per patient declined from 0.28 units in 1999 to 0.11 in 2005. Particularly in total hip and knee replacements the effects are clear, changing from 2.0 units per patient in 1999 to 0.35 in 2005. Today, fractures and not orthopaedic operations account for almost all RBC use in surgery.

Conclusion: Combination of blood management measures can induce a huge allogenic transfusion reduction.

1. Slappendel R, et al. Acta Orthop Scand 2003;74:569-75.
2. Goldberg MA, et al. Am J Orthop 1996;25:544-52.

TRANSFUSIEKLAPPER: VAN TEKST NAAR HYPERTEKST

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Doel: het vergroten van de toegankelijkheid en het vergemakkelijken van het beheer van de bloedtransfusieklapper van het Onze Lieve Vrouwe Gasthuis. De papieren klapper, op A5 formaat, dateerde uit 1998 en was daarmee verouderd. Verspreiding in de kliniek en raadpleging door de doelgroep liet te wensen over. Bij de inhoudelijke herziening van de klapper in 2005 werd daarom in de bloedtransfusiecommissie besloten dat ook de vorm aan vernieuwing toe was. Het digitaal aanbieden van de inhoud lag voor de hand. Er werd besloten dit niet in de vorm van één of meer PDF(portable document format) documenten te doen, maar de onderwerpen van de bloedtransfusieklapper als webpagina's in HTML indeling vanuit het documentbeheersysteem DKS-E (Infoland) toegankelijk te maken op intranet.

Materialen en methoden: de geschreven tekst werd in korte fragmenten (ter grootte van 1 schermpagina) verdeeld om scrollen te voorkomen. Deze fragmenten werden in DKS-E in een zodanige boomstructuur opgenomen dat de juiste pagina met zo min mogelijk muisklikken toegankelijk wordt vanaf een startpagina. Deze startpagina is ontworpen met oog op de intuïtieve en ongeduldige gebruiker, maar staat ook het raadplegen van de klapper als naslagwerk toe. Hiertoe zijn een zogenaamde vluchtstrook en een standaardroute opgenomen. Op de vluchtstrook zit de vaak met spoed gezochte informatie direct achter een knop. Op de standaardroute is de transfusieketen chronologisch te benaderen.

Resultaten: de vertaling naar HTML en het toepassen van hyperlinks voor het koppelen van gergelateerde tekst binnen DKS-E heeft doel gerichte navigatie binnen de grote hoeveelheid informatie sterk vereenvoudigd. Door het gebruik van DKS-E is het beheer geborgd en wordt het up to date houden van de informatie mogelijk. De elektronische bloedtransfusie klapper is onlangs officieel in de kliniek geïntroduceerd en de eerste reacties zijn zeer positief.

HEMOVIGILANTIE IN EEN GROOT ALGEMEEN ZIEKENHUIS

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OLVG

Inleiding: het registreren van bijwerkingen van transfusie vraagt niet alleen goede procedures en beschrijvingen maar ook promotie van het hemovigilantiesysteem. In het OLVG, waar jaarlijks +/- 13000 bloedproducten worden getransfundeerd, zijn 2500 medewerkers werkzaam. Hoe bereik je diegenen die bij transfusie betrokken zijn?

Materiaal en methode: Bij ieder uitgegeven bloedproduct is een **sturend** transfusie- en transfusiereactieformulier bijgesloten, waarop de benodigde acties in de verschillende situaties zijn aangegeven. Bij uitgifte van de eenheden wordt het formulier toegelicht. De getransfundeerde eenheden en het bijgesloten transfusieformulier worden na transfusie van de afdelingen opgehaald om er zeker van te zijn dat de eenheid getransfundeerd is. De eenheden worden verder administratief in het LIS verwerkt. De hemovigilantie medewerker verzorgt samen met de transfusieverpleegkundige de nascholing en de instructie op iedere afdeling. Korte lijnen naar de medewerkers op de afdelingen worden daarmee gerealiseerd.

Resultaten:

- grote bereidheid tot melden van reacties/problemen bij transfusie.
- toename in aantal meldingen.

jaar	aantal	transfusie	type (%)			
			mild	matig	ernstig	overig
2003	27	12498	0.10	0.04	0.01	0.06
2004	61 (1)*	12992	0.19	0.14	0.04	0.01
2005	66 (1)*	12161	0.23	0.08	0.08	0.15

* *TRALI*

Conclusie: Sturend transfusie-transfusiereactieformulier in combinatie met advies en nascholing leveren zichtbare bijdrage in het melden van transfusiereacties en toenemende kennis over de transfusie bij laboratorium en verpleging.

WHAT PARAMETERS PREDICT CHANGES IN USE OF RED BLOOD CELL CONCENTRATES

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Background: The past ten years the use of red blood cell concentrates (RBC's) in the region Northeast has been decreased with 28%.

Goal: In 2005 The Sanquin Bloodbank northeast performed an investigation to the changes in the use of RBC's, with the ultimate goal to identify parameters that can improve the prediction of use of erythrocytes in the future.

Material en methods: The use of RBC's per year has been plotted for each individual hospital in the period 1996-2005. These data together with a questionnaire were sent to the transfusion laboratories of the hospitals in the region northeast. The transfusion laboratories were asked to relate, if possible, the changes in the use of blood products with 1) implementation of blood saving policies of techniques, 2) changes in the number of beds in the hospital 3) changes in the number of medical specialists in the hospital.

Results: Respons: 16 of the 28 hospitals have participated in the investigation by returning the questionnaire (57%).

No relation has been found between changes in the number of hospital beds and the use of blood products. In 8 of the 16 hospitals, the number of medical specialists and/or the production level of the operation rooms (OR's) are changed in the period 1996-2005. In 7 of the 8 hospitals a direct relationship is observed between these changes and the use of blood products. In 7 hospitals that introduced blood saving policies or techniques, a relation can be observed between introduction of specific policies and the use of RBC's. Especially the introduction of the Hb 4-5-6 flexi-rule (mmol/l) for transfusion of erythrocytes, resulted in a decrease of the use of RBC's of 10-20%. A significant part of the overall decrease in use of RBC's is probably caused by an increasing 'awareness' and combination of multiple blood saving techniques and policies

Conclusion: Knowledge of planned introductions of the Hb 4-5-6 flexi-rule in hospitals and knowledge of expected changes in the number of medical specialists and/or the OR production level, may lead to a better prediction of the use of blood products in the future.

THE CLINICAL SEGMENT OF THE TRANSFUSION CHAIN: HEMOVIGILANCE AT THE FRONTLINE

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Introduction: Being at the frontline, physicians and paramedics play a key role in crucial elements of the transfusion chain. Close collaboration with the clinical segment of the chain is necessary to make haemovigilance work. **Method:** the local transfusion chain was described and translated into 4 clear hospital instructions: 1) prescription, 2) product request, 3) selection and delivery and 4) transfusion and registration. The instructions were set up in collaboration with hospital workers of clinical wards. Implementation of the new instructions in the internal ward was realized by: 1) inventory of attitude, knowledge and skills of workers involved, 2) informing hospital workers by e-mail and posters, 3) feedback of the inventory results to work spot management and 4) introduction of a new transfusion registration form. Target after the implementation project: 1) compliance with the instructions, 2) reduction of sampling errors and transfusion incidents. During a 12 month period a zero measurement, an in-between measurement and an end measurement were performed **Results:** compliance with the instructions, evaluated by checking correct registration of blood components administered in the medical patient file, increased from 18.7% (zero measurement) to 53% (end measurement). Correct use of the new transfusion form increased from 13% (zero measurement) to 37% (end measurement). During the implementation period one sampling error was registered (end point) and one transfusion incident was reported. This incident was not related to incorrect handling of procedures in the clinical ward involved. However, the need to monitor pulse and tension before, ten minutes after starting the transfusion and after completing the transfusion resulted in an increase in workload of 10-15 minutes per transfusion. **Conclusion:** the implementation strategy performed in the internal medicine ward resulted in increased compliance with the hospital instructions. This however resulted in increased workload, which may limit the workability in wards transfusing many blood components.

INCREASED REPORTING OF ADVERSE TRANSFUSION EVENTS STARTS WITH COLLABORATION AND EDUCATED IMPLEMENTATION

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Introduction: in order to make a haemovigilance process work at each level, and with all people involved in the transfusion chain it is essential to create close and constructive collaboration between all participants. Physicians and paramedics play a key role in crucial elements of the transfusion chain.

Method: the local transfusion chain was translated into 4 clear hospital instructions, accessible by the intranet quality web (KWINT): 1) prescription, 2) product request, 3) selection and delivery and 4) transfusion and registration. Implementation was initiated by: 1) informing all hospital workers by announcement in a hospital newsletter, 2) informing quality officers of all clusters by educational sessions, 3) accompanying and supporting implementation strategies in hospital wards by a haemovigilance project group. After one year we evaluated the effect of the implementation strategies on the number and type of adverse transfusion events (ATE) reported. **Results:** the number of reported ATE's increased from 43 in 2004 to 71 in 2005. The percentage of reported allergic transfusion reactions related to platelet transfusions increased from 8% in 2004 to 27% in 2005. The percentage of reported allergic transfusion reactions related to plasma transfusions decreased from 85% in 2004 to 68% in 2005. After performing the implementation project the number of adverse events reported by the outward department, increased from 0 in 2004 to 9 in 2005.

	ATE (n)	RBC-related ¹		Platelet-related		Plasma-related	
		n	n/1000 ²	n	n/1000	n	n/1000
2004	43	21	1,0	9	2,8	13	2,9
2005	71	33	1,7	18	4,8	20	4,9

¹RBC: Red Blood Cells

²ATE's per units transfused

Conclusion: implementation of the new hospital instructions in close collaboration with all participants involved resulted in an increase of reported ATE's. This might be due to an increased alertness of hospital workers at the frontline of the transfusion chain induced by educated implementation.

IMPUTABILITEIT VAN KOORTS NA BLOEDTRANSFUSIE

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Aanleiding: Ruim 45% van de meldingen aan TRIP betreffen temperatuurstijging $\geq 2^{\circ}\text{C}$ (niet-hemolytische febrile (transfusie)reacties of temperatuurstijging van $1\text{-}2^{\circ}\text{C}$). In twee ziekenhuizen bestond de wens in een case-controlestudie te zoeken naar patiëntfactoren die het optreden van deze reacties in de hand kunnen werken. Bij de studie van klinische informatie bleek dat de beoordeling of temperatuurstijging te wijten was aan de transfusie, soms anders uitviel dan de oorspronkelijke inschaling.

Methode: In twee ziekenhuizen werden retrospectief statussen beoordeeld van patiënten waarbij (1-1-2003 tot 31-5-2005) een niet-hemolytische koortsreactie (temperatuurstijging $\geq 2^{\circ}\text{C}$) aan TRIP was gemeld. Patiëntkarakteristieken werden geïnventariseerd, en laboratoriumuitslagen en temperatuurscurves van de dagen voor en na de koortsreactie werden beoordeeld. Uit alle informatie werd de toeschrijfbaarheid aan de transfusie (imputabiliteit) bepaald, en vergeleken met de oorspronkelijke beoordeling.

Resultaten: Het onderzoeksbestand bestond uit 76 meldingen en wij hebben in totaal van 64 meldingen oorspronkelijk klinisch materiaal kunnen bestuderen. Bij herbeoordeling van de imputabiliteit werd 36 keer (56%) de toeschrijfbaarheid minder waarschijnlijk gevonden en 8 keer (12%) meer waarschijnlijk. De belangrijkste reden om de imputabiliteit bij te stellen was de temperatuurscurve van de patiënt, waarbij op dagen voor de transfusie ook temperatuurstijging optrad in de namiddag of avond. Wegens het grote aantal meldingen waarbij een lage imputabiliteit werd gevonden, is afgezien van de oorspronkelijke opzet van het zoeken naar predisponerende patiëntfactoren. Bij koortsreacties met imputabiliteit waarschijnlijk of mogelijk ($n=38$) betrof de bijbehorende transfusie in 32 gevallen (84%) een erythrocytenconcentraat, in 5 (13%) een trombocytenconcentraat en in 1 (3%) zowel een erythrocytenconcentraat als vers bevroren plasma.

Conclusie: Bij de beoordeling van klinische informatie van patiënten die na transfusie koorts vertoonden, is bij 56% de relatie tot transfusie minder waarschijnlijk ingeschat dan op de oorspronkelijke melding en bij 12% juist meer. Na signalering van koorts in aansluiting op een bloedtransfusie dienen zowel laboratoriumuitslagen als het totale temperatuursverloop bij de patiënt bestudeerd te worden om een juiste indruk te krijgen van de relatie tot de transfusie.

NON-INVASIVE MEASUREMENT OF PLATELET CONCENTRATE pH WITH A FIBER OPTIC FLUORESCENCE DETECTOR

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Background: Metabolism or bacterial contamination can change the pH of platelet concentrates (PC) upon storage. Many blood banks use pH for quality control of PC, but not every bag is checked since measurement requires the bag contents to be sampled with risk of contamination. A non-invasive method was developed to measure pH of PC.

