Posters: Session A, Monday, 12 July 2004, 17.00–18.00

P1 Donor recruitment and retention

P 1.1 Donor selection among the European Union countries
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Aim: Early in 2002 a federation of transfusion medicine societies (TMS) throughout the EU – EuroNet-TMS – was constituted with the agreement of the 15 EU countries as well as Switzerland and Norway. One of the aims is to collect information within the EU, to draw maps of the current situation and to make proposal for more coherence throughout Europe. The aim of this presentation is to show how the procedure of donor selection is performed. Data of medical circumstances involved in donor selection was not included in the survey.

Materials and methods: Within EuroNet-TMS there are currently nine committees each focusing on their own specialist topic. The donor selection committee designed a questionnaire asking information concerning regulation of donor selection, qualification required for the person responsible for selecting, the possibility of self-deferring, assurances of confidentiality, possibility of be remunerated, intervals between two whole blood donations, donors guidelines. The questionnaires were sent to representatives of TMS of 17 countries and were revised during year 2002.

Results: All the countries answer. This is one of the few times that we can offer data concerning the ‘quality’ of the procedure of donor selection. Results of the survey will be presented.

Conclusions: The figures of the EU countries are quite homogeneous, but some differences exits. Changes should be introduced by all countries to adopt the requirements of the new directive.

P 1.2 Safety of an abbreviated donor history questionnaire (ADHQ)
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Background: Surveys have revealed donor dissatisfaction with the duration of the donation process and repetitive questioning. An ADHQ can increase donor satisfaction, but must not increase risk for blood recipients. We studied the effectiveness of an ADHQ for experienced donors.

Materials and methods: A 35-question (decreased from 53) ADHQ was developed (FDA approved). An additional HIV/hepatitis risk question was used. Travel, medication and health history questions were decreased by 17. Donors were eligible for ADHQ if they had successfully completed three donor suitability assessments including one in the previous 6 months. The experimental group (24,040 donors) utilized ADHQ. Control group (44,090 donors) was eligible for ADHQ but utilized the full questionnaire (FHDQ). History and vital sign deferrals, viral marker rates, postdonation information and donor satisfaction were monitored.

Results: Thirty-seven per cent of donors were ADHQ-eligible. There were no differences in medical history deferrals (ADHQ, 0.38%; FHDQ, 0.43%), viral marker rates (ADHQ, 0.01%; FHDQ, 0.03%) and disqualifying postdonation information (ADHQ, 0.16%; FHDQ, 0.18%). There was a difference in vital sign deferrals (ADHQ, 7.9%; FHDQ, 9.0%). Twenty per cent more ADHQ than FHDQ donors rated the overall donation process excellent and 8% more ADHQ donors indicated they would give blood again within 12 months.

Conclusion: ADHQ increases donor satisfaction and does not change indicators of safety. Blood donation can be further streamlined.

P 1.3 Single-donor two-unit red blood cell collection: evaluation of a new blood separator
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Modern blood separators collect 2 U of RBCs from one donor-reducing manual separation of blood components. The aim of our study were to evaluate a new 2 U RBC blood separator; to test recruitment criteria; to assess the effect on blood count and iron stores. Baxter ALYX is a continuous processing, intermittent draw/return, single-needle blood cell separator collecting two leuko-depleted RBC units. Thirteen male donors were selected according to weight ≥70 kg, Hgb ≥15 g/dl and ferritin 240 ng/ml. All procedures were successfully concluded (data are showed in Tables 1 and 2). Hgb loss after donation was higher (3.0 g/dl) in donors with weight <80 kg. Recovery of Hgb after 1 month was almost complete. Ferritin loss after 30 days was 65% (41–87), with seven donors having <30 ng/ml. Perioral tingling was the only side effect in two cases. RBC collection using ALYX is a safe, fast, low-risk procedure. Hgb values are only temporarily decreased, with full recovery after 30 days. Significant reduction in ferritin suggests that the evaluation of predonation iron balance must be included in the selection criteria.

Table 1. Procedures

| Processed volume (ml) | RBCs volume (ml) | Time (min) | ACD used (ml) |
|----------------------|-----------------|------------|--------------|
| Median               | 987             | 361        | 27           | 124          |
| Range                | 801–1059        | 360–382    | 23–36        | 105–134      |

Table 2. Donors

| Weight (kg) | Hgb baseline | Hgb after collection | Hgb difference | Ferritin baseline | Ferritin difference |
|-------------|--------------|----------------------|----------------|-------------------|---------------------|
| Median      | 82           | 15.6                 | 2.6            | 101               | 25                  |
| Range       | 70–116       | 15.1–16.8            | 2.5–13.7       | 14.1–16.6         | 40–300              |

P 1.4 Rights of blood recipients should supersede any asserted rights of donors
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Background: Asserting the right to donate blood, banned donors are pressuring collection centers to accept their blood. The men who have had sex with men since 1977 deferral (MSM rule), instituted prior to HIV testing, has recently been described as homophobic. Since blood is now tested, some believe that donor screening is unnecessary. Illiterate rhetoric, the disruption of blood drives, and the urging of donors to lie in the interview has ensued.

Conclusion: The AABB favors reducing the MSM restriction to 1 year. The American Red Cross favors no change and the U.S. Food and Drug Administration has not budged. Although blood centers have responsibilities to potential donors and should treat them fairly and compassionately, they have a duty to provide the safest blood available to transfusion recipients. These competing interests have clashed in the past. In the early 1980s, U.S. collection centers were reluctant to question donors regarding sexual behavior, largely in deference to their gay donor base. They were also initially averse to surrogate testing, partly because they did not want to stigmatize their gay donors. Two recent studies indicate that changing the MSM rule would increase transfusion transmitted HIV. A desire to satisfy prospective donors is laudable, but not at the expense of making the blood supply less safe.

Conclusion: Decisions regarding who can donate should be based upon science and focused upon the needs of the recipient, not the donor.

P 1.5 A pilot study for the implementation of a multicomponent apheresis program in mobile drives
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Background: Multicomponent apheresis (MCP) processes are very productive and show advantages for patients. The aim of the study was to evaluate the feasibility of implementing an apheresis program in mobile drives (MD).

Material and methods: We selected regular MD for MCP. Blood donors were recruited by phone, among those with more than five previous donations. We used Trima Accel and a small ABX blood cell counter for PLT counting. Donors satisfaction, platelets quality and productivity were evaluated.

Results: We programmed 56 Apheresis MD (AphMD). We contacted 1105 donors and arranged 142 appointments. A total of 89 donors finally donated (8% of contacts) The deferral rate was 34%. For 78% this was their first apheresis experience. Adverse effects appeared in 10.34%, higher than in the fixed centre but not statistically significant. Median time per procedure was 48 min. As a productivity: 103.4% rendered PLT, 70.1% plasma and 23% RBC. In 61% of AphMD we performed two apheresis processes.
All PLT complied with quality standards. Donor satisfaction: 94% of donors were willing to donate again in AphMD.

Conclusions: An AphMD programme is very time consuming for donor recruiters. The high deferral rate and the trend to higher adverse effect rate could be biased by the high number of new donors. The donor reaction was very positive and productivity justifies the cost. Better results are expected after a higher donor’s return rate.

P 1.6 Relative safety of first-time, lapsed and repeat donors in Republic of South Africa

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Background: The concept that repeat donors who give frequently (F-Rpt) are safer than first-time (FT) or lapsed repeat donors (L-Rpt) is widely promulgated but poorly documented. We used the sensitive/less-sensitive HIV EIA [S/LS-EIA] strategy, which detects recent seroconverters among seropositive samples, to investigate this issue among donors in the Republic of South Africa (RSA).

Methods: A total of 643 HIV-confirmed seropositive donations, derived from 844 375 RSA donations in 2001-2002, were tested by S/LS-EIA. Prevalence of HIV-seropositive donors, rate of recent HIV infections, and risk of HIV transmission from antibody screened blood (projected ratio of infectious pre-Ah (20.3 days) to S/LS-EIA (111 days) window period) were calculated for F-Rpt, L-Rpt and FT donors.

Results: Donations (9.7%) were by FT, 9.2% by L-Rpt and 81% by F-Rpt donors. HIV prevalence (per 100 units) was 29-fold higher in FT (5.18) and 12-fold higher in L-Rpt (2.18) than in F-Rpt donors (1.8). Based on S/LS-EIA testing, rate of recent HIV infections (67 per 100 k units) in FT; 47 in L-Rpt; 12 in F-Rpt and risk of HIV transmission (4.4 per 100 k FT; 3.0 in L-Rpt; 0.8 in F-Rpt), were 5.4-fold higher in FT and 3.8-fold higher in L-Rpt than in F-Rpt donors.

Conclusions: These findings confirm that donations from F-Rpt donors are safer than L-Rpt and FT donations. Efforts to maximize frequent donations by repeat donors are justified both from blood safety and availability.

P 1.7 Quantifying losses to the supply of donated blood due to donor deferral and under/over collection

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Background: The loss of donors and donations for reasons other than disease-marker screening has not been well quantified in the USA.

Methods: We combined three datasets from Blood Centers of the Pacific, San Francisco, CA to estimate the number of donors and donations that are lost in each step of the donation process.

Results: In the year 2000, 13.7% of potential donors were deferred at presentation; of these 68.5% received short-term deferrals, 21% long-term and 10.5% permanent. The most common short-term deferral was low Hgb, particularly for women. For long-term deferral, the most common reasons were tattoo/piercing and travel to malarial areas. For multiple year or permanent deferrals the most common reasons were emigration from malaria areas and travel to the UK (vCJD risk). The prevalence of deferral and the trend to higher adverse effect rate could be biased by the high number of new donors. The donor reaction was very positive and productivity justifies the cost. Better results are expected after a higher donor’s return rate.

Conclusions: Ann AphMD programme improved blood components production, especially of platelet concentrates. This depends on the characteristics of the manufacturing process and on the good acceptance of the procedures by our donors that produced a wide increase in DI. However, a MCC program requires a good organization and an adequate number of blood separators to develop its potential in achieving self-sufficiency.

P 1.9 Neopterin test in donors for an additional prevention of infectious post-transfusion complications

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Neopterin blood levels, alongside with specific infectious markers and ALT, were determined in 489 blood donors. Increased neopterin levels (<10 nmol/l) was evident in 3.23%, The ratio of anti-HIV-1, 2, anti- HCV, anti-TP and HbsAg-positive individuals was as high as 0.26, 1.84, 0.61, 0.81%, respectively. Excessive ALT activity was found in 7.15% cases. Elevated neopterin blood levels or increased ALT figures with concomitant detection of one or two types of anti-infectious antibodies was documented in 0.61 and 2.45% of blood donors. The donors with high neopterin blood levels and ALT activity underwent repeated laboratory investigation in 4-24 weeks. Seventy-eight per cent cases with initially excessive neopterin levels were found to harbour an infection or any other condition (neoplasia), while those with high ALT activity had a concurrent disease diagnosed on serial clinical evaluations in only 30.4% of patients. Thus neopterin levels is a more specific marker than ALT activity and may be used as an additional blood donor selection criterion. The assessment of the marker may be considered a cost-effective method, particularly when the shortage of blood donor is an issue.

P 1.10 How is donor selection performed in European Union (EU)? Results of a recent survey

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Aims: To assess the similarities and discrepancies in the practices of blood donor selection in the EU, in the perspective of the enforcement of the directive 2002/98/CE.

Materials and methods: Questionnaire sent to the relevant institutions of the 15 EU countries (c.) + Switzerland, including (c) questions on interviewing practices and contraindications (CI) before homologous blood donations (d.), regarding non-specific risks to donors and recipients; identified risks to donors; infectious, bacterial, viral, parasitic and prionic risks to recipients; non-infectious risks to recipients; CI questions about exclusion period for each CI to d.

Results: Pre-d. interviews are supported by written questionaires in 13/16 c. Detection of identified risks for donors: before d. body weight is measured in 16 c., blood pressure in 14 c., heart rate in 13c., and haemoglobin (all donors) in 11 c. Detection of infectious and noninfectious risks for recipients: the questions asked are mostly similar in each c. with a few exceptions. But analysing ineligibility times reveals wide differences: 1 day–1 week for dental care, 2–12 months for endoscopy, 6 months permanent for transfusion.

Conclusions: The results of that survey have revealed some differences between c. in the questions asked/measurements and above all in the ineligibility times. Continuing this work could significantly contribute to a better harmonization of blood donor clinical selection, a major factor of patients’ transfusional safety.

P 1.11 Autologous donors perceptions of donation related risk, costs and benefits

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Aim: The aim of this investigation was to determine the characteristics, motivations and beliefs of a cohort of autologous donors.
Materials and methods: Consecutive autologous donors (from 7/03–12/03) were asked to complete a short questionnaire. This data was merged with existing blood centre donor databases.

Results: Of the 255 final respondents: 48% had donated homologous blood; 37% more than once; 40% had family members who had donated autologously and 29% had received a transfusion. Overall, 52% of patients requested to bank their blood, for 73% their doctor recommended it. Those who requested it are more concerned about acquiring infectious diseases through blood (e.g. hep C 74% vs. 38%, P = 0.0001). Donors predicted that the risks of acquiring HIV, Hep C, or WNV, having a transfusion reaction or postoperative infection to be higher for allogenic vs. autologous blood. They also predicted the cost of allogenic blood is higher. If all infectious agents could be eliminated from donated blood, 76% of donors would still prefer autologous donation.

Conclusion: Autologous donors are very familiar with blood donation; they requested to donate prior to surgery and their doctors recommended it; they have significant concerns regarding risk of disease and perceive that autologous donation is low-risk and low cost. These results call for education of donors and their physicians regarding the costs and risk associated with various blood options.

P 1.12

Double dose apheresis red cells for thalassaemia patients

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A total of 44 thalassaemia patients were enrolled to receive 2 U transfusions from double dose red cell (DDRC) apheresis procedures, three weekly. The aim was to see if reliable DDRC donors could be recruited and if use of DDRC for thalassaemia patients would keep haemoglobin (Hb) within narrow limits. A total of 400 males >70 kg, haematocrit (Hct) >42, were recruited to donate DDRC six monthly. A total of 354 (89.9%) donors remain on the panel. A total of 46 withdrew, due to illness (13) no time/move (12) unwell/failed procedure (10) low Hct (2) retired (1) unknown (7). A total of 245/354 (69.2%) donated two to five times. Attendance was unreliable 2/6/03–18/8/03. 64% attended; 9% called in advance to delay appointments. Then donors were sent information about the thalassaemia transfusion recipients. From 10/1/03–26/1/04, 74% attended; 24% called in advance to delay appointments. A total of 894 DDRC donations have been collected. Volume and Hct of apheresis red cell units (258–305 ml, 0.57–0.61, n = 328) varies little compared with standard red cell units (181–415 ml, 0.54–0.6 n = 10 154). A total of 43 of 44 patients preferred DDRC, due to consistent volume and Hb rise. Keeping Hb within narrow limits is difficult as attendance is irregular. Pretransfusion Hb varied by 5–16 g/l for eight consecutive transfusions in nine more regular attenders. Transfusion of DDRC to thalassaemia reduces donor exposure by 50%, increasing safety. Consistent rise in patient Hb is achieved using a standardized apheresis product. Hb may be kept within narrow limits if patients attend regularly.

P 1.13

Predicting blood donor arrival

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Background: To maintain a stable pool of repeat donors; each donor’s donation experience should be as enjoyable as possible. Avoiding excessive waiting times at donation is important in this respect. At our centre, fixed appointments are used, and few donors arrive without appointments. On average, 59% of scheduled donors arrive, but day-to-day variations are large. Methods for predicting the number of donors that will arrive on a given day would be valuable in reducing waiting times, and in optimizing the use of blood bank resources. We present a statistical model for predicting blood donor arrival, based on information about each appointment and each donor’s donation history.

Methods: Information about candidate predictor variables was collected for all appointments made in a 971-day period (17 912 appointments). A generalized additive model (GAM) was fitted for exploratory purposes, and a generalized linear model (GLM) was fitted based on the GAM.

Results: The GLM used 18 predictor variables. The most important were the contact medium used; time from appointment making to appointment date; the donor’s number of no-shows, arrivals and deferrals during the preceding 2 years and the donor’s age and total number of previous donations. Compared with a model taking only the average arrival rate into account, prediction intervals were halved.

Conclusion: Statistical modeling can provide useful estimates of blood donor arrival, allowing for better planning of donation sessions.
Conclusion: The third method is simple, accurate, fast, cost effective, safe and convenient as a rough Hb determination method in potential blood donors. Likewise, it is convenient for the determination of anemia degree in anemic patients, thanks to rapid easy reading of values <80 and >140 g/l.

P 1.17
Risk behavior among blood donors who donate for HIV test in Shiraz
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Introduction: There has been concern that some individuals may donate blood primarily motivated by the access to HIV testing and that such donors may represent a risk to the transfusion service.

Materials and methods: This is a cross-sectional study utilizes a questionnaire. The selection was made using systematic random sampling the questionnaires include: (i) Personal characteristics; (ii) reason for blood donation; (iii) risk factor of HIV. Chi-square, logistic regression and analysis of variance were done.

Results: Mean age of respondents was 34.6 ± 11.3 SD; 82.4 were male and 68.8% female. The mean number of blood donation was 6.7 ± 3.2 S.D. A total of 14.8% respondents reported to have donated blood in order to check up. The tendency for HIV check-up was higher in men, single donor and first time blood donor (P < 0.05). There were no correlations between job, education, age and HIV check-up (P > 0.05). The risk factor for them of HIV were 38.3% sexual contact, 18.7% contact with person who suspicious to HIV; 3.9% drug use; 2.8% tattoo and 36.5% unknown.