Methods: Platelet storage bags were prepared with an integrated probe, consisting of an optically clear plastic window and a small membrane disc impregnated with a pH-sensitive fluorescent dye to the PC side of the window. A fiber optic based spectrometer was used to illuminate the dye through the window with a light emitting diode. The ratio of fluorescence emission at 2 wavelengths was measured and used to calculate pH. In a paired study standard platelet concentrates in plasma (derived from pooled buffy coats) were stored in either a licensed platelet container (Fresenius Hemocare; steam sterilized) or in a new platelet container with the integrated probe (BCIS; ethylene oxide sterilized). During storage, some *in vitro* quality markers were analyzed. The pH in sequential samples was measured with a blood gas analyzer (Rapidlab 860, Bayer) and for the bags with probe also with the fluorescence detector (BCSI).

Results. A good correlation was found between pH measured at day 2 till day 11 by blood gas analyzer and fluorescence detector ($r^2 = 0.9489$). The mean difference from the average pH by both methods was 0.045 (n=32, SD 0.058). No significant differences were found between the two containers tested (n=4; paired) with respect to changes in pH, CD62P expression, PS-exposure, morphology (Kunicki score and swirling) during storage for up to 8 days. Also the metabolic status of the PC, as characterized by nucleotide content (ATP, ADP and AMP) and mitochondrial membrane potential (measured with JC-1) did not differ between the two containers tested.

Conclusion: Integration of the newly developed probe in a platelet storage container did not compromise platelet quality during storage. The pH readings obtained showed a good correlation with the measurements by blood gas analyzer. Thus, the integrated probe allows for multiple (and even continuous) non-invasive pH measurements of PC during storage.

EFFECT OF EXTRACELLULAR pH ON PLATELET CONCENTRATES

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Background. During storage of platelets, among other changes (known as “platelet storage lesion”), a gradual decrease of pH as a result of lactate production takes place. So far, it is unknown if the pH decrease as such induces the “platelet storage lesion”. Therefore, we decided to apply various pH values to platelets, resuspended in additive solution (to avoid plasma effects) and investigated several biochemical parameters during storage. The experiments were repeated in 30% plasma/70% additive solution to test if the results could be applied to blood bank conditions.

Methods. To remove plasma, platelets prepared in 30 % plasma were washed in a buffer supplemented with acid citrate dextrose (ACD) and the pellet was resuspended in additive solution containing 40 mM phosphate or 30 % plasma at various pH (range 6.15 – 7.50; if applicable with 30 % plasma). Samples were stored in culture flasks under 5% CO₂, in the presence of 10 mM glucose and 12 mM bicarbonate. At various times during storage, samples were taken to analyze pH, blood gases, platelet count, glucose, lactate, mitochondrial membrane potential (with JC-1), PS exposure (with AnnexinV) and CD62P expression.

Results. An applied pH between 6.8 and 7.2 (at 37°C) was found to be optimal, with at lower or higher pH values increased rates of glycolysis and CD62P expression. The applied pH values were well maintained during storage for up to 6 days, except for the low pH values as all glucose was converted to lactate. The increase of PS exposure was similar at all pH values tested during the first 3 days. At day 6, the PS exposure was clearly increased for those samples with low pH in which the glucose was depleted. The JC-1 signal was relatively constant over the first 3 days, independent from applied pH, and collapsed on day 6 only in the samples without glucose. In the presence of plasma, platelets were able to partially counteract the applied pH. An applied pH of 6.15 resulted in a pH around 6.7 within hours, which was in contrast with washed platelets, in which the pH only increased up to 6.4. Platelets incubated in the presence of plasma were clearly much better (higher pH, lower percentage CD62P and PS positive cells, higher JC-1 signal) than in the absence of plasma over the whole pH range.

Conclusions. Our results indicate that human platelets during storage at pH < 6.8 and > 7.15 do change with respect to some, but not all characteristics of the “platelet storage lesion”. The enhancing effect of low pH on glycolysis represents a potentially deleterious forward loop during storage in plasma or plasma/additive solution mixture. An interesting finding is that at low pH an acceleration of the glycolysis was measured (in contrast with literature data).

MCS+ APHERESIS PLATELETS: 7-DAY STORAGE IS FEASIBLE

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Objective and Design: Whole blood buffy coat derived, white blood cell (WBC) reduced platelet (PLT) concentrates can be stored in plasma for at least 7 days after donation provided that bacterial screening is performed. In this study we evaluated the in vitro quality of apheresis WBC reduced PLT concentrates with a storage period of 8 days after donation. **Methods:** After receiving consent from the donors, 12 apheresis procedures were performed with the MCS+ (Haemonetics; disposable 994CFE next generation, with butyryl-trihexyl-citraat (BTHC) storage bag of 1.0 liter, software version C.5). WBC reduction was achieved by an inline filter incorporated into the disposable. The volume, the numbers of PLT, WBCs and red blood cells (RBCs) of the product were tested on day 0 after donation, as well as pH, glucose, lactate, CD62P expression and swirling effect on days 0 and 8. A sample for bacterial screening was taken at day 8 after donation.

Results (n=12):

	Mean ±	SD		
PLT (x10 ⁹ /unit)	382 ±	45		
Volume (mL)	311 ±	19		
PLT concentration (x10 ⁹ /unit)	1.2 ±	0.1		
WBC (x10 ⁶ /unit)	0.2 ±	0.3		
RBC (x10 ⁶ /unit)	0.9 ±	0.9		
	Day 0		Day 8	
	Mean ±	SD	Mean ±	SD
pH (22°C)	7.14 ±	0.03	7.07 ±	0.1
Glucose (mmo/L)	22.0 ±	1.3	15.5 ±	1.7
Lactate (mmol/L)	1.3 ±	0.5	12.0 ±	1.9
CD62P (%)	8.8 ±	4	24.7 ±	6.7
Swirling score	3+		3+	

All products complied with the requirements as mentioned in the national guidelines. Bacterial screening of all products showed no growth. **Conclusion:** At day 8 after donation, all WBC-reduced apheresis platelet concentrates are within the specifications of the national guidelines. We conclude that 7-day storage of MCS+ derived WBC reduced apheresis platelet concentrates is feasible.

REGULATION OF PHOSPHATIDYLSERINE EXPOSURE BY THE AMP-DEPENDENT KINASE IN HUMAN PLATELETS

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Introduction: One of the properties of platelets is the ability, upon activation, to expose phosphatidylserine (PS), providing a negative surface necessary for the docking of the blood coagulation complexes. Many events underlying PS exposure are still unknown despite intensive research over the last 20 years. The involvement of a phosphorylation event is suggested and therefore the AMP-dependent kinase (AMPK) could be a candidate involved in the cascade leading to PS exposure.

Methods: Platelets were washed free of plasma by three consecutive wash steps in substrate-free medium, preventing aggregation during the first two steps by acidification of the platelets with acid citrate dextrose (ACD). Platelets were subsequently incubated with different metabolic substrates. The nucleotide profiles of the platelets were analyzed by HPLC, PS exposure via flow cytometry using AnnexinV-FITC as label and phosphorylated AMPK (reflecting activation of AMPK) was determined via SDS-PAGE and immuno-blotting using antibodies against phosphorylated AMPK (P-AMPK).

Results: Under substrate-deprived conditions, AMPK becomes phosphorylated but in the presence of mitochondrial substrates (pyruvate, acetate), AMPK was phosphorylated to a lesser extent. Hardly any phosphorylation was found when glucose was present. With both mitochondrial and glycolytic substrates present, an increased P-AMPK was seen with respect to the glucose situation. The analysis of the nucleotide profiles showed a good correlation between phosphorylated AMPK and AMP/ATP ratios. Interestingly, PS exposure showed a strong correlation with AMP/ATP ratios and the phosphorylation of AMPK.

Conclusion: In human platelets, activation of AMPK strongly correlates with PS exposure, suggesting a role for AMPK in the induction of PS exposure.

PLATELET COUNTING USING VARIOUS HEMATOLOGY CELL ANALYZERS

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Background: Platelets (PLT) can be counted using impedance, optical or immunologic methods on various cell analyzers. For counting PLT in whole blood these analyzers have been tested thoroughly. In this study, various cell analyzers were tested for counting PLT in platelet concentrates (PCs), thus in absence of erythrocytes.

Material and Methods: From leukoreduced PCs, concentration series were prepared in Isoton and counted in three series in tenfold. Used levels and analyzers are shown in the table. Linearity (r^2), CV and accuracy (% within 10% of expected) were determined. Data were compared using ANOVA with $p < 0.05$ as significant.

Results: See table (mean \pm SD, n=10).

Analyzer	Expected ($\times 10^9$ PLT/L)			
	800	1100	1400	1700
AcT 8, i	819 \pm 17 ^{abcde}	1137 \pm 22 ^{abcde}	1358 \pm 24 ^{abc}	1527 \pm 9 ^{abcd}
CD, i	1010 \pm 11 ^{afghi}	1428 \pm 17 ^{afghi}	1806 \pm 39 ^{adfg}	2197 \pm 52 ^{aefgh}
CD, o	712 \pm 8 ^{bfjkl}	1013 \pm 15 ^{bfjk}	1302 \pm 28 ^{bdh}	1617 \pm 41 ^{beijk}
CD, imm	656 \pm 3 ^{cgjmn}	1008 \pm 16 ^{cglm}	1341 \pm 19 ^e	1616 \pm 26 ^{cfilm}
Onyx, i	770 \pm 11 ^{dhkm}	1069 \pm 15 ^{dhjln}	1311 \pm 31 ^{cfi}	1493 \pm 15 ^{gjln}
Sysmex,i	770 \pm 17 ^{eilm}	1095 \pm 17 ^{eikmn}	1394 \pm 23 ^{ghi}	1726 \pm 37 ^{dhkmn}

CD: Cell-Dyn 4000, Sysmex: Sysmex K4500, i: impedance, o: optical, imm: immunological (CD61), ^{a-n}significant for the specific level.

All series showed $r^2 > 0.99$. CV was $< 5\%$ for all analyzers and levels. Accuracy was below 80% for Act 8 and Onyx at 1700×10^9 PLT/L, for CDi at the whole range and for CDo and CDimm at 800×10^9 PLT/L.

Conclusion: None of the analyzers had comparable results for the tested range. Linearity and CV were acceptable for all analyzers. Sysmex K4500 was the only analyzer with $> 80\%$ accuracy for the whole range.

Of the tested analyzers the Sysmex K4500 had the best results for platelet counting in absence of erythrocytes.

REDUCED APC CATALYZED INACTIVATION OF FACTOR Va BOUND TO STORAGE INDUCED MICROPARTICLES

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Goal: Platelet concentrates contain microparticles (MP) and their numbers increase during storage. We established in a prothrombinase assay that factor V(a) was present on the surface of storage induced MP. In this study we compared activated protein C (APC) catalyzed inactivation of this MP-bound FVa to inactivation of purified plasma FVa on phospholipid vesicles and to inactivation of platelet-derived FVa bound to thrombin activated platelets.

Methods: MP were harvested from outdated platelet concentrates. The inactivation of FVa by APC was performed in the presence of either storage induced MP (without added FVa), synthetic PtdSer containing vesicles (with purified plasma FVa) or thrombin-activated platelets (without added FVa). The inactivation of FVa was started by addition of APC (0.5nM). At selected time points, samples of the inactivation mixture were assayed for residual FVa activity in a prothrombinase assay.

Results: Purified plasma FVa was rapidly inactivated in the presence of synthetic vesicles, with only 5% \pm 4% residual FVa activity after 20 min. APC-catalyzed inactivation of MP-bound FVa resulted in 38% \pm 2% residual FVa activity and residual activity of FVa bound to thrombin-activated platelets was 25% \pm 3% (N=3). Addition of synthetic vesicles to MP or platelet bound FVa resulted in a residual activity of 5-10%. Furthermore, the velocity of Arg506 and Arg306 cleavage on MP by APC is reduced when compared to synthetic lipids, whereas on the platelet surface only the Arg506 cleavage site is compromised.

Conclusions: We observed that FVa bound to storage induced MP is more resistant to inactivation by APC than purified plasma FVa on a synthetic lipid surface. We hypothesize that there might be a difference in the binding of FVa on different surfaces e.g. synthetic vs platelet (derived) surfaces, rendering it more resistant to cleavage by APC. The APC resistance of factor Va bound to storage induced MP may have a beneficial clinical effect in patients who need haemostatic support (and lack sufficient platelets).

EFFECT OF MICROPARTICLES FROM PLATELET CONCENTRATES ON HAEMOSTASIS IN A THROMBOCYTOPENIC PERFUSION MODEL

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Goal: During storage platelets release microparticles (MP). In non-flowing plasma, we demonstrated that these MP support haemostasis by enhancing the propagation phase of blood coagulation via anionic phospholipids and membrane dependent activation of FXI by thrombin. We investigated whether transfusion MP 1) adhere to collagen, fibrinogen, von Willebrand factor (VWF) or surface immobilized platelets, 2) support further platelet deposition and 3) enhance surface dependent blood coagulation in a flow model with thrombocytopenic blood. **Methods:** MP ($5 \times 10^9/L$) were added to thrombocytopenic blood ($< 20 \times 10^9$ platelets/L) and perfused after recalcification through capillaries coated with collagen, fibrinogen, platelets (shear rate of 100 s^{-1}) or VWF (shear rate of 1000 s^{-1}). MP (CFSE labelled) and platelet adhesion was detected by phase contrast and fluorescence microscopy. At the outlet of the capillary thrombin-antithrombin (TAT) complex formation was determined as a measure of thrombin formation. **Results:** Platelet-derived MP attain the capacity to firmly adhere to collagen, VWF, fibrinogen and platelets. The integrin $\alpha_{IIb}\beta_3$ blocker abciximab prevented stable adhesion of MPs on all surfaces. Yet, the addition of MP to thrombocytopenic blood did not significantly influence platelet adhesion. On the other hand, MP strongly stimulated TAT complex formation when blood was perfused over a fibrinogen surface. In the control situation TAT was detected after 9 ± 2 (N=3) min, while in the presence of MP, TAT complex formation occurred within 2 ± 1 (N=3) min. **Conclusions:** At physiologic shear rates ($100\text{-}1000 \text{ s}^{-1}$) transfusion MP adhere to haemostatic proteins and platelets via integrin $\alpha_{IIb}\beta_3$ dependent interaction. Subsequently, they support haemostasis through stimulation of blood coagulation.