Conclusion: In this survey 14.8% of people donate blood for HIV check-up and this can be dangerous for blood safety. The most risk factor that reported was sexual contact. For safe blood supply we have to educate people in order to not donate blood for health check-up and discussing population about residual risk of HIV transmission through blood and importance of blood donor for blood safety.

P 1.18
A study of motivation factors in Iranian blood donors
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Our main goal was to determine the major motivating factor for blood donation among Iranians. The motivating factor in first time donors was then compared to that in repeat donors.

Method: This study was performed in 16 provinces during the fall and winter of 2003. A questionnaire was prepared asking about the donors’ motivation and demographic data. Then the questionnaire was given to blood donor physicians at a workshop, where they were instructed to only record the donors’ first reply as the primary motivation. Blood donors entered into this study were chosen by systematic, random sampling.

Results: A total of 4623 blood donors were entered into this study. Voluntary donors were 92%, 7% made replacement donations, and the remaining 1% was not identified; 23% of all donors were first time donors; 35% gave a history of previous donation and 37% were repeat donors. Humanitarian motivation was the most often reason (47%) for blood donation; 13% of cases donated for the purpose of maintaining a healthy dose; 7% of cases donated blood for a needing relative or friend; 6% of donors perceived blood donation as a check-up or wished to be tested for viral diseases. Another 6% had other motives, mainly motivated by the access to (HIV) testing and that such donors may represent a risk to the transfusion service.

Conclusion: A humanitarian motivation was the major reason for blood donation in our country.

P 1.19
Is the printed blood donor information sufficient in European blood banks?
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The aim was to review the printed information given to blood donors in the EU area compared with the proposal for the requirements in the European Blood Directive. These requirements state content for such information. Participants of the study were 17 blood centres from EU/EEA countries, which are members of the European Blood Alliance (EBA) and two from other EU countries (16 countries together). A questionnaire was sent on topics of the proposal. Blood centres were also asked to deliver printed material they gave to blood donors before the Directive. The material was then reviewed with the proposal. The study was run during spring 2003 by the Finnish Red Cross Blood Service in cooperation with the EBA. None of the material per a blood centre fulfilled all the technical requirements. Some showed severe lack in contents. The topics most defectively covered were: donors right to withdraw at any time of donation, donors responsibility to inform on relevant issues and donor privacy protection. The EU aims at high level of protection of public health, one target being delivery of safe and high-quality blood products. One factor for safe blood is healthy donors. This is best secured by efficient pre-donation interview and health check and by informing and ensuring the understanding of donors so that self-deferral can take place when necessary. The study showed that donor information material needs to be improved. EU level regulations seem to be necessary to ensure this to happen.

P 1.20
Switching traditional blood donors to multicomponent collection (MCC): a feasibility study
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Introduction: MCC has been referred as an effective strategy to increase the productivity of a blood collection center.

Aims: To prospectively evaluate blood donors eligibility and their acceptance to MCC.

Methods: All donors referring to blood center from 14/02/01 to 31/07/03 have been evaluated. Age [18–60 years] and weight [≥60 kg] were general criteria for eligibility. The hematological parameters for eligibility to the different MCC were:

| Parameter       | Limit       |
|-----------------|-------------|
| Hb (g/dl)       | ≤15.0       |
| Serum ferritin  | ≥20         |
| Plt (x10^12/l)| ≥300         |

212 donors were selected on January 2002. All eligible donors were informed about MCC and, in case of acceptance, a written consent was obtained.

Results: Total evaluated donors were 21 841 (17 701 males, 4140 females). 70% of them (15 341/21 841) were eligible according to clinical parameters (75% of male donors, 51% of female donors). 82% of eligible donors declared their acceptance to undergo MCC procedures. Eligibility (as percentage of total eligible donors in agreement) were distributed as follows:

| Parameter       | Limit       |
|-----------------|-------------|
| Hb (g/dl)       | ≤15.0       |
| Serum ferritin  | ≥20         |
| Plt (x10^12/l)| ≥300         |

76% of donors were included in the first group of donors (only 18% refused). A MCC programme can also improve the RBC and Plt production (eligibility to 2RBC and RBC + Plt were 21 and 34%, respectively).

Conclusions: A MCC program is feasible (eligibility > 70%) and well accepted by donors (only 18% refused). A MCC programme can also improve the RBC and Plt production (eligibility to 2RBC and RBC + Plt were 21 and 34%, respectively).

P 1.21
Analysis of productivity and safety of a multicomponent collection (MCC) program
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Introduction: MCC allows to tailor a collection programme to the specific needs of a transfusion service, with minimal donor’s discomfort. Red blood cells (RBC) and platelet concentrates (Plt) requirements are very high in our hospital.

Aims: To compare the MCC and the standard donation (SD) productivity and to monitor the adverse reactions.

Methods: MCC procedures performed and the priority of choice were: (i) 2RBC; (ii) 2RBC + Plt; (iii) RBC + plasma (RBC + FFP); (iv) 2Plt (since January 2002); (v) FFP + Plt. The only adverse events observed were tinglings and vasovagal reactions occurring in 11/1989 (6%) donors. A total of 1816 2RBC, 1633 Plt, and 525 FFP were collected. The numbers of blood components collected by MCC have been compared with the numbers of components that would have...
Suitability of apheresis donors for multicomponent apheresis

M C C represents an alternative to traditional whole blood donation. In addition to blood component standardization and resource optimization, one main advantage of M C C is tailoring donation procedures according to donor characteristics. From March to May 2003 we evaluated blood donor eligibility for different M C C procedures (as listed in the table) according to these criteria: Hgb >14 g/dl (>15 g/dl if 2 U) and ferritin>30 ng/ml (>40 if 2 U) for RBC; platelets >150 x 10^{11} /l (>250 x 10^{11} /l if 2 U) for PLT; proteinemia >6.0 g/dl and normal protein electrophoresis for plasma (PLS). We evaluated 356 donors (74% male, 26% female): 312 (88%) were eligible for M C C. Ductility, that is eligibility for more than one procedure, was higher in male than in female (90% vs. 60%). Causes of noneligibility were low weight for female and age >60 years for male. Criteria for 2-U RBC donation were fulfilled in 32.3% of males. PLT–PLS collection was only acceptable procedure for 43.6% of females. In conclusion, M C C is suitable for nine of 10 donors. Ductility permits a better design of blood collection enrollment according to individual donor characteristics and blood center requirements.

Tab. Percentages of eligibility for different M C C procedures: (i) two RBC units; (ii) RBC + PLT; (iii) RBC + PLS; (iv) two PLT units; (v) PLT + PLS; (vi) RBC + PLT + PLS.

| Procedures | 1,2,3,4,5,6 | 1,2,3,5,6 | 2,3,4,5,6 | 2,3,5,6 | 1,3,4,5 | 3,5 |
|------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Male (n = 254) | 11.0 | 21.3 | 13.8 | 39.0 | 1.2 | 5.9 | 2.4 | 5.5 |
| Female (n = 55) | 0.0 | 0.0 | 1.8 | 12.7 | 1.8 | 40.0 | 0.0 | 43.6 |

Blood: from donor to recipient

We carried out 166 MCA of 106 BD (88 M, 13 F); 2 TU RBC (n = 37), 2 TU PLT (n = 51). ITU PLT+ FFP (n = 78). Collected volumes were 7–12% of BD. 13% were by women with TBV 3998 – 4300 ml. Total processed volume (TPV) was higher by donors with TBV 4300 ml.

Conclusions: Our stated entrance criteria for MCA donors are suitable for the practice. We must consider wider entrance criteria (e.g. the relation between predonate platelet count, TBV). The direct apheresis influence on BD with lower TBV will be the subject of our following study.
Blood collection in disaster conditions (proposal of a model)

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Background: In disaster conditions blood donation demand increases very sharply, even there is not a real blood need. This is mostly because of share the problem of the public by the community.

Aim: The aims of this proposal are providing fast, comfortable, effective and satisfactory blood donation conditions to the donors and providing necessary basic conditions to the blood bank to work in satisfactory conditions.

Methods: Blood centres/blood banks are mostly not suitable to provide effective service to the highly increased blood donation demand due to their limited working area. Accepting blood donations at arenas (closed sport centres) in disaster conditions can be the most effective solution to those problems. Before the disaster will happen; (i) the arenas should be identified according to their size, localization and other basic facilities such as parking area, etc. (ii) standard operating procedures (SOP) for each step of the system and guidelines of whole system should be completed. The evaluation of the system should be tested and evaluated by periodic exercises.

Conclusion: Under the experience of last two decades we have concluded that accepting the blood donations at the arenas is the best way both for the donors and the blood banks. By this way both donor satisfaction and blood bank service efficiency will be higher then accepting the blood donations at blood centres and/or banks.

Blood donor recruitment and the problem of outdated contact information

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Finnish red Cross Blood Services responsible for the blood programme in Finland. Hospitals or other organizations do not collect blood. In 2003 we sent 427 000 personal invitation cards and over 33 000 electronic invitations to blood donors in order to collect 300 000 U of whole blood. A prerequisite to effective use of invitations is availability of correct contact data of blood donors. We have used an automated mail address service supplied by the Finnish Post Corporation for 2 years. This service identifies blood donors with their personal donor number and updates the addresses of blood donors to whom we had sent a card with outdated mailing address. 4.5% (19 000) of the addresses of active donors were outdated in our register in 2002. In 2003 the frequency was even higher (6.0%, 26 000 addresses). Most striking was that the amount of outdated contact data of new donors had increased from 119 (2002) to 494 (2003). These donors have visited the blood service within last 3 months. With SMS messages the problem of outdated phone numbers has recently become easier because when people change mobile operator they can keep their phone number. 10-20% of e-mails sent to blood donors are returned due to incorrect e-mail addresses. This data shows that effective methods for updating donor contact information are necessary for blood donor recruitment.

The attitudes of young blood donors in Cyprus – a follow-up study

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Cyprus depends totally on voluntary blood donation. The absence of scientific data on the attitudes of young blood donors towards the frequency of blood donations and the factors affecting it were obstacles in sustaining and enlarging this group of donors. A survey among a stratified sample of 249 young donors (male and female) from the district of Limassol studied their beliefs and attitudes on blood donation. The data was analyzed using linear logistic regression. Results indicated that 32% of the young people are frequent donors (at least six donations in 10 years), 51% have donated less than five times and 17% one time; therefore, as frequency increases, participation decreases. Gender and place of living affect the frequency of blood donation. Young male donors donate more frequently than young female donors, and inhabitants of rural areas more often than those of urban areas. The frequency...
is mainly affected by neglect [63%], health related reasons [13%] and the busy schedule of blood donors [10%]. On the contrary, fear and ignorance are minor factors. Young donors [86%] express their willingness to donate in the future and the intention could be higher if motivation was given (i.e. a thank you certificate).

Blood donation is considered a voluntary contribution and not an act of monetary reward. Blood donor recruitment and sustainability of young donors can be improved through improved planning, public relations and communication techniques.

P 3.2

Incidence of transfusion transmitted viral infections in Italy
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Incidence rate (IR) of transfusion-transmitted infections (TTI) in blood donors is essential for monitoring blood safety. This study is based on data of the Italian Surveillance System of TTI. From 1999 to 2001, IR for HIV, HCV and HBV were calculated. To estimate the total number of person-years at risk, a sample of Italian repeat donor population was done. A two-phase stratified sampling was performed: in the first phase two transfusion structures (TS) from each of the 20 Italian regions; and in the second phase 150 repeat donors, from each sampled TS, were randomly selected. Crude and adjusted IR for seroconverted donors was calculated. Further adjustment for HBsAg IR was done, taking into account the Italian vaccination policy and the different pattern of antigenemia after primary infection. The number of estimated person-years was 2 100 000 for HIV and HCV and 2 357 394 for HBsAg. Adjusted IR per 100 000 person-years was 1.75; 9.26 and 44.12 for HIV, HCV, and HBV respectively [95% CI: 3.15–3.19; 9.20–9.32 and 43.81–44.43]. HIV IR is low, in the same order of magnitude of industrialized countries. For HCV and HBV great differences are observed comparing with other countries, reflecting the endemicity of these infections in some areas of Italy. At present HCV IR and the consequent residual risk are reduced by the introduction, in June 2002, of NAT testing. The ongoing policy for HBV vaccination is also relevant for the prevention of this TTI.

P 3.3

Haematological malignancies in patients with hepatitis C virus (HCV) infection
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Introduction: HCV infection represents a major health problem worldwide, often related to blood transfusions. Since HCV is also lymphotropic virus, its association with different haematological disorders such as non-Hodgkin lymphoma (NHL) and mixed cryoglobulinemia (MC) has been reported.

Aim: This retrospective study aimed to record the haematological malignancies in patients with HCV infection.

Materials and methods: We report detailed information on 24 patients referred to the Haematology Divisions among 1838 HCV(+) individuals admitted to our tertiary hospital, during the last 7 years. HCV serum antibodies were assayed using EIA (AxSym, Abbott), while anti-HCV positivity was confirmed by an immunoblot method (Inno-Lia, Innogenetics). All 24 patients were negative for HBsAg and anti-HIV 1.2.

Results: Of the 1838 HCV(+) patients, 24 cases were related to haematological malignancies (M/F = 11/13, aged 48 ± 20 years). Previous blood transfusions were recorded in six of them (25%). Eight of 24 suffered from NHL, seven from other lymphoproliferative disorders (LPD), while the remaining nine cases included non-lymphoid malignancies. In three of five NHL patients and in two of four with LPD, cryoglobulins were found in their sera.

Conclusion: The prevalence of haematological malignancies among patients with HCV infection was 1.31%. There is no evidence of a predominance among NHL, LPD and non-lymphoid malignancies in HCV(+) individuals. Cryoglobulin production is a frequent finding in patients with NHL and LPD.

P 3.4

Residual risk of transfusion-transmitted HIV, HCV and HBV infections in France and impact of NAT
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Background: Trends in residual risk (RR) of transfusion-transmitted viral infections were analysed over nine overlapping periods of 3 years from 1992. The last estimates (2000–2002) were compared with the results of HIV-1 and HCV NAT implemented in France in July 2001.

Method: Residual risks were estimated by multiplying incidence rates (IR) by the durations of the window periods (WP). For the first seven periods, IRs were calculated from data collected by the blood centres of the Transfusion-Transmissible Agents Working Group which collect more than 50% of blood donations, and for the two last periods, on the overall blood supply. Results: RR [00–02] without NAT were estimated at one in 1 400 000 for HIV, 1 in 1 000 000 for HCV and at 1 in 400 000 for HBV. By a reduction of the WP, predicted RR with minipool NAT became nearly two times lower for HIV (1 in 2 500 000) and at 1 in 1 000 000 for HCV and at 1 in 400 000 for HBV. By a reduction of the WP, predicted RR with minipool NAT became nearly two times lower for HIV (1 in 2 500 000) and seven times lower for HCV (one in 6 600 000). Of the 6 million donations screened with NAT until December 2003, two HIV-1 and two HCV were remote thanks to the NAT. These results are consistent with the NAT expected yield for HIV but not for HCV [five expected].

Conclusion: Without NAT, the overall RR for HIV, HCV and HBV decreased from one in 65 000 [92–94] to one in 235 000 [2000–2002]. Since NAT implementation, this RR is one in 325 000 [28% less than without NAT]. NAT results confirm the validity of RR theoretical estimates and the limited benefit of genomic screening due to the very low level of RR at the time of NAT implementation.

P 3.5

Health outcomes and mortality in HTLV-I and HTLV-II infected former blood donors
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Objectives: Uncertainty about disease outcomes complicates the counseling of HTLV-I and -II infected blood donors.

Methods: We enrolled 151 HTLV-I, 387 HTLV-II, and 799 matched seronegative blood donors into a prospective cohort study in 1990–1992, followed by biannual medical histories and examinations. We calculated multivariable odds ratios (ORs) by logistic regression and hazard ratios (HRs) by survival analysis.

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Results: Compared with seronegatives, HTLV-II subjects had a significantly increased incidence of acute bronchitis (OR = 1.68), bladder or kidney infection (OR = 1.55), arthritis (OR = 2.66) and asthma (OR = 2.88), and a borderline increase in pneumonia (OR = 1.82). HTLV-I subjects had a significantly increased incidence of bladder or kidney infection (OR = 1.82) and arthritis (OR = 2.84). Definite or probable HTLV-associated myelopathy was diagnosed in six (3.7%) HTLV-I and four (1.0%) HTLV-II subjects. After a median follow-up of 8.6 years, there were 45 deaths in the cohort, and all-cause mortality was significantly increased for HTLV-II (HR = 2.3, 95% CI 1.1–4.9) but not HTLV-I donors. No single cause of death predominated.

Conclusions: HTLV-II infection is associated with respiratory and urinary tract infections and asthma, and both HTLV-I and -II are associated with arthritis. HTLV-associated myelopathy is a serious, infrequent outcome of both HTLV-I and HTLV-II infection. The finding of increased all-cause mortality associated with HTLV-II requires further confirmation.

P 3.6 Prevalence of transfusion-transmitted infections in the National Blood Transfusion Service – Kenya

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Background: The National Blood Transfusion Service (NBTS) was established in 2001. In the year 2000 the hospital-based blood banks collected 12 000 U of blood with HIV prevalence among donors of %7 and heavy dependence on family replacement donors. The NBTS targets low risk volunteer donors, especially secondary school students through donor outreach sessions.