SHEDDING OF PROCOAGULANT MICROPARTICLES BY INTEGRIN-MEDIATED DESTABILIZATION OF ACTIN CYTOSKELETON IN PLATELETS

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Background and Objectives: Platelet activation by potent agonists can result in shedding of microparticles that are active in coagulation. Aim of this study was to investigate by which mechanism platelet preparations stored under standard blood bank conditions can produce microparticles. **Design and Methods:** Platelets were allowed to produce microparticles by storage up to 24h in plasma at room temperature. Microparticles were quantified by flow cytometry and their procoagulant activity was evaluated by measuring thrombin generation. **Results:** Here we report that stored platelets also form procoagulant microparticles in the apparent absence of agonists and with minimal activation. This microparticle formation was not due to autocrine activation and relied on integrin α IIb β 3 activity. It was enhanced, in an integrin-dependent way, by destabilisation of the actin cytoskeleton, and involved integrin outside-in signaling. **Interpretation and Conclusions:** This integrin-dependent 'spontaneous' microparticle formation is likely to be of clinical relevance, because these high amounts of procoagulant microparticles enter the patient during platelet transfusion.

EFFECT OF PROPHYLACTIC PLATELET TRANSFUSION ON HAEMOSTASIS: PLATELET FUNCTION IN SEVERE THROMBOCYTOPENIC BLOOD

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Background and Objectives: Aim of this study was to evaluate the haemostatic effect of platelet transfusion in blood from severe thrombocytopenic patients. **Design and Methods:** The suitability of two novel methods for measuring platelet function under physiologically relevant conditions, specifically at very low platelet counts ($<60 \times 10^9$ platelets L^{-1}), was studied. Adhesion and aggregate formation on a collagen surface were measured under flow in a whole-blood perfusion assay. Coagulation was measured in platelet-rich plasma in a thrombin generation assay triggered with low tissue factor. We used both methods to determine haemostatic function of transfused platelets in thrombocytopenic blood. **Results:** Flow and thrombin generation conditions were determined at which platelet adhesion/aggregate formation on collagen and respectively coagulation were linearly related to low platelet concentrations. Improvement of haemostatic activity was evaluated in blood from 36 thrombocytopenic patients who received a platelet transfusion. In blood from almost all thrombocytopenic patients we found that platelet adhesion/aggregate formation was increased after transfusion. Moreover, transfusion with platelets also resulted in increased thrombin generation. **Interpretation and Conclusions:** We conclude that at very low platelet count ($<20 \times 10^9$ platelets L^{-1}), transfusion with platelets is necessary to improve the haemostatic activity of thrombocytopenic patients by increasing platelet adhesion/aggregation and thereby enhancing coagulation. Although time-consuming, these assays provide an alternative method for the CCI in predicting transfusion efficacy.

HET BEWAREN VAN TROMBOCYTENCONCENTRATEN IN VERSCHILLENDE TROMBOCYTEN POOLSYSTEMEN

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Doel: Het vergelijken van leukocyten gedepleteerde trombocytenconcentraten (LD-TCs) in plasma, gemaakt van buffycoats van overnacht bewaard volbloed, bereid en bewaard in 4 trombocyten poolsystemen van verschillende fabrikanten.

Methoden: Er werden 20 buffycoats, afkomstig van overnacht bewaard volbloed, en 4 plasma's gepoold, en verdeeld over de trombocyten poolsystemen van Baxter (A), Fresenius (B), Terumo (C) en Pall (D). De buffycoatpool werd gecentrifugeerd en het plaatjes rijke plasma werd handmatig overgeperst via het inline filter met een plasmaklem. Van de TCs werden op dag 1, 5, 7 en 9 monsters genomen voor het meten van pH, glucose, lactaat, CD62, aantal trombocyten (PLT) en aantal leukocyten (WBC). Daarnaast werden swirl en volume bepaald. De LD-TCs werden bewaard bij kamertemperatuur op een trombocytenschudder. Resultaten werden vergeleken met ANOVA (repeated measurements) en $p < 0,05$ werd als significant beschouwd.

Resultaten: Zie tabel (gem. \pm SD; n=5).

	Dag	A	B	C
Volume (ml)	1	326 \pm 11 ^a	312 \pm 16	303 \pm 17 ^{ab}
PLT (*10 ⁹ /E)	1	435 \pm 42	435 \pm 58	437 \pm 32
WBC (*10 ⁶ /E)	1	0,14 \pm 0,26	0,15 \pm 0,13	0,02 \pm 0,01
pH (22°C)	1	7,16 \pm 0,04 ^c	7,17 \pm 0,04 ^{de}	7,15 \pm 0,02 ^d
pH (22°C)	9	7,20 \pm 0,03	7,12 \pm 0,20	6,74 \pm 0,49
Glucose (mmol/l)	9	13,0 \pm 0,8	11,8 \pm 2,5	8,3 \pm 5,1
Lactaat (mmol/l)	9	18,1 \pm 2,1 ^f	20,1 \pm 5,5	24,1 \pm 5,7 ^f
CD62 (%)	9	30 \pm 6	27 \pm 6	39 \pm 26

^{a-f} $p < 0,05$

De swirl bleef $\geq 2,5$ tot en met 7 dagen bewaren. Op dag 9 was de swirl $\leq 1,5$ tweemaal voor LD-TCs in zak C en eenmaal voor LD-TCs in zak D. Een swirl ≥ 2 wordt beschouwd als goed. Eén LD-TCs in zak C gaf op dag 5 een pH $< 6,8$. Op dag 7 werd tweemaal in zak C en eenmaal in zak D een pH $< 6,8$ waargenomen en op dag 9 was dit eenmaal in zak B, tweemaal in zak C en eenmaal in zak D. Op dag 9 werd geen significant verschil gevonden in pH, glucose en CD62 tussen de verschillende zakken.

Conclusie: LD-TCs bereid met trombocyten poolsystemen A, B, C en D resulteren in LD-TCs met een hoog aantal PLT, een laag aantal WBC en voldoende glucose. LD-TCs in zakken A en B voldoen op dag 9 beter aan swirl (≥ 2) en pH ($> 6,8$) dan LD-TCs in zakken C en D.

EFFECTS OF NON-AGITATION ON IN VITRO PARAMETERS OF PLATELET CONCENTRATES STORED IN COMPOSOL-PS

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Background: Leukoreduced platelet concentrates (LR-PCs) should be stored with continuous agitation at room temperature. This study investigated the effects of interruption of agitation on in vitro parameters of LR-PCs in Composol-PS (with 35% plasma).

Method: This study was conducted as a paired in vitro study by pooling and dividing four LR-PCs in Composol-PS. These LR-PCs were stored for 7 days (after preparation) under the following conditions: pool A, continuously agitation; pool B, agitation interrupted until 16 hours after preparation; pool C, agitation interrupted until 20 hours after preparation; pool D, agitation interrupted until 24 hours after preparation. After the period of non-agitation, the LR-PCs were continuously agitated until 7 days after preparation. Various in vitro parameters were determined on 0, 16 (A and B), 20 (A, B and C), 24, 114 and 162 hours after preparation. We required that $pH_{37^{\circ}C}$ was >6.8 and swirl was present at end of storage. The difference between non-agitated LR-PCs and reference was tested with ANOVA Dunnett's test. $P < 0.05$ was used to indicate a statistically significant difference.

Results: See table (t=162 h; mean \pm SD; n=10) * significant difference.

	A, reference	B (16h)	C (20h)	D (24h)
Swirl present	10-Oct	10-Oct	10-Oct	10-Oct
$pH_{37^{\circ}C}$	7.05 \pm 0.02	6.85 \pm 0.09 *	6.68 \pm 0.15 *	6.59 \pm 0.20 *
$pH < 6,8$	0/10	01-Oct	06-Oct	09-Oct
Glucose (mM)	3.8 \pm 0.3	2.0 \pm 0.7 *	1.1 \pm 1.0 *	0.7 \pm 0.9 *
Lactate (mM)	10.3 \pm 0.5	13.6 \pm 1.1 *	15.0 \pm 1.7 *	15.9 \pm 1.5 *
MPV (fL)	8.6 \pm 0.3	8.8 \pm 0.3	9.0 \pm 0.5	9.2 \pm 0.5 *
CD62p expression (%)	18.9 \pm 2.6	21.4 \pm 3.1	27.5 \pm 9.1	35.3 \pm 18.4 *

Conclusion: The quality of LR-PCs decreased without agitation. After 16 hours non-agitation, the platelets conformed to the requirements until 162 hours after preparation. Initial non-agitation for a period of \pm 20 hours leads to unacceptable changes at the end of storage.

COMPARISON OF IN VITRO BLOOD COMPONENT QUALITY USING TWO DIFFERENT DESIGN BUTANE-1,4-DIOL COOLING SYSTEMS

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Objective: A new system (CompoCool-II) to rapidly cool and keep whole blood (WB) at room temperature during a 20-24 hours period was developed by Fresenius HemoCare. The system consists of a closed box and a cooling cartridge filled with butane-1,4-diol, it is smaller and lighter than the system currently in use in our blood bank (Compocool-I). The cooling cartridge is placed vertically in the middle of the transport box and WBs are placed upright on both sides of the plate. We compared both CompoCool systems.

Materials and method: Units of WB were collected and immediately cooled to 20-24°C using either the CompoCool-II or the Compocool-I system. After overnight storage (16-20 h after collection) the WB was centrifuged by use of a hard spin and separated into a red cell concentrate in SAGM (RCC), a plasma (PL) and a buffy coat (BC) using the Compomat G4. The RCC was leukoreduced (LR) with the integrated in-line filter. Various in vitro measurements were performed. The LR-RCCs were stored at 2-6°C and sampled until day 42.

Results: See table (mean \pm SD, n=23 in both groups). The composition of the blood components was similar in both groups. There were no significant differences in storage parameters for LR-RCCs. LR-RCCs and PLs conformed to the national requirements.

Conclusion: No significant differences could be detected between the Compocool-I and CompoCool-II groups. Both systems are suitable for cooling and overnight storage of WB with maintenance of in vitro quality.

LR-RCC	Compo-cool-I	Compo-Cool-II	Requirements	p value
Volume, mL (day 1)	268 \pm 16	271 \pm 24	>245	n.s.
Hemoglobin, g (day 1)	47 \pm 4	49 \pm 7	>40	n.s.
Hemolysis, % (day 42)	0.4 \pm 0.2	0.4 \pm 0.3	<0.8	n.s.
ATP, μ mol/g Hb (day 42)	2.9 \pm 0.4	3.0 \pm 0.4		n.s.
pH (day 42)	6.38 \pm 0.04	6.36 \pm 0.03		n.s.
BC				
Volume, mL	48 \pm 1	48 \pm 2	45-55	n.s.
Hematocrit, %	42 \pm 3	41 \pm 3	35-45	n.s.
Platelets, $\times 10^9$	102 \pm 26	99 \pm 24	>75	n.s.
Plasma				
Volume, mL	291 \pm 16	288 \pm 24	>225	n.s.
Factor VIII, IU/mL (day 42)	1.09 \pm 0.28	1.01 \pm 0.16	>0.7	n.s.

INVESTIGATION OF MIXING OF WHOLE BLOOD UNDER VARIOUS FLOW RATES USING THE HEMOLIGHT PLUS COLLECTION MIXER

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Background: Blood collection mixers are used to thoroughly mix whole blood with anticoagulant during blood collection. It was our aim to investigate the mixing capacity of the HemoLight Plus (Fresenius HemoCare) blood collection mixer at various flow rates.

Methods: Whole blood was mimicked by a 25% v/v glycerol solution with a specific gravity equal to that of whole blood (1.060 g/mL). A blood collection system was prepared for the study by removing the CPD anticoagulant, staining the CPD with 0.32 mM toluidin blue (TB), and returning the TB-CPD into the collection system. The imitated whole blood was warmed to 37°C and pumped into this collection container at a flow rate of 30, 60 and 90 mL/min. The solutions were then continuously mixed by the swinging tray of the mixer. After 500 mL was added, the procedure was terminated. The bag was very carefully moved upright and placed in a plasma extractor. The tubing was cut and fractions of 10 mL were collected in tubes. The extinction of each of the fractions was measured at 620 nm. All samples were thereafter pooled to give a "100% sample". The extinctions of the individual samples were calculated as percentage of the "100% sample". Experiments were performed in triplicate.