Methods: Written records of all blood donations and infectious disease screening results are maintained on standardized forms. Donations are screened for HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis. The screening records of 39 184 blood donations from October 2002, through September 2003, were analyzed.

Results: Total annual donations from the NBTS increased to 40 000 while reducing replacement donations from 83–40%. The HIV prevalence rate among donors was 1.3% vs. 9.4% in pregnant women nationally; the prevalence of HBV was 3.2% vs. a 13% carrier rate in the general population. Syphilis reactivity was 0.5%, while the HCV prevalence was 1.0%. A marked variation in regional and monthly infection prevalence rates was noted.

Conclusions: The NBTS has dramatically increased blood supply and improved safety through a shift from replacement to volunteer donors. Challenges currently facing this service include formulation of strategies for donor retention and motivation, persistently high HBV prevalence and high regional and monthly variations in infection rates among blood donors.

P 3.7 Serological diagnosis of the HCV infection in Romanian blood donors during 1995–2002

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Background: The screening of blood for anti-HCV has been introduced in January 1995. We report here the results on 3 124 780 blood donations, up to the end of 2002.

Methods: Repeatedly reactive cases referred to the NITH were tested in all the currently used EIAs and confirmed reactive sera were further tested in Deciscan or RIBA 3.0; samples reacting against at least two different antigens in any of the immunoblots were considered positive; negative, indeterminate or weak positives were further tested in the other immunoblot; a follow-up of minimum 6 months was requested for nonpositive cases. A lot of randomly selected new blood donors (rsNBD, n = 3227) were screened for HCV core antigen also.

Results: The prevalence in new blood donors varied between 0.1 and 5.4% in different parts of the country, whereas the incidence in repeat blood donations has dropped from 0.25–2.27% in 1995 to 0.0–0.21% in 2002. The balance of the results in immunoblot is: 79.2% positives, 16.3% indeterminates, 4.9% negatives; 8.5 and 0.8% of the positive cases displayed weak and evolutive patterns respectively. In rsNBD, beside 7% anti-HCV positive and 18 indeterminate cases, three HCV. Antigen and pcr (AmpliCoc HCV v2.0) positive/anti-HCV negatives were found (1/1076).

Conclusions: The percentage of confirmed positives is significantly higher when compared with low prevalence areas. After 8 years of screening the residual risk for post-transfusion HCV infection although greatly reduced, remains substantial.

P 3.8 Occurrence of infections among blood donors in Germany is not influenced by monetary compensation

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Background: The German Transfusion Act allows monetary compensation for blood or plasma donations in recognition of effort. We studied the impact of compensation for whole blood donations on the occurrence of transfusion transmissible infections in blood donation services (BSD).

Methods: In 2001 a survey of all BSD was conducted to identify those offering compensation for donors. Results were linked to the national blood donor surveillance data regarding infectious disease markers (HIV, HCV, HBV and syphilis). The current analysis was restricted to whole blood donations. Logistic regression was used to determine a possible association of monetary compensation with occurrence of infection, adjusted for factors like donor type (first time or repeat donor), structure and size of the BSD and demographic data.

Results: Of all 89 BSD, about 40% offered compensation (ranging from 5 to 28 per donation) and collected roughly 20% of the more than 4 million whole blood donations. Infections occurred in 85% of all centres among first time donors and in 54% of all centres among repeat donors, respectively. Early results of multivariate analysis indicate that compensation is not associated with an increased occurrence of infectious diseases in BSD for both first time and repeat donors.

Conclusion: These data support that limited financial compensation for whole blood donors in Germany does not negatively affect the high level of safety of blood components.

P 3.9 Epidemiology of HIV, HCV, HBV and syphilis infections in blood donors in Germany in 2002

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Background: Nation-wide surveillance of transfusion-relevant infections among blood donors has become mandatory with the enactment of the Transfusion Act since July 1999. The data can contribute to improve donor selection strategies and screening methods.

Methods: In 2002 data were collected from all 131 blood donation services on the number of first time and repeat donations, type of donation, gender, age group and number of confirmed HIV, HCV, HBV and syphilis infections. Additional data from positive donors, e.g. information on the possible way of infection, were available. Results were compared with data from previous years.

Results: More than 6.6 million blood donations and blood samples of prospective donors were screened for infections. Of these 70% were whole blood, 23% plasmapheresis and 2% platelet donations. A total of 86 HIV, 654 HCV, 1019 HBV and 295 syphilis infections were diagnosed. Compared with previous years the prevalence of HCV and HBV infections did not change significantly among first time donors but an increase in HIV prevalence from 3.5/100.000 in 1999 to 8.6/100.000 in 2002 was noted. In the group of repeat donations we found a small increase in HIV seroconversions especially among plasmapheresis donors.

Conclusion: The surveillance of infections among blood donors in Germany demonstrates low prevalence and few seroconversions of transfusion relevant infections. We will continue to carefully investigate the recent rise in HIV infections.

P 3.10 Hepatitis B prevalence and risk factors in blood donors in Ghazvin, Iran

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Iran Blood Transfusion Organization – Research Center

The prevalence of hepatitis B is 1–5% in different provinces of Iran. It is necessary to know the common routes of transmission of hepatitis for prevention of it. In this study, we evaluate the risk factors of hepatitis B by comparing the hepatitis B patients with healthy blood donors. We assessed 39 598 volunteer blood donors for hepatitis B and C. Risk factors were obtained from 186 patients and 186 healthy donors. Independent risk factors were determined, using logistic regression analysis. Prevalence of HBV was 1.08%. Female sex (P = 0.05, OR = 2.4), education level under diploma (P < 0.001, OR = 2.5), being married (P = 0.001, OR = 3.01), and age more than 35 years old (P < 0.001, OR = 2.7), were risk factors in univariate analysis. Logistic regression showed that only duration of marriage (P = 0.01, OR = 1.04), contact with an icteric person (P < 0.0001, OR = 23.62), extramarital sexual contact (P = 0.03, OR = 10.46),
history of sexual transmitted diseases (P = 0.0007, OR = 5.37) and high risk jobs (P = 0.01, OR = 2.2) are independent risk factors for prediction of hepatitis B infection. Risk factors, which were addressed in this study, covered 95.7% of the patients. In conclusion, Ghaiezvin is one of the low prevalent regions for hepatitis B in Iran. Prevalence of hepatitis B is decreasing in comparison with last decades. Horizontal mode is more important than vertical transmission in this region of Iran. Screening programmes, education and vaccination, specifically in high risk groups is essential for prevention of new cases.

P 3.11
Screening for anti-HTLV in Romania – evidence for a distinct HTLV-I endemic area
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Background: HTLV-I was identified locally in 1990–1992. We report here the results of screening a total of 1 390 237 blood donations collected during October 1999–December 2003 and ATL cases hospitalized in Bucharest.

Methods: The confirmation of all EIA-repeatable reactivities was performed at the NIHIB observing the HERIN criteria. For 23 seropositive cases PBMCs DNA was extracted and analyzed by HTLV specific real-time PCR, the envelope andLTR regions were amplified, sequenced and analyzed in a phylogenetic tree, at the Robert Koch Institute.

Results: Anti-HTLV-I was confirmed in 148 repeat and 164 new blood donors. HTLV-I was confirmed in 75% of EIA-reactives. No positive regular blood donor has been identified after 2001, whereas seropositive new donors are continuously detected – a prevalence above 0.1% has been found in three different areas. In the Brasov (0.1%), Bucharest (0.25%), and Constanta (0.09%) area one early seroconversion could be documented and in six other cases the EIA + WB reactivities suggest a recent infection. Forty-three HTLV-I positive ATLs, have been confirmed in Bucharest: 2–16 per year. Vertical transmission over three generations has been documented, whereas no import case has been identified. All the samples analyzed by PCR were found tax-positive: in all but one sequences were closely related to each other and clustered with a sequence from a Romanian ATL-patient (K122-Rum) described in 1997.

Conclusions: The results point to the existence of at least one endemic area.

P 3.12
HIV-positive blood donors in Kwazulu-Natal (KZN)
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Background: Donors are grouped into four risk categories (R1–4) based on HIV prevalence in donor cohorts: R1-very low risk, R2-low risk, R3-intermediate, R4-high risk. Lower risk blood is preferentially released. Understanding donor behaviour may further reduce risk.

Method: All HIV-positive donors in KZN are notified and requested to contact the blood centre. The records from November 2001–November 2003 were reviewed.

Results: A total of 474 donors (M249; F225) with a mean age of 30.37 years (range 16–61) tested HIV-positive. A total of 262 were first time donors, 109 lapsed and 103 repeat donors. The risk breakdown was R1–3, R2–30 to R3–33 and R4–408. Concurrent other infections (laboratory testing, health-history screening and self-deferrals) applied before blood donation, a small risk of infectious disease transmission still exists. Although these residual risks are small, there is a need to improve the screening donation process. We have implemented a computer-based subjective deferral system (SDS) to exclude a blood unit from transfusion when interviewers have doubts about the risk behaviors of the candidates.