Results: All fractions conformed to the requirement of a relative extinction within 20% of the "100% sample", so, at all 3 flow rates, the imitated whole blood was well mixed with the TB-CPD. At flow rates of 60 and 90 mL/min the median relative extinction was between 96 and 101% and at flow rates of 30 mL/min the median relative extinction was between 88 and 106%.

Conclusion: The HemoLight Plus collection mixer ensures good mixing of the whole blood with anticoagulant, both at normal (60 mL/min), very high (90 mL/min) and very low (30 mL/min) blood flow speeds. At very low blood flow speeds (30 mL/min) mixing of the solutions was not optimal but the degree of homogeneity still conformed to our requirements.

INVESTIGATION OF COMPOYIELD, A WHOLE BLOOD FILTER THAT DOES NOT REMOVE PLATELETS

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Background: Fresenius HemoCare has developed a platelet saving filter to filter whole blood (WB).

Aim: To investigate 1) the leukocyte removal capacity and the platelet yield of the CompoYield WB filter 2) the composition of the platelet concentrates (PCs) made from the leukoreduced (LR) WB 3) the storage parameters of the LR-PCs.

Methods: WB (500 mL) was collected in routinely used collection systems and stored overnight under butane-1,4-diol plates. CPD was removed from an experimental CompoYield system. WB was transferred to the experimental system and filtered with the platelet-saving leukoreduction filter within 20-22 hours of collection, and the LR-WB was stored for 2 hours before further processing. LR-WB was separated by centrifugation and subsequently processed on an automated expessor. LR-BCs were stored at room temperature for 2 hours before further processing. LR-PCs were made from 5 AB0 identical LR-BCs and 1 LR-PL in a pooling kit without filter (CompoYield group) and compared with routine LR-PCs made in a pooling-kit with filter (Control group). BC pools were centrifuged and separated using routine procedures. LR-PCs were stored on a flat bed shaker at 22 °C and sampled on day 1, 6 and 8. Various in vitro measurements were performed.

Results: See table (mean \pm SD). PCs in both groups met requirements for LR-PCs, although the number of platelets in the CompoYield group was $<250 \times 10^9$ /unit in 3/16 PCs. In vitro storage characteristics of the LR-PCs in the CompoYield group were good and, due to the lower platelet numbers, reflect better storage conditions than LR-PCs originating from routine procedures.

Conclusion: PCs made from WB after filtration by CompoYield were leukoreduced and conformed to European requirements. In vitro storage characteristics were comparable to routinely produced LR-PCs. Platelet yield needs further optimisation.

LR-PC	CompoYield	Control	Requirements	p value
Volume, mL (day 1)	326 \pm 11	372 \pm 16	>200	<0.001
Platelets, $\times 10^9$ (day 1)	307 \pm 65	409 \pm 69	>250	<0.001
Leukocytes, $\times 10^6$ (day 1)	0.27 \pm 0.25	0.05 \pm 0.03	<1	<0.01
pH at 37 °C (day 8)	7.07 \pm 0.05	6.68 \pm 0.44	>6.8	<0.001
CD62P expr., % (day 8)	17.8 \pm 4.9	29.7 \pm 23.6		n.s.

EFFECT SLANGLENGTE OP HET FILTREREN VAN VOLBLOED TEN BEHOEVE VAN LEUCOCYTEN DEPLETIE

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Inleiding: Om filtratie van volbloed te optimaliseren is de slanglengte tussen filter en afnamezak gevarieerd. Het doel is het onderzoeken van het effect van de verschillende slanglengten op kwaliteit van het gefiltreerde volbloed.

Methode: Twaalf overnacht bewaarde volbloed eenheden werden met verschillende slanglengten (zie tabel) gedockt aan een filter. Eenheden zijn bemonsterd voor en na filtratie. Volume, aantal leukocyten (WBC), aantal trombocyten (PLT) en filtratieduur zijn bepaald. Resultaten zijn vergeleken met de ongepaarde T-toets met $p < 0,05$ als significant.

Resultaten: zie tabel (gemiddelde, \pm SD, $n = 6$).

Systeem		1	2
Slanglengte boven filter (mm)		450	250
Slanglengte onder filter (mm)		800	800
Tijdsduur filtratie (min: sec)		12:18 \pm 1:27*	16:38 \pm 3:14*
Volume (ml)	voor filtratie	569 \pm 3	566 \pm 5
	na filtratie	529 \pm 3	526 \pm 4
% verlies		7.0 \pm 0.1	7.1 \pm 0.04
Ht (%)	voor filtratie	36.1 \pm 2.0	37.5 \pm 3.0
	na filtratie	35.7 \pm 2.0	37.6 \pm 3.2
WBC ($\times 10^6$ /eenheid)	voor filtratie	2616 \pm 659	3521 \pm 894
	na filtratie	0.61 \pm 0.82**	0.58 \pm 0.42**
Log reductie		3.95 \pm 0.55**	3.93 \pm 0.43**
PLT ($\times 10^9$ /eenheid)	voor filtratie	79 \pm 27	98 \pm 21
	na filtratie	19 \pm 16	22 \pm 10
% opbrengst		24.6 \pm 14.7	21.8 \pm 9.0

* $p < 0.05$, ** $n = 5$

Voor beide systemen is bij 1 op de 6 volbloed eenheden het WBC aantal na filtratie $> 1.0 \times 10^6$ /eenheid.

Conclusie: Het effect van slanglengte is voornamelijk te zien in filtratie duur. Hoe langer de slang boven het filter, hoe korter de filtratietijd. Geen significant verschil is gezien in de overige parameters.

IN VITRO EVALUATION OF RED CELL CONCENTRATES AFTER FILTRATION OVER THE NEW COMPOSAFE Pr SYSTEM (system for prion reduction from leukocyte depleted RCC)

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Background: Although the risk for contamination of RCC with prions is unknown, several companies are developing filters for prion reduction of blood components. The prion filter for RCC recently developed by Pall (LAPRF), was combined with leukodepletion in a Fresenius whole blood system and tested for the *in vitro* quality of RCC during storage for up to 42 days.

Methods: Whole blood was collected in standard Fresenius in line systems with integrated whole blood filter. The whole blood was either leukoreduced after overnight storage at 22°C (group I; n=8) or within 4 h (group II; n=8) and subsequently separated into plasma and RCC in SAGM. For group I the RCC units were immediately filtered over the prion filter (Composafe Pr) and for group II the RCC were filtered over the prion filter after overnight storage at 4°C. After filtration over the prion filter all RCC were stored at 4°C and sampled at various time points during storage.

Results. The CompoSafe Pr filter had no effect on the total amount of protein in the supernatant of the RCC, but removed factor IX from the supernatant (before 0.14 IE/ml, after < 0.01 IE/ml) which has been indicated as a pseudomarker for prion removal. The RCC in both groups contained 54 ± 4 g Hb (mean \pm SD), with a combined loss due to the whole blood and RCC filtrations, including losses in tubes and bags by transfer, of about 20 g Hb. The CompoSafe Pr filtration causes an additional loss compared to whole blood filtration of about 7 g Hb. The filtration over the CompoSafe Pr filter induced a slight increase of hemolysis (about 0.03% increase), but this did not result in a more rapid increase during subsequent storage: after 42 days 0.26% hemolysis for group I and 0.16% for group II were detected. For group I at day 42 1.3 % of the red cells were positive for AnnexinV (representing PS-exposure), whereas for group II this was 0.9 % (comparable to standard leukodepleted RCC in SAGM). The amount of ATP was predicted to be above 2.7 μ mol/g Hb at day 35, with about 15 mM glucose remaining at day 42.

Conclusion: Despite the double filtration step in the total procedure (one to leukoreduce the whole blood and one to remove prions from RCC), the remaining RCC met the European requirements for Hb content. The amount of Hb in the final RCC is similar to that in leukodepleted RCC prepared from whole blood after buffy coat depletion. The *in vitro* quality after 42 days was similar to those of standard leukodepleted RCC, prepared from whole blood after buffy coat depletion.

EVALUATION OF THE IN VITRO QUALITY OF FROZEN AND THAWED RED CELLS PROCESSED WITH THE HAEMONETICS® ACP®215

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Background: Haemonetics has developed an automated closed system, the ACP215 system, which allows for glycerolization and deglycerolization of certain red blood cell (RBC) products. Recently, a larger bowl, 325 mL, has been developed that should allow the processing of larger RBC units. The aim of this study was to compare the freeze-thaw-wash recovery obtained with the normal (275 mL) bowl ("N") and the large bowl ("L") and to investigate the post-thaw quality of previously frozen, deglycerolized red cells resuspended in the additive solutions SAG-M or AS-3. **Methods:** A total of 69 units of leukoreduced red blood cell concentrates (RCC) were frozen with 40% glycerol and stored at -80°C for at least 14 days. The thawed units were deglycerolized with the ACP215 with a 275-mL ("N", n=24) or 325-mL disposable bowl ("L", n=45). The deglycerolized (325-mL bowl) were stored in SAGM or AS-3 at 2-6°C for 21 days and assayed for hematological and metabolic parameters. Values are expressed as mean±SD. **Results:** The freeze-thaw-wash recoveries were 73±8% (N) and 83±5% (L). The deglycerolized products contained 39±6 g (N) and 43±4 g (L) hemoglobin with a volume of 295±3 mL (N) and 355±3 mL (L). The average hematocrit was 42±6 (N) and 40±4% (L). Units stored in SAGM exhibited significantly greater hemolysis than those stored in AS-3. Hemolysis remained below 0.8% for 2 days in SAGM, and for 14 days in AS-3. The ATP content of red cells stored in AS-3 declined faster than those stored in SAGM. The faster decline in ATP was accompanied by a lower consumption of glucose and production of lactate in AS-3 as compared to red cells in SAGM. **Conclusions:** Deglycerolization of previously frozen, leukoreduced RCCs using the ACP215 equipped with the disposable 325 mL bowl yields products with a higher recovery and higher hemoglobin content as compared to the 275-mL bowl. The deglycerolized units meet the acceptance criteria (Council of Europe) for volume (>185 mL), hemoglobin content (>36 g) and levels of supernatant hemoglobin (<0.2 g). With both bowls, the hematocrit of the deglycerolized units was below the European recommendation (0.50-0.65). The integrity of deglycerolized cells, as measured by hemolysis, is better maintained during storage in AS-3 than in SAGM. The rapid decline in ATP in AS-3 could be due to the lower pH of the cells, resulting in lower glycolytic activity and therefore lower production of ATP.

EFFECT OF THE pH ON THE ATP CONTENT AND GLYCOLITIC ACTIVITY OF THAWED RED CELLS

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Background: Cryopreservation of RBCs is a valuable approach for managing an inventory of rare RBC units. A major limitation of the use of thawed RBC units may be the shelf life of only 24 h when a functionally open system is used for deglycerolization. The Haemonetics® ACP® 215, an automated, functionally closed system for glycerolization and deglycerolization of human RBC units, allows for longer storage after thawing. It has been shown before that the integrity of thawed cells is better maintained in AS-3 as compared to AS-1 and SAGM. However, during storage in AS-3, the ATP content of the red cells rapidly declines which might be due to the relatively low pH of cells resuspended in AS-3. Therefore we investigated the effect of pH manipulation on the glycolytic activity, the ATP content and the integrity of thawed cells during storage in AS-3. **Methods:** Leukoreduced red blood cell concentrates (RCC) were frozen with 40% glycerol and stored at -80°C for at least 14 days. The thawed units were deglycerolized using the ACP215 with saline-glucose (Baxter, pH 5.2), phosphate-buffered saline with glucose (Bioluz, pH 6.7) or PBS, pH 7.4. The deglycerolized cells were resuspended and stored in either normal AS-3 (pH 5.9) or AS-3 pH 7.6 at 2-6°C for 21 days and assayed for internal pH, hematological and metabolic parameters. Values are expressed as mean±SD of at least 3 independent experiments. **Results:** Hemolysis during storage in AS-3 was comparable for all 3 washing solutions. Cells washed with PBS had a higher internal pH (6.50 ± 0.03) as compared to the glucose-containing washing solutions: 6.35 ± 0.04 (Baxter) and 6.20 ± 0.01 (Bioluz). In addition, during storage, the ATP content remained higher in the cells washed with PBS: $45\pm 7\%$ of the value at day 0 after 3 weeks of storage. After washing with the Bayer and Bioluz solution this was $32\pm 5\%$ and $24\pm 3\%$ resp. Storage of the cells in AS-3 pH 7.6 resulted in an even higher internal pH (6.84 ± 0.045) and a better maintenance of the ATP content ($64\pm 2\%$ after 3 weeks). At this higher pH, the production of lactate was increased, indicating higher glycolytic activity. **Conclusions:** Increasing the internal pH of thawed, deglycerolized cells by increasing the pH of the washing solution results in a better maintenance of the ATP content of the cells during storage in AS-3. This effect could be enhanced by an increase of the pH of AS-3. Since there is a correlation between ATP content and *in vivo* survival of red cells, it is important to maintain ATP levels during storage. The use of a washing solution with higher pH for deglycerolization is an easy way to achieve this.

EXPLORING THE POSSIBILITIES OF CRYOPRESERVATION OF CORD BLOOD STEM CELLS BEFORE AND AFTER EX VIVO EXPANSION.

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AIM: Umbilical cord blood (CB) stem cells are increasingly used for hematopoietic stem cell transplantation. The limited number of stem cells that can be obtained from CB and the delayed hematopoietic recovery after transplantation, may however hamper the use of CB in adult patients. Ex vivo expansion of CB stem cells is one approach to increase the number of cells in the graft. Moreover, we have recently shown that ex vivo expansion for 10 days with thrombopoietin (TPO) accelerates platelet recovery in NOD/SCID mice. In this study, we have investigated whether it is feasible to cryopreserve the ex vivo expanded CB stem cells. This would not only simplify the logistics for transplantation, but most important this would increase the safety of the graft, e.g. possible higher expansion rates, the patient is guaranteed to receive a successfully expanded product, and testing for contamination after expansion is possible.

METHODS: In our experiments we compared the clonogenic capacity (HPC-GEM and CFU-Mk) and the expansion potential (10 day culture with 50ng/ml TPO) of fresh and cryopreserved CB stem cells. After culturing with TPO, we analyzed the clonogenic capacity of the expanded cells. In addition, the expanded cells were subjected to a freeze/thaw cycle and the viability and clonogenic capacity of the thawed expanded cells was analyzed and compared to non-frozen expanded cells.

RESULTS: Our results show that the recovery of the cells after cryopreservation is $77\pm 11\%$ with a viability of $87\pm 15\%$. No difference in recovery and viability was observed between the expanded and non-expanded cryopreserved cells. Ex vivo expansion of stem cells with TPO appears to be higher with fresh cells (15 ± 11 fold) compared to cryopreserved stem cells (8 ± 4 fold). This effect was reflected in the CFU-Mk cultures; 32 ± 20 colonies/ 10^3 WBC for the fresh cells vs. 23 ± 16 colonies/ 10^3 WBC for the cryopreserved stem cells. In contrast, there was no adverse effect of cryopreservation on the HPC-GEM cultures (338 ± 96 vs. 313 ± 32 colonies/ 10^3 WBC respectively). Cryopreservation of ex vivo expanded CB stem cells appeared to have no effect on the colony formation in the CFU-Mk and HPC-GEM cultures.

CONCLUSION: These results show that ex vivo expansion of fresh CB stem cells is preferred over expansion of cryopreserved CB stem cells. After expansion, there appears to be no adverse effect of cryopreservation on clonogenic capacity. Therefore, these results seem promising for a strategy to ex vivo expand (part of) the CB stem cells before storage.

THE ATREUS 2C+ SYSTEM FOR AUTOMATED SEPARATION OF WHOLE BLOOD

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Objective: This study aimed to assess yield and *in vitro* quality of blood products, obtained using an early prototype (beta 2.0) of the fully automated Atreus WB 2C+ system and compared with products obtained with our semi-automated routine procedure.

Methods: Whole Blood (WB) was collected in top and bottom bags and randomly selected to be processed by either current routine (control) or the Atreus prototype system (test). For the Atreus group (n=26) the WB was transferred into a processing bag, placed in the device and after a hard spin separated into a red cell concentrate in SAGM (RCC), a buffy coat (BC) and a plasma (PL) unit. The RCC was then leukoreduced (LR) with a filter. For the control group (n=34) the WB was centrifuged by a hard spin, separated into a RCC, BC and PL using a Compomat. The RCC was leukoreduced with the integrated inline filter. Various *in vitro* measurements were performed. The LR-RCCs were stored at 4°C and sampled until day 42.

Results: See table (mean \pm SD). LR-RCCs had similar composition in both groups on day 1. Hemolysis was higher at day 42 in the test group, but well within specifications; ATP values were higher in the test group over the whole course of storage. The test-BC contained fewer platelets. Test-PL had a >100-fold lower red cell (0.37 ± 0.29 vs. $72 \pm 60 \times 10^6$; $p < 0.001$) and WBC contamination (0.03 ± 0.02 vs. $4.0 \pm 3.5 \times 10^6$; $p < 0.001$) as compared to the controls.

Conclusion: The prototype Atreus WB 2C+ system yielded LR-RCCs with similar *in vitro* quality as controls, produced BCs with a lower platelet content (suggesting further system optimization is needed) and plasma with very low levels of leukocytes and red cells.

LR-RCC	Atreus	Control	requirements	p value
Volume, mL (day 1)	295 \pm 23	293 \pm 17	>245	n.s.
Hemoglobin, g (day 1)	54 \pm 6	54 \pm 5	>40	n.s.
WBCs, $\times 10^6$ (day 1)	0.11 \pm 0.09	0.14 \pm 0.26	<1	n.s.
Hemolysis, % (day 42)	0.37 \pm 0.12	0.25 \pm 0.11	<0.8	<0.001
ATP, μ mol/g Hb (day 42)	3.0 \pm 0.4	2.7 \pm 0.4		<0.01
pH (day 42)	6.35 \pm 0.05	6.37 \pm 0.04		n.s.
BC				
Volume, mL	53 \pm 2	50 \pm 3	45-55	<0.001
Hematocrit, %	42 \pm 6	41 \pm 3	35-45	n.s.
Platelets, $\times 10^9$	82 \pm 18	102 \pm 21	>75	<0.001

MONOCYTE COLLECTION WITH MCS+

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Objective and Design: Cellular immune therapy holds great promise for the treatment of a wide variety of diseases (cancer, autoimmune disease and organ transplantation). Robust, closed system production methods under cGMP conditions to obtain dendritic cells are crucial. The aim of our study was to develop a leukocytapheresis method to obtain monocytes from individuals not stimulated with G-CSF, using the MCS+ (Haemonetics®). To enable further processing of the monocyte-rich blood component, the following specifications were defined: volume >150 mL, Ht <0.05 L/L, WBC >5x10⁹/unit, monocytes >1x10⁹/unit, and granulocytes <1x10⁹/unit.

Methods: After informed consent of voluntary blood donors, in an ongoing study, leukocytapheresis procedures were performed with the MCS+, applying the PBSC protocol and a modified 971E disposable. Donor full blood counts (pre- and post-donation) and composition of products were determined on an automated impedance counter (Sysmex, XT1800i).

Results: Up to now, after 21 procedures to achieve the optimal parameter settings, 5 procedures with final settings were performed. The results of the procedure time and the composition of the product (mean ±SD) of the latter 5 procedures are shown in the table.

	Mean (± SD)
Time (min)	120 ± 18
Processed volume (mL)	4,154 ± 634
Product volume (mL)	249 ± 11
Ht (L/L)	0.02 ± 0.01
WBC (x10e9/unit)	5.4 ± 2.6
Monocytes (x10e9/unit)	1.1 ± 0.6
Granulocytes (x10e9/unit)	0.5 ± 0.6
Granulocytes (%)	8 ± 7

Conclusions: We were able to develop a leukocytapheresis method for the MCS+ to obtain a monocyte-rich product with low red cell and granulocyte contamination within a reasonable procedure time.

PREVENTING THE “SPURTING RESERVOIR PHENOMENON” DURING PLASMAPHERESIS

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INTRODUCTION:The Autopheresis-C system (Baxter) is a fully automatic machine for the collection of human plasma. Plasma is separated from blood cells in a rotating filter (called separator). The blood cell concentration is temporally stored in a small reservoir before it is given back to the donor (return phase). The opening to this reservoir has a coarse filter to be sure that the filling is not turbulent. In a small (but unknown) percentage of plasma donors the blood cells concentration flows in a turbulent way and it spurts against the wall of the reservoir (hence de term “spurting reservoir”). When this occurs, some of the erythrocytes are destroyed by mechanical haemolysis. It is presumable that free haemoglobin is infused to the donor when red cells are returned. Free haemoglobin is known for its renal toxicity.

The probable cause of the spurting reservoir is the activation of donor trombocytes in the separator filter. This can cause clogging in the tubing that connects it with the reservoir and in the coarse filter inside the reservoir. Due to the pressurisation of the bloodline crossing the blood pump and the decrease in capacity of the filter in the reservoir, blood is squirting out though the remaining openings of the filter.

The cause of the activation of the trombocytes is yet unknown. The spurting reservoir could be donor related. When it occurs it is most likely to recur in the next donation. Other factors like a longer procedure time, low plasma flow, high haemoglobin level and a high level of trombocytes could also contribute to the problem. It is unknown which role, if any, is played by the donor plasma coagulation factors that contribute to the spurting reservoir problem. In every plasma collection procedures the extra-corporeal donor blood is partially de-coagulated with the use of sodium citrate. In the Netherlands the concentration sodium citrate is used in a 4% ratio (4ml sodium citrate on 100ml whole blood). This study is performed to investigate the effect of a higher AC-ratio on the occurrence of the “spurting reservoir”.

METHOD:In January and February 2006, 984 plasmacollections were performed in the collection centre in Delft on 10 different Autoferesis-C machines. During the study 5 machines used the 4% ratio and simultaneously 5 machines used a 6% ratio. Only donors who signed an informed consent were accepted. They were blinded for the procedure chosen.

Additional to the donor and collection data standard forms and administrative computer input, data was collected concerning clinical (donor) complications and the presence of absence or spurting reservoir on a special study form.

RESULTS

	NUMBER OF DONATIONS	NUMBER OF SPURTING RESERVOIR	ML CITRATE USED BY DONATION	LIGHT (C1) CITRATE TOXICITY	C2 and C3 CITRATE TOXICITY	OTHER ADVERSE REACTIONS	DONATION TIME (min)
4% citrate	469	3	94	0	0	0	40,7
6% citrate	513	1	131	9	0	0	39,3
% onbekend	2	0		0	0	0	
TOTAL	984	4		9	0	0	

CONCLUSIONS:Spurting reservoir happens in 0.64% of the plasma donation procedures with the Autopheresis-C machine if a citrate ratio of 4% is used for partial extracorporeal anticoagulation. The use of a citrate ratio of 6% during plasmapheresis may help to prevent some of the cases of spurting reservoir but our research found no statistical difference (to find a statistical difference with the low incidence reported a sample size of more than 4000 for each citrate group is required). Donors receive approximately 39% more citrate in their bodies, which leads to a slight increment of citrate toxicity. Although the differences in number of citrate toxicity are statistically very significant, the severity of the reactions is mild.

VALIDATION OF THE TRANSFUSIO-THERM 2000 MICROWAVE FOR THAWING FRESH FROZEN PLASMA

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Aim: To compare our standard method (water bath 37°C, 18 minutes) for thawing fresh frozen plasma (FFP) to a new fast method (Transfusie-Therm 2000, Biotest, 5-14 minutes depending on the number of FFP).

Method: The experiment was divided into 2 parts.

Experiment 1.

Five FFP's were thawed in a water bath (WB), then sampled and divided into paediatric units (PU) of approximately 80 ml. These PU's were frozen at -30°C. Then one PU was thawed in a WB and 1 was thawed in the Transfusio-Therm 2000 (TT).

Experiment 2.

Initially, 10 FFP's were either thawed in a WB (n=5) or in the Transfusio-Therm 2000 (n=5), sampled and frozen again at -30°C. Then the WB thawed FFP's were thawed for the second time in the Transfusio-Therm 2000 (WB -> TT) and the TT thawed FFP's were thawed for the second time in the water bath (TT->WB).

All samples were immediately frozen at -70°C.

Total Protein, IgG, Factor V, Factor VII, Factor VIII, Factor IX, Fibrinogen, PT, APTT, von Willebrand activity, D-Dimers and complement activity (classical pathway) were measured simultaneously in all samples.

The freeze/thawing effect was calculated by dividing the result of the second thawing by the result after the first thawing.

Results and Conclusions: Compared to the standard water bath method no significant decrease in activity or concentration in the tested parameters was found when using the Transfusio-Therm 2000.

As expected there was a freeze/thawing effect for FVIII and von Willebrand activity (82 and 106%) in both methods. The freeze/thawing effect of all other parameters is between 91 and 106% in both methods. Thawing paediatric units in the Transfusio-Therm 2000 resulted in slight turbidity in the plasma's which could not be confirmed by decreased activity of the parameters measured.

FIBRIN GLUE PREPARED FROM SINGLE DONOR ALLOGENOUS PLASMA

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Background: Fibrin Glue prepared from human plasma with the CryoSeal-1 device (CS-1, Thermogenesis), consists of two syringes in a sterile overwrap. One syringe contains thrombin and the other syringe contains cryoprecipitate. When thrombin and cryoprecipitate are sprayed together over a wound, the thrombin converts fibrinogen into fibrin. This will facilitate clot formation and can reduce postoperative bleeding. Until now, mainly autologous plasma has been used for this purpose. Our aim was to validate this method using single donor allogeneous quarantain plasma.

Methods: Fibrin Glue was produced from quarantain plasma using the CS-1. The plasma that had been stored frozen for at least six months, was thawed in a 37°C water bath until 4-8°C and was kept refrigerated (4-8°C) until processing. The fibrin glue was characterized by yield (ml fibrin glue), clotting time (seconds) and clot stability (qualitative factor XIII test). Reproducibility was tested by pooling and splitting two thawed plasma units in equal portions before processing, with 9 paired procedures.