Materials and methods: We have evaluated the prevalence of infectious disease markers (IDM) among donors excluded by SDS during August 2002 and January 2004. Of the 10 803 donors, 151 were discarded due to positive SPD. In 26/151 showed positive results in serology screening (seven/syphilis, 12/anti-HBc, six/anti-HIV 1 + 2, two/AgHBs, two/anti-HCV, one/anti-HTLV I/II, one/Chagas).

Conclusion: Donors excluded by positive SDS showed higher prevalence (P < 0.05) to the following IDM: syphilis, anti-HBc, and anti-HIV 1 + 2. These results represent a important improvement for donor screening, specially for those donors at high risk factors related to sexual behavior.

P 3.13
Comparison of three anti HBC tests – a useful screening parameter for blood donations
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Background: After the introduction of PCR testing the expected residual transfusion risk for hepatitis B virus infection is estimated to be 1:5000 and about 40 times higher than for HIV or HCV. The main reason for the elevated transfusion risk are chronic anti-HBc-positive, HBsAg and PCR-negative donors with low virus load.

Material and methods: Three anti-Hbc assays (Abbott PRISM®HBc, Abbott AxSym® CORETM and Abbott PRISMAg® HB core) were assessed for agreement by testing 10 000 blood donors at our blood donation center.

Results: Multiple time donors (2.2%) were reactive for anti-HBc, whereas only 1.8% of first time donors were reactive for anti-HBc. Compared with the present PRISM®Hbc test system, the new PRISMAg® HB core and the AxSym® CORETM test system showed 0.4% less reactive samples for first-time donors and 0.2% less-reactive samples for multiple time donors. The average age was 33 ± 12 and 46 ± 12 years for first time and multiple time donors, respectively.

Conclusion: The differences between first-time donors and multiple-time donors may be explained by different average age of the donors. The new PRISMAg® HB core test system is more specific than the PRISMAg® HBc test. Further examinations are necessary to confirm initial reactive results, to rule out false-positive measurements and to estimate the infection risk of initial anti-HBc positive blood donors.

P 3.14
Evaluation of a computer-based subjective deferral system to reduce infectious disease transmission
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Background: Blood safety precautions have been implemented to restrict potentially infected individuals from donating blood. Despite the extensive safety measures (laboratory testing, health-history screening and self-deferrals) applied before blood donation, a small risk of infectious disease transmission still exists. Although these residual risks are small, there is a need to improve the screening donation process. We have implemented a computer-based subjective deferral system (SDS) to exclude a blood unit from transfusion when interviewers have doubts about the risk behaviors of the candidates.

P 3.15
Cellular immune response against HCV proteins in HCV+ blood donors and their female sexual partners
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Background: Cellular immune response against hepatitis C virus has recently been described in anti-HCV negative but HCV exposed individuals.

Aims: To evaluate cellular immune response against HCV core and NS3 proteins in anti-HCV+ blood donors and their female sexual partners.

Methods: We collected PBMC from 12 former blood donors with chronic hepatitis C (CHC), eight anti-HCV negative female partners, eight patients with resolved HCV infection, and 13 healthy controls. Cellular immune response against HCV core and NS3 proteins was evaluated by ELISPOT-IFNg. Results were expressed as IFNg-producing cells/100,000 cells (IFNg-PC).

Results: Significant differences were found between healthy controls and patients with resolved infection for both NS3 and core (median NS3 IFNg-PC: 4 vs. 19, P = 0.003; core: 0 vs. 4, P = 0.034, respectively) but not in CHC patients (median NS3: 5; core: 1).

Conclusion: No significant differences were found between healthy controls and patients with resolved infection for both NS3 and core (median NS3 IFNg-PC: 4 vs. 19, P = 0.003; core: 0 vs. 4, P = 0.034, respectively) but not in CHC patients (median NS3: 5; core: 1).

Materials: Three anti-HBC assays (Abbott PRISM®HBc, Abbott AxSym® CORETM and Abbott PRISMAg® HB core) were assessed for agreement by testing 10 000 blood donors at our blood donation center.

Results: Multiple time donors (2.2%) were reactive for anti-HBc, whereas only 1.8% of first time donors were reactive for anti-HBc. Compared with the present PRISM®Hbc test system, the new PRISMAg® HB core and the AxSym® CORETM test system showed 0.4% less reactive samples for first-time donors and 0.2% less-reactive samples for multiple time donors. The average age was 33 ± 12 and 46 ± 12 years for first time and multiple time donors, respectively.

Conclusion: The differences between first-time donors and multiple-time donors may be explained by different average age of the donors. The new PRISMAg® HB core test system is more specific than the PRISMAg® HBc test. Further examinations are necessary to confirm initial reactive results, to rule out false-positive measurements and to estimate the infection risk of initial anti-HBc positive blood donors.
Counseling of donors with positive microbiological markers – an ignored issue in developing nations

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Background: Postdonation counseling of blood donors with positive microbiological markers is not a routine practice in India and universal guidelines are not available, unlike the developed world. This effort aimed to counsel hepatitis B [HbsAg] positive blood donors to reduce the risk of disease transmission in the community.

Methods: Questionnaires were used to determine donors’ preferred mode to communicate their test results. A protocol for post donation counseling was developed using the NBS, Colindale, UK guidelines.

Results: In spite of 16% deferral due to history of jaundice, the prevalence of hepatitis B among the blood donors is 3.2% at our center. Most donors (96%) were keen on knowing the test results, 58% preferred information by mail, 30% by direct personal contact and 12% wanted to be informed on the telephone. On call 89% (57/64) donors turned up for postdonation discussion. One of the donors, his three siblings and mother, excluding father were positive for HbsAg, HbeAg and HBV DNA with high ALT levels. The transmission seemed vertical through mother who has developed hepatocellular carcinoma. The family is being managed at the specialist clinic.

Conclusion: Uninformed, blood donors positive for microbiological markers remain potential source of onward disease transmission. The issue merits global attention and universal guidelines need to be developed and mandated. Understanding the origin of infection can identify areas to improve blood safety.

Performance characteristics of the qualitative and quantitative bead capture- TaqMan WNV NAT assay

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Background: The year 2003 registered the highest number of reported cases for West Nile Virus. There is a critical need for nucleic acid detection of low WNV viral titers. Here we describe an assay based on Bead capture-Taqman technology for qualitative and quantitative detection of WNV RNA.

Materials and methods: A magnetic bead based protocol was used to isolate WNV RNA and a related internal control RNA in a single tube in a semi-automated method from 0.5/ml of plasma. The target capsid region was amplified by Taqman technology on the beads. A blind panel of 25 WNV samples distributed by Blood Centers of the Pacific (BCP) was tested. We have also used the quantitative Bead Capture-TaqMan assay to quantitate WNV positive samples from hamsters and vero cells and to track the epidemiology of WNV in donated blood.

Results: The analytical sensitivity with the BCP WNV panel for the Qualitative Bead Capture-TaqMan assay indicate 100% detection of 30 Cps/ml, with an improved assay detecting 10 Cps/ml. The assay has very high specificity and tolerates a variety of anti-coagulants, interfering substances, and plasma from pathological conditions. The assay has been used at a reference laboratory to confirm 656 of the 3141 possible WNV positives between July and November 2003. The Quantitative assay estimates WNV in the range of 102–1010 Cps/ml.

Conclusion: The Bead Capture-TaqMan WNV assay is a rapid, sensitive, user-friendly, accurate assay for WNV RNA detection and quantitation.

Patient-related blood donors: safe or not?

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Background: Patient-related blood donors contribute to a significant proportion of the blood units collected in hospitals. In many developing countries the prevalence of patient-related blood donors coexists with the constant lack of blood.

Aim: To determine the safety of patient related compared with the voluntary blood donors.

Materials and methods: We have compared infectious disease markers [HbsAg, anti-HCV, anti-HIV] between patient-related and voluntary donors who donated whole blood in our hospital, during a 4-year period.

Results: During the period under study 6503 donors gave whole blood units, of which 2188 (33.6%) were provided by patient-related donors. There were 108 (4.9%) confirmed virus-reactive donations of which 16 (0.7%) anti-HCV, 92 (4.2%) HbsAg and zero anti-HIV. Regarding the first-time donors, 9.4% of them are virus-reactive, compared with repeated donors who are positive in 3.3% cases. Virus-reactive voluntary donors are 81 (1.9%): 30 (0.9%) anti-HCV, 51 (1.2%) HbsAg and 0 anti-HIV.

Discussion/conclusion: There is significant difference between patient-related and voluntary donors concerning the reactivity to infectious disease markers, which are 2.5 times more frequent in patient-related donors. Our results support the assumption that patient-related donors represent an increased risk of infectious diseases, more frequently than voluntary donors. Healthy patient-related blood donors should be encouraged to become voluntary nonremunerated donors.