Results: Of the 44 procedures, 39 were successful. From one unit of plasma (281 ± 15 ml, $n=39$) we obtained 12.4 ± 3.2 ml (range 5.0-19.2) fibrin glue in about ninety minutes (67 ± 5 min on CS-1 plus about 20 min hands-on time). Only three procedures resulted in less than 8.0 ml fibrin glue. The average clotting time was 2.9 ± 0.9 s and the clots did not resolve within 24 hours, indicating enough factor XIII. Reproducibility of paired fibrin glue products resulted in a difference between the two products of 1.8 ± 1.2 ml (range 0.1-4.0) in yield and of 0.8 ± 0.7 s (range 0-2) in the clotting test. The unsuccessful procedures were due to a clot in the plasma (1x), cryopoor plasma that was not removed from the cryoprecipitate (2x), and a batch defect (2x).

Conclusion: Fibrin glue can be easily obtained from single donor allogeneous quarantain plasma with in general high yields (> 8.0 ml), sufficient short clotting times (< 10 s), stable clots and good reproducibility.

HIGH VARIABILITY IN COAGULATION CONTENT OF FRESH FROZEN PLASMA

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Goal: Fresh frozen plasma (FFP) is transfused in patients with prolonged clotting times or with apparent bleeding. However, current approaches for FFP use in the clinic are not evidence-based. Furthermore, the clinical effectiveness in patients is variable and adverse events can occur. To be able to correct haemostasis in patients and to prevent undesired effects, an exact knowledge of the content of our FFP is warranted.

Methods: In 20 FFP units we determined the level of coagulation factors II, V, VII, VIII, IX, XI and the protease inhibitor antithrombin. We also measured IgG, IgM and albumin levels. In 10 additional FFP units, we measured the number and cellular origin of microparticles (MP). We also determined the ability of FFP to generate thrombin after recalcification in the presence of 1pM tissue factor (TF).

Results:

N=20 FFP	FIII	FV	FVII	FVIII	FIX	FXI	AT
Mean	125	123	110	114	123	124	98
Sd	13	21	24	29	23	21	7
Min	94	86	78	60	84	87	85
Max	154	165	178	168	193	186	110
%CV	11	17	21	25	19	17	7

The levels of coagulation factors varied widely between the different products (see Table, results are expressed as % of a commercial normal plasma). Total Ig content (IgG and IgM) was 8.9 ± 1.5 g/L (range 6-12 g/L) and albumin was 38.4 ± 2.5 g/L. In addition, the number and cellular source of MP differed substantially. All ten units contained PtdSer positive MP (range 1229-23373 MP/ μ L), 8 units contained leukocyte derived MP (range 9-16991 MP/ μ L), 7 units contained platelet derived MP (range 314-23660 MP/ μ L) 6 units contained endothelial cell derived MP (range 44-2440 MP/ μ L) and 4 units contained TF positive MP (range 228-1109 MP/ μ L). Thrombin generation yielded peak thrombin values between 10-90 (mean 39 ± 24) nM IIa. The total amount of thrombin generated correlated well with the number of PtdSer exposing MP ($R=0.81$).

Conclusions: We conclude that there is large variation in procoagulant lipid content and coagulation factor levels between different FFP units. This may, at least partially, account for the variability in clinical outcome found after transfusion.

VIRUSRISICO'S VAN UIT PLASMA BEREIDE GENEESMIDDELENM.P. Janssen¹, T.W. Kuijpers², C.L. van der Poel², J. Over²¹Julius Centrum voor Gezondheidswetenschappen en Eerstelijns Geneeskunde, UMC Utrecht, ²Stichting Sanquin Bloedvoorziening.

Het voorkomen van de transmissie van virusinfecties door uit plasma bereide geneesmiddelen is sinds de overdracht van HIV en HCV in het verleden een belangrijk aandachtspunt voor fabrikanten, regelgevers en patiëntenverenigingen. Op dit moment wordt in nieuwe Europese regelgeving (EMEA richtlijn CPMP/BWP/269/95) voor plasmaproducten een kwantificering van het restrisico vereist voor HBV, HCV, HIV, Parvo B19 en HAV. Het kwantitatieve risicomodel dat voor de plasmamedicijnen van Sanquin is opgesteld met bijbehorende modelaannames, gevoeligheden en beperkingen zal worden toegelicht. Daarnaast zal worden aangegeven in welke mate de diverse modelparameters het restrisico beïnvloeden.

Voor het berekenen van de kans op virale besmetting van een eindproduct (het restrisico) zijn alle individuele stappen van het productieproces gemodelleerd. Het restrisico wordt met behulp van Monte-Carlo simulaties doorgerekend. Door de gekozen manier van modelleren is het mogelijk om variabiliteit en onzekerheden ten aanzien van procescondities mee te nemen in de bepaling van het restrisico. Variabiliteit en onzekerheden hebben onder meer betrekking op de donorepidemiologie, donatie-intervallen, uitgevoerde tests, omvang van de quarantainevoorraad, grootte en samenstelling van de productiepool, doorlooptijd van het product, virusreducerende capaciteit van het productieproces, productopbrengst en grootte van de afvulling.

Uit de studie blijkt dat het restrisico vooral wordt bepaald door de virusincidentie (seroconversies) in de donorpopulatie in combinatie met de gevoeligheid van uitgevoerde tests, de virusreducerende capaciteit van het proces en de productopbrengst. Het type donatie (afereze of volbloed donaties) heeft geen, en het verhogen van de "inventory hold period" slechts een beperkte invloed op het restrisico. De simulaties laten zien dat de spreiding in het geschatte restrisico groot is (2 tot 6 logs). Deze spreiding wordt voornamelijk veroorzaakt door de onzekerheid die bestaat ten aanzien van de virustiter bij een besmette donor en de exacte waarde van de virusreducerende capaciteit van het productieproces.

Met behulp van Monte-Carlo simulaties, kan het restrisico van plasmaproducten worden berekend. Daarbij moet wel rekening gehouden worden met de specifieke kenmerken van het productieproces. Deze probabilistische aanpak maakt het, in tegenstelling tot de conventionele methoden die gebruikt worden voor het bepalen van het restrisico, mogelijk om ook meer complexe beslissingstrategieën binnen het productieproces te modelleren. Tevens kunnen daarbij onzekerheden die bestaan over de exacte waarde van verschillende modelparameters, zoals bijvoorbeeld incubatietijd, lengte van de windowfase of het verloop van virustiters in de tijd bij besmette donors, worden meegenomen en het effect van deze onzekerheden worden geëvalueerd. Op deze manier kan inzicht worden verkregen in de invloed die verschillende factoren hebben op het restrisico van het eindproduct en kan de effectiviteit van veiligheidsmaatregelen efficiënt worden geëvalueerd.

RISK FACTORS FOR RHESUS-D-IMMUNIZATION DESPITE POST- AND ANTENATAL ANTI-D-PROPHYLAXIS

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Background. To prevent RhD immunization, since 1969 a national prevention programme with postnatal administration of 1000 IU of anti-D is implemented in the Netherlands. To further prevent RhD immunization by ongoing fetomaternal haemorrhage (FMH) in the 3rd trimester of pregnancy, a single gift of 1000 IU anti-D is administered in the 30th week of the first pregnancy. According to guidelines anti-D is additionally administered in cases of assumed or proven increased exposure to fetal red cells (e.g. Kleihauer test or flowcytometry). Despite post- and antenatal anti-D-prophylaxis RhD-immunization still occurs. Identification of risk factors for RhD-IEA may provide clues to improve the current preventive strategies.

Objective. To determine risk factors for Rh-D-immunization despite antenatal and postnatal anti-D-prophylaxis.

Methods. Case control study. Cases: 41 RhD-immunized primiparae (one foregoing delivery), detected by first trimester routine antibody screen from 1999-2004, who received ante- and postnatal anti-D-prophylaxis in first pregnancy. Controls: 342 RhD-negative or -positive parae-1 with a negative IEA screening in 2002 and 2003. Data on the obstetric and medical history were collected. Factors with a p-value < 0.20 in univariate analysis were analyzed by multivariate logistic regression analysis.

Results. All women received the standard dose of ante- and postnatal anti-D. No anti-D was given on indication. Risk factors for RhD-immunization in multivariate analysis were: age (risk reduction 0.865/year; 95%-CI: 0.77-0.97), complicated assisted delivery (OR (Odds Ratio) 50.8; 95%-CI: 1.2-2153), postmaturity (\geq 42 weeks duration of pregnancy) (OR 4.0; 95%-CI: 1.1-14.3), caesarean section (OR 2.9; 95%-CI: 1.1-7.7). In 59% of the cases none of the last three risk factors was present.

Conclusion. In about 40% of cases increased FMH, as associated with artificial delivery, or insufficient antenatal prophylaxis late in pregnancy, may contribute to failure of anti-D-prophylaxis. More strict compliance to existing guidelines concerning determination of FMH and accordingly adjusted anti-D-prophylaxis or routinely administration of extra anti-D after artificial delivery, might further decrease RhD-immunization. Administration of 2 doses of 500 IU in week 28 and week 34 might contribute to sufficient levels of anti-D in postmature pregnancies.

WAT VINDEN ZWANGERE VROUWEN VAN DE SCREENING OP IRREGULAIRE ERYTHROCYTENTANTISTOFFEN (IEA)?

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Achtergrond. Sinds 1-7-1998 worden alle vrouwen in Nederland in het 1e trimester van de zwangerschap gescreend op Irregulaire Erythrocyten Antistoffen (IEA). De screening beoogt tijdige opsporing en behandeling van kinderen met risico op ernstige Hemolytische Ziekte van de Pasgeborene (HZZ) door non-Rhesus-D-IEA. Een al dan niet bevestigde positieve screeningsuitslag kan echter vragen oproepen en onrust veroorzaken bij zwangere vrouwen.

Onderzoeksvragen. Voelen zwangeren zich goed geïnformeerd over de IEA-screening en vinden zij de belasting opwegen tegen het nut?

Methoden. Observationale studie onder vrouwen met negatieve IEA-screening uit (1) 1^e en (2) 2^e lijn (3) met een niet-geconfirmeerde positieve screening, (4) met klinisch niet-relevante IEA, en met klinisch relevante non-RhD-IEA (KR-IEA) al (5) dan niet (6) at risk (vader positief) voor HZZ. Twee gestructureerde vragenlijsten werden ingevuld rond week 20 van de zwangerschap en 2 weken post partum. Algemene angst werd gemeten met de Spielberger State Trait Anxiety Inventory (STAI), een gevalideerde vragenlijst en met specifieke vragen over de IEA-screening. Periode: april 2004 – september 2005.

Resultaten. Beide vragenlijsten werden ingevuld door 233 vrouwen.

Achtergrondangst verschilde niet tussen de groepen. De algemene angst tijdens de zwangerschap en post partum was niet wezenlijk verschillend tussen groepen. Vrouwen at risk voor HZZ (6) maakten zich bij meting in de zwangerschap meer zorgen; bij meting 2 weken post partum was dit verschil verdwenen. Alle groepen vinden het nut van screening opwegen tegen de belasting. Controles uit de 1^e lijn (1) zijn tevreden over de hoeveelheid ontvangen informatie, alle andere groepen wensen meer informatie oplopend tot 69% van de vrouwen met IEA en risico op HZZ (6). Info is gewenst over de gevolgen van IEA voor kind (37%), moeder (33%), en volgende zwangerschap (22%). De informatie over klinische diagnostiek was voldoende. Aan schriftelijke informatie was de meeste behoefte (37%).

Conclusies. Zwangeren vinden het nut van de IEA-screening ruimschoots opwegen tegen de belasting ervan. Meer informatie is gewenst na een positieve screening over de gevolgen voor moeder en kind, voor een volgende zwangerschap en over het bloedonderzoek.

AFKAPWAARDEN VOOR NON-RHESUS-D-IEA VOOR TITER EN ADCC-UITSLAGEN TEN BEHOEVE VAN DE IDENTIFICATIE VAN ZWANGERSCHAPPEN MET EEN HOOG RISICO OP ERNSTIGE HEMOLYTISCHE ZIEKTE VAN DE PASGEBORENEN.

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Achtergrond: Sinds 1-7-1998 worden Nederlandse zwangeren (200.000 p/j) vroeg in de zwangerschap gescreend op irregulaire erythrocyten antistoffen (IEA). IEA van de IgG klasse kunnen de placenta passeren en de bloedbaan van het kind bereiken en tot afbraak van de kinderlijke erythrocyten leiden, waardoor hemolyse met geelzucht en anemie kan optreden (hemolytische ziekte van de pasgeborene (HZZ)). Ernstige foetale anemie kan al tijdens de zwangerschap ontstaan en maakt soms intra-uteriene bloedtransfusies (IUT) noodzakelijk. Na de geboorte kan hyperbilirubinemie wisseltransfusies (WT) noodzakelijk maken. Door middel van het vervolgen van de IEA titer en uitslag van de antibody-dependent cellular cytotoxicity (ADCC) test wordt tijdens de zwangerschap de ernst van eventueel ontstane HZZ geschat om zo een beeld te krijgen van de klinische toestand van het kind. Als de laboratoriumtesten een verhoogd risico aangeven, kan klinische diagnostiek (echo, Doppler, Liley-index) uitgevoerd worden. In geval van Rhesus D antistoffen zijn de grenswaarden voor titer en ADCC uitslag waarboven HZZ met noodzaak tot intra-uteriene of wisseltransfusie verwacht kan worden in eerder onderzoek bepaald.

Doel van dit onderzoek: Voor non-Rhesus-D-IEA het afkappunt voor titerwaarde en ADCC uitslag bepalen ten behoeve van identificatie van zwangerschappen met een hoog risico op klinisch relevante HZZ.

Methoden: Laboratorium-en klinische gegevens van cases uit het OPZI (Opsporing en Preventie van Zwangerschapsimmunisatie)-onderzoek uit de periode 1-9-2002 tot 1-10-2004, waarbij het kind positief was voor het antigeen waartegen de moederlijke IEA gericht waren, zijn verzameld. Ernstige HZZ werd gedefinieerd als die gevallen waarbij IUT, WT of een bloedtransfusie in de eerste levensweek noodzakelijk was. Receiver operator characteristics (ROC) curves werden geconstrueerd voor alle cases en naar IEA specificiteit, voor de IEA titer uitslag en de ADCC test uitslag, waarbij gestreefd werd naar het minimaliseren van het percentage misclassificaties, met als uitgangspunt een zo hoog mogelijke sensitiviteit in combinatie met een aanvaardbare specificiteit.

Resultaten. 260 cases werden ingesloten, waarvan 16 met ernstige HZZ; 12 door anti-c, 4 door anti-K, 1 door anti-E en 1 door anti-e. Naar IEA specificiteit werden ingesloten: anti-c: n=72; anti-E: n=78; anti-K: n=14, anti-Fy(a): n=31; anti-Jk(a): n=23; rest: n=42.

Uit de ROC curves konden de volgende optimale afkapwaarden geconcludeerd worden: 1:16 voor de titer en 30% voor de ADCC bepaling. Wanneer de met de ROC-curves bepaalde optimale afkapwaarden worden gehanteerd hebben de IEA titerbepaling en de ADCC-test dezelfde sensitiviteit van 87,5% (95% BI: 0,617 - 0,985). De specificiteit van de ADCC test is daarentegen hoger dan die van de IEA-titerbepaling: 93,9% (95% BI: 0,901-0,965) tegenover 77,9% voor de IEA titer van 1:16 (95% BI: 0,721 - 0,829). Gegeven de afkapwaarde van een titeruitslag van $\geq 1:16$ bleek de positieve voorspellende waarde van de test 20,6% is en de negatief voorspellende waarde 99%. Voor de ADCC-uitslag zijn, gegeven de afkapwaarde van $\geq 30\%$, de positief voorspellende waarde 48,3% en de negatief voorspellende waarde 99,1%. De resultaten per IEA specificiteit waren geen aanleiding om per niet-rhesus-D-IEA specificiteit de afkapwaarden anders te kiezen.

Conclusie: Het vervolgen van titer en ADCC-uitslagen om zwangerschappen met een hoog risico op ernstige HZZ te identificeren kan met de vastgestelde afkappunten doelmatiger worden uitgevoerd.

ADSORPTION OF ANTIBODIES USING DIFFERENT ADSORPTION TECHNIQUES

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Background: A serum sample can contain several alloantibodies or autoantibodies. Therefore, it may be desirable to adsorb out the unwanted ones or to extract the wanted ones. The most common technique for adsorption is the saline (PBS) adsorption. The saline technique is a sensitive but time consuming method. This prompted us to compare the score before and after adsorption of the antibodies and time after using different adsorption techniques.

Material and Methods: Serum samples (n=25) with different alloantibodies were adsorbed against red cells with the corresponding antigens and without the corresponding antigens (control) using four different adsorption techniques (PEG-GAMMA; PEG-CLB; LISS-diamed; Saline). Before and after adsorption the score of the antibodies, the frequency of adsorption steps and the incubation time needed per adsorption were analyzed. Data were compared using ANOVA and Tukey-Kramer as post-test. Scores before versus after adsorption were analyzed using a paired t-test. $P < 0.05$ was used as significant.

Results: See table (mean \pm SD, n=25).

Adsorption	Results				
	Score Before ^a	Score After ^b	Recovery % ^c	Adsorption steps ^d	Time min ^e
PEG-Gamma (A)	42 \pm 23	25 \pm 18	57 \pm 24	1.1 \pm 0.3	22 \pm 6
PEG-CLB (B)	46 \pm 21	27 \pm 19	54 \pm 26	1.2 \pm 0.4	17 \pm 6
LISS-Diamed (C)	45 \pm 24	36 \pm 24	78 \pm 23	2.0 \pm 1.4	29 \pm 21
Saline (D)	42 \pm 23	30 \pm 20	70 \pm 25	2.3 \pm 1.7	104 \pm 74

^a ANOVA $p=0.03$, ns in post test; ^b A-C and B-C $p < 0.001$; ^c A-C and B-C $p < 0.001$, A-D and B-D $p < 0.01$; ^d A-C and B-C $p < 0.01$, A-D and B-D $p < 0.001$; ^e A-D, B-D and C-D $p < 0.001$.

Scores before and after adsorption were for all methods significant ($p < 0.001$).

Conclusion: LISS-Diamed showed the best recovery and comparable incubation time with PEG-Gamma and PEG-CLB. Thus LISS-Diamed is preferable above the other tested methods for adsorption of alloantibodies. However, for adsorption of autoantibodies more investigation is needed.

HET KCAM ANTIGEEEN: EEN RECENT ONTDEKT ERYTHROCYTEN ANTIGEEEN IN HET KNOPS BLOEDGROEPEN SYSTEEM

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Inleiding: Voor de specificiteitsbepaling van High Titer Low Avidity (HTLA) antistoffen, die gericht tegen antigenen behorende tot het Knops bloedgroepen systeem maken, wij gebruik van de Monoclonal Antibody Immobilisation Erythrocyte Antigen (MAIEA) test. Bij deze bepaling komt het soms voor dat wel wordt vastgesteld dat de antistoffen gericht zijn tegen het CR1 eiwit (drager van de Knops antigenen) maar dat de specificiteit anders is dan gericht tegen de tot nog toe bekende knops. De meeste patiënten die dit soort antistoffen hebben, hebben daarbij ook een duidelijk verminderde expressie van CR1 op de erythrocyten. Recent is door Moulds et al (1) een nieuw antigeen met een hoge frequentie, gelegen op CR1, beschreven. Dit antigeen is KCAM genoemd. Patiënten die dit antigeen missen hebben veelal een verminderde expressie van CR1. Deze publicatie was de aanleiding om te onderzoeken of de CR1-reactieve antistoffen die we in het verleden hadden gevonden, maar waarvan de specificiteit nog niet hadden vastgesteld, gericht waren tegen KCAM.

Materiaal en methode: Serum van acht patiënten met antistoffen tegen CR1 zonder duidelijke zijn in de MAIEA techniek getest met KCAM-negatieve erythrocyten van de in de publicatie vermelde patiënt (verkregen via SCARF). Voor zover de ABO bloedgroep het toeliet en er erythrocyten bewaard (-196oC) waren zijn ook de erythrocyten van de acht patiënten getest in de MAIEA met anti-KCAM van de originele patiënt (verkregen via SCARF).

Resultaten: Van de 8 geteste sera waren in de MAIEA 6 niet reactief met KCAM-negatieve erythrocyten, maar wel met verschillende erythrocyten die een ander antigeen van het Knops systeem missen. Van 3 van deze patiënten bleken de erythrocyten niet reactief te zijn met anti-KCAM maar wel reactief met andere Knops-antigeen antisera.

Conclusie: De 6 hierboven beschreven patiënten hebben allemaal antistoffen met de specificiteit anti-KCAM, en negativiteit voor het KCAM-antigeen kon voor 3 patiënten worden vastgesteld. De uitbreiding van het MAIEA cel panel met KCAM-negatieve testerythrocyten zal er toe leiden dat de specificiteit van HTLA antistoffen nog vaker vastgesteld zal worden.

(1) Moulds et al, Transfusion, S82-040F, 2005;45,Supplement

EEN PATIËNT MET AUTOANTISTOFFEN TEGEN EEN HOOG-FREQUENT ANTIGEEN, EEN SPECIAAL TRANSFUSIE ADVIES

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Voor een 59-jarige man, opgenomen met een acute nierinsufficiëntie, postinfectieus, gecompliceerd door Guillain-Barré polyneuropathie werd ivm symptomatische anemie een transfusie aangevraagd. De IEA screening en kruisproeven waren positief. Bij onderzoek door Sanquin Bloedbank ZW, bleek het serum reactief met alle testerythrocyten, met een wisselende reactiesterkte. De titer van de antistoffen liep lang door, passend bij HTLA-antistoffen. Een transfusie met ongekruste fenotypisch-compatibele erythrocyten moest na 15 min worden gestaakt ivm koorts, koude rillingen, hypertensie en dyspnoe. Posttransfusie hemolytische parameters waren niet afwijkend. Gezien de ernst van de reactie en de transfusiebehoefte werd aanvullende immunohematologische analyse uitgevoerd door het nationaal referentie laboratorium van Sanquin Diagnostiek Amsterdam. Met Lu(a-b-) cellen van het In(Lu) type (verminderde expressie van Lu-antigenen, P1, het Indian systeem en het AnWj-antigeen) en AnWj – negatieve erythrocyten werden negatieve reacties verkregen. In het eluaat werd anti-AnWj agetoond. Anti-AnWj komt voor als allo- of auto-antistof. Alloantistoffen zijn zeer zeldzaam. Autoantistoffen gaan gepaard met onderdrukking van de expressie van het AnWj-antigeen, zoals in deze casus, het geen een uiting is van een onderliggend ziektebeeld. Bij transfusie van AnWj-positieve erythrocyten is de overleving sterk verkort, met de mogelijkheid op een acute transfusie reactie[‡]. Directe familieleden werden getest als mogelijke donor, maar bleken positief in de kruisproeven. Patiënt werd met hoge doses EPO behandeld. Bij een Hb van 3,0 mmol/l en ischemische ECG afwijkingen werd, omdat er geen AnWj-negatief bloed beschikbaar was, geadviseerd om erythrocyten van het In(Lu) type uit de bloedbank van de Raad van Europa te transfunderen. Dit verliep ongecompliceerd met een Hb stijging naar 4,6. Twee vervolg transfusies met erythrocyten van het In(Lu) type gaven een vergelijkbare goede opbrengst.([‡]) Marsh WL et al Transfusion 1983;23:128

MULTI-ETHNIC DONOR BLOOD GROUP GENOTYPING ON COMMON SINGLE NUCLEOTIDE POLYMORPHISMS NOT RELIABLE

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To provide typed red blood cells (RBC) to alloimmunized patients, part of the Dutch donor population is serologically typed for the clinically most relevant blood group antigens besides AB0 and RhD. For patients with rare combinations of antibodies or antibodies against high frequency antigens (HFAs) a frozen stock of red cell units is kept at the Blood Bank of the Council of Europe. Maintenance of, or expanding this extensively serologically typed donorpool and/or extending the numbers of typed antigens per donor, is very labor intensive and not feasible because typing sera are rare or of variable quality. The emerging medium/high throughput genotyping techniques may solve these problems and, moreover, even enable preventive matching. Therefore, correct predictions of the RBC phenotype are essential. We developed RBC genotyping assays with the Pyrosequencing technique. Genotyping results were evaluated among four different ethnic groups, (i.e. blacks from South-Africa (BSA), asians from South-Africa (ASA), blacks from Ethiopia (BE) and caucasians from The Netherlands (CN)), by comparing genotyping results with serological results.

Multiplex PCR and multiplex Pyrosequencing RBC assays have been developed to type for KEL1/KEL2 (T698C), KEL3/KEL4 (T961C), MNS1/MNS2 (C59T, G71A, T72G), JK12 (G838A) and FYX (C265T). Single assays are FY1/FY2 (G125A) and LU1/LU2 (A230G).

No typing discrepancies were found for K and k (127 BSA, 123 ASA, 99 BE, 49 CN) and KpaKpb (126 BSA, 125 ASA, 107 BE, 49 CN). For MN 13 discrepancies were found indicating the presence of MNS1 alleles in M-negative donors (2/108 BSA, 3/114 ASA, 6/89 BE, 2/39 CN). Indications for the presence of JKnull-alleles were also found (1 JK2null/127 BSA, 2 JK2null/97 BE, 1 JK1null/114 ASA) and in 2 Lub negative BE (n=96) LU2 Single Nucleotide Polymorphisms (SNPs) were detected, whereas 4 LU1 SNPs were detected in Lua negative BSA (n=118). In addition, discrepancies in Fyb typing that may be explained by the GATA mutation were found in 76/88 BSA, 7/94 ASA and 25/50 BE. FYX was detected in 1/50 CN and in 1/98 BE.

Conclusion: in total (all groups) 2490 genotypings were performed of which 23 results indicated the wrong phenotypes (FY excluded), i.e. 0.92%.

Genotyping on the SNPs for KEL1/KEL2 (T698C), KEL3/KEL4 (T961C), FY1/FY2 (G125A), FYX (C265T) in combination with GATA-FY to predict Kk, KpaKpb and FyaFyb phenotypes seems reliable in a multi-ethnic society. For the MN, Jk and Lu systems it needs to be evaluated whether the common genotyping approach needs to be adjusted for reliable prediction of these phenotypes.