NAT screening in Europe

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Background: The european authorities introduced since 1999 NAT test for HCV RNA on plasma for fractionation. Afterwards in the different european countries was decided to apply this methodology to all blood donations, but the decision has not been adopted in the same way. For this reason the European Network of the Transfusion Medicine Societies (EuroNet-TMS) promoted an european survey to know the situation in Europe.

Materials and methods: A questionnaire was sent to the Societies that originally founded the Euronet-TMS. The first part of the questionnaire regards NAT testing on plasma for fractionation and the second part NAT screening for all blood donations.

Results: In all countries NAT for HCV has been introduced on plasma for derivatives between 1998 (France and Sweden) and 2002 (Greece and Belgium). In France, Switzerland and Netherland NAT testing for HIV is also mandatory. NAT on each hemo component has not been implemented in Greece, Denmark and, formally, in Sweden where, in fact, it has been introduced. A total of quite 41 million of blood units has been tested for HCV RNA in Europe with a general incidence rate of 1.3/106 of only NAT positive cases. More than 24 million of units was tested for HIV RNA with a total incidence rate of 0.3/106.

Discussion: Some differences among the different countries are yet present in Europe and a lot of scientific and also socio-economic efforts are to be done to obtain comparable conditions of transfusion safety in Europe.

Development of a real-time multiplex assay for detection of members of the Flaviviridae virus family

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Roche Molecular Systems Inc., Alameda, CA, USA

Background: Given the increasing complexity of emerging pathogens threatening the blood supply, multiplexed assays for related families of viruses provide an efficient means to counter this threat. Our goal is to develop a single assay capable of detecting West Nile virus (WNV), Kunjin virus, the St Louis, Japanese, and Murray Valley encephalitis viruses, Dengue fever virus, and yellow fever virus.

Materials and methods: Primers and probes corresponding to well-conserved regions of the Flaviviridae genomes were designed and combined into a single TaqMan assay. Reactions consist of equal volumes of a 2X master mix and template. The master mix contains primers, probes, Thermus species Z05 DNA polymerase, dATP, dCTP, dGTP, dUTP, Mn(OAc)2, uracil-N-glycosidase, glycerol, DMSO, Tricine, and KOAc. A total of 62 amplifications cycles were done in the COBAS TaqManTM instrument. Synthetic RNA templates representing target viruses were used to evaluate performance of the prototype assay.

Results: The prototype multiplex assay is capable of detecting ≤100 copies of RNA representing WNV (lineages I and II), Kunjin virus, Japanese encephalitis virus, Murray Valley encephalitis virus, St Louis encephalitis virus (lineages IA, IB, IIA, IIB, and IIC), Dengue virus types 1–4, and yellow fever virus.

Conclusions: We have developed a prototype assay for the detection of Flaviviridae virus family members which should aid in the prevention of transmission via blood products and/or transplantation.
P5 Bacteria

P5.1 Routine bacterial monitoring of platelets
S Pearce, M Hayward, G Rowe, C Scott, D Williams and R Ahya
Welsh Blood Service

Background: The frequency of transfusion associated bacterial sepsis is greater than that of any transfusion transmissible viruses currently tested for in the UK. The risk of bacterial contamination of platelets may be as high as one in 2000 platelet concentrates.

Aims: Implementing a continuous, monitoring method to reduce mortality and morbidity caused by bacterial contaminated platelets.

Method: From February 2003 the Welsh Blood Service has been monitoring all platelet components produced for bacterial contamination, using a BacT/ALERT blood culture system linked electronically to the blood stock management system. 17 ml samples are taken on day 1 from every platelet for aerobic and anaerobic cultures. Updates to the status of each individual platelet occur every 12 min with the ability to prevent contaminated platelets being issued immediately and instigate a recall if required, at any time. Time expired, unused platelets are also similarly resampled after day 5.

Results: By December 2003, 8927 platelets have been tested. Forty-three were screen positive (0.48%) and five confirmed positive (0.05%). Testing of time expired platelets revealed 1 unit that had tested negative on primary culture but found to be positive on repeat day 6 culture. There has been no confirmed bacterial transfusion reaction in any recipient during this period.

Discussion: The BacT/ALERT is proving to be an effective bacterial contamination monitoring method for all platelet components.

P5.2 Bacterial screening of whole blood derived platelets: effect of disinfection and deviation bag
D de Korte*, J Curvers, WL Kort de, JJ Marcelis en and CL der Poel van
Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

Introduction: Previous investigations indicated that contaminating bacteria are predominantly present in the first millilitre of a donation. We evaluated nationwide the effect of diversion of the first 20 ml as well as various skin cleansing methods in the presence of 100% bacterial screening foruffy-coat derived pooled platelet concentrates (PC).

Material and methods: PC were cultured with an automated system (BacT/Alert). Data on bacterial contamination of PC were acquired at two periods in time. From January to September 2002, skin cleansing was done with variable methods, from October 2002, standardized double cleansing with 70% isopropyl alcohol was performed. In addition, one blood bank used a sample bag (Composaver Sample), for diversion of the first 20 ml over the whole period.

Results: No significant difference before and after introduction of standardized double cleansing was found. The percentage of contaminated pooled platelets was significantly reduced with the diversion bag (P < 0.001; Pearson Chi-Square).

| Old skin disinfection | No diversion | Deviation | Total |
|-----------------------|-------------|-----------|-------|
| No. tested            | 42600       | 4362      | 46962 |
| No. initially positive | 400 (0.94) | 22 (0.50) | 422 (0.90) |
| 70% Isopropyl alcohol |             |           |       |
| No. tested            | 20123       | 2058      | 22181 |
| Initially positive (%) | 180 (0.89) | 10 (0.49) | 190 (0.86) |

Conclusions: The use of a deviation bag for collecting the first 20 ml for screening tests reduces incidence of bacterial contamination in the final buffy-coat derived PC in routine practice. No significant effect of skin cleansing methods was found.

P5.3 Evaluation of TPHA to identify possible window-period donations in South Africa
MC Ferreira* and G Joubert
SANBS, RSA; Department of Biostatistics, UFS, RSA

Background: Syphilis testing may have value as surrogate marker for other infectious diseases that may be acquired similarly. It also prevents syphilis transmission through blood products. SABTS estimated that 105/100 000 undetected HIV-positive donations in new and 261/100 000 in regular donors may have entered the blood supply during 1996/1997 and TPHA was considered useful because of the positive relationship with HIV.

Aim: The study re-evaluates TPHA as surrogate marker for sexually and blood-transmissible infectious diseases, namely HIV, HBV and HCV.

Method: A single centre, retrospective analysis was performed, based on data for 2 741 052 donations made from 1998 till 2002.

Results: Sensitivity (SI): 152 of 3645 HIV-positive donations (S = 4.2%), 24 of 1272 HbsAg-positive donations (S = 1.9%) and 9 of 378 anti-HCV-positive donations (S = 2.4%) tested TPHA positive. Positive predictive value (PPV) of: 2144 TPHA-positive tests 152 were HIV-positive (PPV = 7.1%), 24 of 2016 TPHA-positive donations tested positive for HbsAg (PPV = 1.2%) and nine of 2001 TPHA-positive donations were also anti-HCV positive (PPV = 0.4%). The estimated number of infectious window-period donations was: 116 (HIV), 153 (HBV) and 364 (correction factor of 2.38 applied) and 53 (HCV). Of these, 4.8 (HIV), 2.7 or 6.4% (HBV) and 1.2 (HCV) would have been removed due to a positive TPHA-test.

Conclusion: TPHA does not add significant value in identifying possible window-period donations infected by HIV, hepatitis B or C.

P5.4 Mirasol® PRT performance against a methicillin resistant strain of Staphylococcus aureus
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Objective: Evaluate performance of the Mirasol Pathogen Reduction Technology (PRT) System against an antibiotic resistant clinical isolate strain of Staphylococcus aureus.

Mirasol PRT is a photoinactivated treatment of platelets products with riboflavin solution to reduce the pathogen load occurring in normal donor blood products.

Materials and methods: Staphylococcus aureus (ATCC 700328) was isolated from a blood sample from a patient with a fatal case of bacteremia in 1998. The strain has shown a reduced suscceptibility to vancomycin, and resistance to methicillin. Four apheresis platelet concentrates were collected on a Trima Automated Blood Collecting System and spiked with an average of 6.0 log/ml S. aureus. All products were prepared with a constant 30 ml of 500 µM riboflavin, a platelet volume range of 170-190 ml and a platelet concentration range of 1000-1100 x 10^7/ml. Platelet products received 5.1 J/ml of light. Samples were prepared to measure bacterial titer before and after treatment.

Results: Reduction of this antibiotic resistant, clinical isolate strain of S. aureus was 5.0 ± 0.6 log/ml at treatments of 5.3 J/ml.