TWEE POPULATIES CELLEN BINNEN EEN. DONOR: HET AANTONEN VAN EEN TETRAGAMETISCH CHIMERISME

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Inleiding: Bij een routine bloedgroepbepaling van een vrouwelijke donor werd, naast een normaal aanwezig A antigeen, een "mixed field" reactie waargenomen bij het B antigeen. Een dergelijke reactie, waarbij naast een antigeen-positieve, ook een antigeen-negatieve celpopulatie wordt aangetoond, was ook aanwezig bij het D, C, E, Jk^b en s antigeen. De donor had nooit een transfusie of een beenmergtransplantatie ondergaan. Het aantreffen van gemengde celpopulaties wijst op een chimerisme. Chimerisme van uitsluitend het bloedvormende weefsel kan ontstaan door uitwisseling tijdens een tweelingzwangerschap, wanneer de beide kinderen verschillende bloedgroepantigenen hebben. Een tetragametische chimeer ontstaat door samensmelting van meer dan twee voortplantingscellen en behalve in de bloedcellen komen de verschillen dan ook tot uiting in andere weefsels.

Resultaten vervolgonderzoek: Twee populaties rode bloedcellen werden serologisch gescheiden. De grootste was bloedgroep A, ccDEE, Jk^b(+) en s(+); de andere was bloedgroep AB, Ccdee, Jk^b(-) en s(-). In het speeksel, dat een weerspiegeling vormt van de bloedgroep allelverdeling in de lichaamsvloeistoffen, was een grote hoeveelheid A en H antigeen aanwezig en een kleinere hoeveelheid B antigeen. Zowel in het DNA geïsoleerd uit de leukocytenfractie als uit het wangslim (niet-hematopoïetisch weefsel) werd een identiek genotype vastgesteld: *O1B, CcDEE, JK^aJK^b, Ss*, . Daarnaast toonde een genotyperingsassay naar 16 single-tandem-repeat allelen, voor 8 systemen de aanwezigheid aan van 3 allelen. De discrepantie tussen de ABO genotypering en de ABO serologie is inherent aan de gebruikte assay, waarbij de aan-of afwezigheid van het A1 allel slechts bij uitsluiting wordt aangetoond.

Conclusie:

Bij deze donor was er sprake van een tetragametisch chimerisme. Als donor dient deze persoon beschouwd te worden als: AB, CcDEE, Jk^b en s positief.

BLOOD GROUP AUTOMATION: BE AWARE OF PITFALLS!

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Introduction: automation in blood group serology is adopted in many transfusion laboratories. This case illustrates that detailed analysis of unexpected results is necessary to detect pitfalls in blood group automation.

Case report: the ABO RhD type of a pretransfusion sample of an 87-year-old male was determined O RhD positive, by using the Ortho Innova system. Because of massive bleeding two units of O RhD negative red blood cells were transfused without cross matching. After transfusion of these 2 units a second blood sample of the patient was analysed for definite ABO RhD typing. Despite receiving only 2 units of O RhD negative red cells the patient's sample showed only weak reactivity with anti-D using the Ortho Innova system. However additional manual testing using the Ortho BioVue column technique resulted in 4+ reactivity with anti-D. Further analysis showed that upon centrifugation RhD negative donor red cells travelled to the bottom of the tube and, as the pipette of the Innova system takes red cells from the bottom of the tube, mainly donor red cells were tested. This resulted in only weak reactivity with anti-D in the Innova system. On the other hand, for manual testing the laboratory technician takes red cells from a higher level in the tube, thus sampling and typing mainly patient red cells in the BioVue method. This resulted in 4+ reactivity with anti-D. Rhesus-phenotype testing of the different cell populations confirmed that the majority of red cells in the lower layer of the sample were of donor origin. The upper part of the sample consisted mainly of patient red cells.

Conclusion: posttransfusion mixing of donor red cells with patient's red cells results in different red cell populations in a blood sample. This may result from differences in mean corpuscular volume, ageing and storage of donor red cells. Depending on the (automated) blood group typing technique used, such mixed samples might result in unexpected or discrepant typing results.

DETECTIE VAN T-ANTIGEEN ACTIVATIE TER ONDERSTEUNING VAN DIAGNOSE HEMOLYTISCH UREMISCH SYNDROOM BIJ KINDEREN

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Het hemolytisch uremisch syndroom (HUS) wordt gekenmerkt door hemolytische anemie, trombopenie en acute nierinsufficiëntie. In 90% van de gevallen wordt HUS voorafgegaan door infectie met E. Coli en is geassocieerd met diarree. Bij de atypische vorm van HUS (10%) treedt geen diarree op en kan het stellen van de diagnose gecompliceerd zijn. Ook de atypische HUS wordt meestal voorafgegaan door een invasieve infectie m.n. door S. Pneumoniae als veroorzaker.

Zowel E. Coli als S. Pneumoniae produceren het enzym neuraminidase. Neuraminidase kan zorgen voor activatie van het T-antigeen dat o.a. voorkomt op erythrocyten, trombocyten en endotheel. Het T-antigeen is een cryptantigeen dat normaal bedekt wordt door neuraminezuur. Door afsplitsing van het neuraminezuur door het enzym neuraminidase wordt het T-antigeen geëxposeerd. Uit de literatuur is bekend dat T-activatie veelvuldig voorkomt bij HUS.

Om te onderzoeken of het aantonen van het T-antigeen op erythrocyten de diagnose HUS kan ondersteunen is bij 4 patiënten met verdenking op HUS de T-antigeen expressie op de erythrocyt en de neuraminidase activiteit in het serum bepaald. De leeftijd van de patiëntjes varieerde van 1 week tot 6 maanden. In alle gevallen was sprake van een pneumococcale meningitis.

In 3 van de 4 gevallen kon de expressie van het T-antigeen op de erythrocyt vastgesteld worden m.b.v. de lectines Arachis Hypogea en Glycine Soja. Deze bevinding was een bevestiging voor de diagnose HUS. In één geval werd geen expressie van het T-antigeen gevonden. Ook klinisch bleek de diagnose HUS bij dit patiëntje niet van toepassing (geen acute nierinsufficiëntie, geen trombopenie).

Neuraminidase activiteit werd in twee van de drie T-antigeen positieve monsters aangetoond.

Bij één patiëntje is de T-activatie gedurende de behandeling met plasmaferese vervolgd. Na drie behandelingen met plasmaferese werd geen T-activatie meer aangetoond.

Conclusie: Bepaling van het T-antigeen op erythrocyten kan gebruikt worden als een diagnostische marker voor HUS.

UTILITY OF LONG-TERM STABILISED BLOOD SAMPLES AS CALIBRATORS FOR FLOW CYTOMETRIC HLA-B27 SCREENING

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Long-term stabilised blood samples as calibrators for flow cytometric HLA-B27 screening may have a number of uses, such as: provide positive and negative control samples, validation of procedures and monoclonal antibodies (mAb), and enhance flexibility in the organisation of external quality assessment (EQA) schemes.

In this study, we compared forward scatter (FSC), sideward scatter (SSC), and fluorescence signals of fresh, short-term, and long-term stabilised peripheral blood and buffy coats from 9 blood donors with various HLA-typings. Commercially available HLA-B27 mAb as FD705, ABC-m3, and GS145.2 have been tested. Data acquisition has been performed 9 times in 1 year (i.e., fresh, short-term stabilised, and long-term stabilised [1 and 2 week(s); 1,2,3,6, and 12 month(s)]). All tests were performed and interpreted as recommended by the manufacturers. Furthermore, an extra EQA send-out with stabilised blood samples of 4 selected blood donors was organised to compare historical EQA data obtained using non-stabilised blood samples from the same donor.

The scatter and fluorescence signals remained acceptable for at least 3 months post stabilisation. There were no major effects on FSC and SSC characteristics of lymphocytes, and the discrimination between HLA-B27 positive and HLA-B27 negative samples remains possible. Moreover, peripheral blood is preferred above buffy-coats.

Whilst stabilised samples performed less well than fresh samples in some circumstances, we have shown that, providing rigorous quality control of stabilised samples is undertaken, they can be successfully used in an EQA exercise or used as negative or positive procedure controls for flow cytometric HLA-B27 screening.

COMPARISON OF TWO BEAD ASSAYS FOR SCREENING OF HLA CLASS I AND HLA CLASS II ANTIBODIES

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For screening of HLA antibodies, various techniques exist based on antigen coated beads and detection of antibody binding using fluorescent second antibodies. Traditionally, fluorescence is measured using a standard flow cytometer (FCM). More recently, tests have been developed using the Luminex 100 system. This robust, medium throughput system offers automated calibration and maintenance procedures. Furthermore, dedicated software programs are available for data analysis. We compared our routinely used FCM-based assay (FlowPRA, OneLambda) with the Luminex based assay (LABScreen mixed (LSM), One Lambda). Sera from 75 patients were tested in both assays for the presence of HLA class I and HLA class II antibodies according to the manufacturers instructions. To validate the cut-off threshold to discriminate between positive and negative test results 40 sera from untransfused males were used. For screening of HLA-class I antibodies 48 sera gave positive reactions both in the FlowPRA and LSM assays; 22 sera were negative in both assays, where discrepant results were observed on 4 occasions: 1x only the FlowPRA was positive and 3x only the LSM was positive; results of 1 serum was not evaluable. For screening of HLA-class II antibodies 31 sera gave positive reactions both in the FlowPRA and LSM assays; 43 sera were negative in both assays; a discrepant results was observed on 1 occasion where only the LSM was positive. We conclude that the results of the LABScreen were highly concordant with those from the FlowPRA assay and thus that both assays are comparable for the screening of HLA class I and HLA class II antibodies. The LSM assay has the advantages of using the Luminex 100 system.

ON SITE VALIDATION OF THE EPO-IMMULITE 2000 ASSAY

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Introduction The glycoprotein hormone erythropoietin (EPO) is produced in the renal cortex and regulates the continuous formation of red blood cells. The EPO concentration in blood varies in certain pathophysiological conditions. In polycythemia vera the EPO level is low in contrast to secondary polycythemia and can therefore be used as a diagnostic tool. In cancer patients EPO can be administered in case of chemo-induced anemia in order to postpone or prevent blood transfusion. EPO levels can then be used as a predictive value for therapy. Here we present the validation results of the EPO-Immuline 2000 assay.

Methods The EPO-assay is based on a two-site immunoenzymometric assay developed by Diagnostic Products Corporation (DPC, Los Angeles, US) performed on a fully automated, continuous random access, Immuline 2000 analyzer that utilizes chemiluminescent detection.

Results The EPO assay on the laboratory site was validated for:

- Imprecision. Healthy blood donors (duplicate, n=25) tested on two days and control standards compared with reference values (duplicate, 14 tests) [Table 1].

Table 1 U/L	Internal	Internal	Internal	Ref.	Ref.
	Mean	Within-run CV %	Run-to-run CV %	Mean	Run-to-run CV %
Donors	-	6	5.7	-	-
Control 1	12,3	5	5.7	14,0	6.4
Control 2	27,2	4.1	5.3	27,2	6.6
Control 3	54,4	4.4	5.3	53,4	6.4

- Comparison. The results of patient samples (n=26), tested with the internal and external EPO assay, showed a correlation coefficient of 0.988.

- Normal range. Testing plasma (Na-heparin) of healthy blood donors (duplicate, n=49) [Table 2].

Table 2	Mean	SD	Perc. 5%	Perc. 95%	Reliab.
U/L	10.1	4.1	4.4	- 15.8 U/L	90%

Conclusion The EPO-Immuline 2000 assay is a reliable and sensitive method for quantification of EPO levels.

EFFECT OF VARIOUS K3-EDTA TUBES ON BLOOD CELL COUNTING RESULTS

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Background: Recently, the LABAS of Sanquin decided to harmonize procedures and decided to use plastic test tubes of Greiner instead of glass tubes of B&D. Investigated is whether various the tubes had consequences on the results of the cell counting in blood products, for quality control purposes.

Methods: The following tubes were compared for cell counting in whole blood (WB): tube A: 4.5 ml Greiner and tube B: 2 ml Greiner, both with sprayed K3-EDTA and tube C: 4.5 ml B&D filled with 0.054 ml liquid K3-EDTA. As usual each tube was filled with 2 ml WB, and cell counts were done (n=6). To concentrate and count cells in plasma (PL) the following tubes were compared: tube D: 6 ml Greiner filled with 0.135 ml liquid K3-EDTA and tube E: 7 ml B&D tube filled with 0.084 ml liquid K3-EDTA. Both tubes were filled with PL to the maximum volume 6 respectively 7 ml. The cells in PL were concentrated 10 times by centrifugation. Cell counts were determined by Sysmex K1000 analyser. Red cells in plasma were counted with the Bürker hemocytometer.

Results: See table

Blood product	Tube	White cells $\times 10^9/L$	Red cells $\times 10^{12}/L$	Hemoglobin mmol/L	Platelets $\times 10^9/L$
WB	A	6.5±1.3	4.02±0.33	7.3±0.7	206±49
WB	B	6.5±1.2	4.01±0.35	7.4±0.7	203±49
WB	C	6.3±1.1*	3.92±0.32*	7.2±0.7*	200±41*
PL	D	n.d.	6.5±3.2×10 ⁹	n.d.	91±30
PL	E	n.d.	6.6±3.3×10 ⁹	n.d.	88±27

*p<0,05; n.d=not determined

White cells, red cells, hemoglobin and platelets in tube A and B were about 3% higher in comparison with tube C with liquid EDTA.

Conclusions

The use of K3-EDTA tubes with sprayed K3-EDTA resulted in about 3% higher cell counts.No effect was observed between the test tubes used for concentration and counting of plasma.