Conclusions: The Mirasol PRT System for Platelets utilizing riboflavin solution and light shows robust performance against this antibiotic resistant blood-isolate strain of S. aureus.

P5.5 Fatal outcome after transfusion of platelet concentrate heavily contaminated with Bacillus cereus
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A patient with AML had a platelet count of 8 x 10^3 /µl when given platelet transfusion. Two hours after transfusion, the patient had severe general symptoms. Despite symptomatic treatment in the intensive care unit, she died within 9 h. Cultures from blood and several organs showed extensive growth of Bacillus cereus. The platelet concentrate was made from four buffy coats 1 day prior to the incident. The buffy coats were stored 24 h at RT before the concentrate was made (Baxter, PL.)
P 5.6
The Pall Bacterial Detection System plus (BDS+): an improved system
CP McDonald*, J Colvin1, R MacDonald1, MC Fernandez1, K Wilkins2, S Robbins1 and
JAJ Barbara1
1National Bacteriology Laboratory, National Blood Service, Colindale, London and 2Pall Europe, Portsmouth, UK

Background: Bacterial transmission constitutes the major proportion of residual microbial risk from transfusion. The Pall Bacterial Detection System (BDS) was developed for the detection of bacteria in platelet concentrates. BDS+ has been modified from the first generation Pall BDS assay by the addition of a tryptic soya broth tablet to enhance bacterial proliferation. Pall BDS incubation conditions were 24 h at 35 °C, followed by subsequent incubation at 22 °C whereas incubation for BDS+ was standardized at 35 °C.

Method: Buffy coat derived pooled platelet concentrates were spiked with nine clinically significant organisms (one species per bag; n = 10) at 100 cfu/ml. Pall BDS+ sample pouches were inoculated with the bacterially laden platelet concentrates and incubated at 24, 30 and 144 h. The oxygen content and bacterial concentration were measured at the end of the incubation period.

Results: Pall BDS+ detected bacteria in 96, 98 and 100% at 24, 30 and 144 h respectively. In comparison, BDS without the addition of the tryptic soya broth tablet detected 84 and 98% of these organisms at 24 and 30 h respectively. All positive pouches contained >106 cfu/ml and overall there was 100 times more bacteria present in the pouch than the mother bag after 24 h.

Conclusion: The modified Pall BDS+ was shown to enhance bacterial detection in platelet concentrates. Additional modifications to the Pall BDS+ have resulted in the formulation of the Pall eBDS.

P 5.7
Evaluation of the Pall enhanced Bacterial Detection System (eBDS) for testing platelet concentrates
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1National Bacteriology Laboratory, National Blood Service, Colindale, London and 2Pall Europe, Portsmouth, UK

Background: Bacterial contamination of platelet concentrates remains a significant problem in transfusion medicine. In the UK 22 cases of bacterial transmission from platelet concentrates (five fatal) have been reported from 1995–2002. Pall BDS detects bacteria by measuring reduction in oxygen content. Pall eBDS is the next generation of this assay incorporating several enhancements to increase sensitivity and improve ease of use.

Method: Pooled platelet concentrates were spiked at 10 cfu/ml with five organisms (one species per bag; n = 6). The organisms tested were E. coli, S. aureus, S. choleraesuis, S. epidermidis and E. cloacae. Pall eBDS pouches were inoculated with the spiked platelet concentrates. After 24- and 30-h incubations at 35 °C the oxygen content was measured. A further set of pouches was taken at 24 h from the inoculated platelet concentrates. Incubation and reading intervals were as for the initial set of pouches.

Results: The Pall eBDS resulted in 100% detection for all organisms tested in the pouches taken immediately post-inoculation. In pouches taken after 24 h incubation of the spiked platelet concentrates, all bacteria were detected apart from Salmonella. Further investigation of the Salmonella spiked platelet concentrates from which the Pall eBDS pouches were negative revealed no viable bacteria present.

Conclusion: Pall eBDS potentially offers a practicable and sensitive system for the bacterial screening of platelet concentrates.

P 5.8
Detection of bacteria in platelet concentrates: comparison of 16S rDNA PCR and automated culturing
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Sanquin Blood Bank North West and Department of Medical Microbiology and Infection Control, VU Univ. Medical Center, Amsterdam, The Netherlands

Background: Platelet concentrates (PCs) are frequently associated with transfusion-transmitted infections. To assess the presence of bacteria in PCs a rapid and dependable method is needed. Based on real-time PCR technology, a 16S rDNA assay was developed. The assay was validated and its performance compared to an automated culture system.

Methods: PCs prepared from five buffycoats in plasma were used. The presence of bacteria in these PCs was routinely assessed in an automated culture system (Bact/Alert). Culturing was conducted until a positive signal was detected or for up to 7 days when remaining negative. The PCR assay was performed with DNA extracted from the same cultured samples with a fully automated method. PCR amplification was performed with a set of universal primers and probe targeting eubacterial 16S rDNA. PCR results were compared with culturing.

Results: 1366 samples were tested by both the PCR assay and culture system. Three specimens were found positive by both methods. Identified microorganisms included Micrococcus spp., Staphylococcus epidermidis and Propionibacterium spp. One sample generated an amplification signal in the PCR but gave no positive signal in the Bact/Alert, probably because of the detection of DNA from a non-viable bacterium by PCR that can not be detected by culture.

Conclusion: The PCR assay enables accurate and sensitive detection of bacterial DNA in PCs. Hence, this method may be a valuable improvement in monitoring bacterial contamination of PCs.
Methods: Upon receipt at the hospital, a 3 ml aliquot was removed from random donor (RDP) and single apheresis platelets (SDP) and introduced into the Pall BDS pouch. The pouch was incubated at 37 °C for 24 h then the oxygen was measured to determine bacterial contamination.

Results: A total of 14 522 RDPs and 354 SDPs have been tested. Bacteria were detected in 10 units (5 RDPs and 5 SDPs), Propionibacterium Acnes (2), coagulase-negative Staphylococcus (2), Bacillus species, Micrococcus species, coryneform bacteria and Staphylococcus aureus were confirmed by manual microbiological technique. The Pall BDS system was easy to use and required <5 min for all manipulations. CCIs measured 1-h post-transfusion were 18 950 ± 9800 (n = 23).

Conclusions: The Pall BDS system permits evaluation of platelets for bacterial contamination. Negative platelets have been successfully transfused in our institution up to 7 days after storage with good CCIs. Apheresis platelets had higher contaminated than RDPs.1 Rock G et al Seven day storage of random donor platelet concentrates. Transfusion 2003; 43: 1374–1377.
component volume showed 99% acceptability with a mean of 25±5 ml, giving an 11 ml mean increase. Haemoglobin acceptability was 98% with a mean of 50.6 g/l, an increase of 4.5 g/l. There was no significant alteration to the leucocyte count with 100% acceptability and a maximum count of 1.0×10^9/l. Platelet pool platelet count gave 98% acceptability with a mean of 346×10^9/l. Interim results suggest a significant increase in red cell component haemoglobin yield using the new backplate whilst giving a slight decrease in platelet component platelet counts. Other NHS Centres may be changed to use of this new backplate producing an improved quality red cell component.

P.8.2 Oxidative red cell injury after gamma irradiation
R Chaudhary*, J Shukla, RB Mishra and RK Hans
Department of Transfusion Medicine, SGPGIMS, Lucknow, India and Industrial Toxicology Research Center, Lucknow, India

Background: Cellular blood components are gamma irradiated to prevent graft vs. host disease (GvHD) in transfusion recipients at risk of this disease, such as recipients of bone marrow transplantation. Because, gamma irradiation can result in the production of oxygen-free radicals (reactive oxygen species, ROS), the role of these ROSs was investigated.

Method: Whole blood from healthy blood donors was exposed to gamma irradiation at 25 and 50 Gy. Plasma Hb, plasma K+, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were measured in normal and irradiated blood by standard techniques. Lipid peroxidation of red cell membrane was measured by studying thin-barbituric acid-reactive substances (TBARS).

Results: There was progressive increase in the mean values of plasma-free Hb, plasma K+ and LDH in gamma irradiated blood compared to control. Gamma irradiation also increased lipid peroxidation (TBARS formation) in a dose dependent manner. TBARS increased from 3.5±0.25 in controls to 4.75±0.25 at 25 Gy and 5.6±0.215 at 50 Gy. Similarly, gamma irradiation also induced generation of ROSs, such as SOD and GSH-PX in dose dependent fashion. SOD decreased from 927±113 in control to 803±150 at 25 Gy and 668±150 at 50 Gy. GSH-PX decreased from 61±2.55 in controls to 47±2.33 at 25 Gy and 42±2.6 at 25 Gy and 47±2.3 at 50 Gy.

Conclusion: Gamma irradiation increases lipid peroxidation of red cell membrane and oxidative injury to the red cells in dose-dependent manner.

P.8.3 Evaluation of the Roche 2,3-DPG assay in whole blood and red cell concentrates
P Cookson*, M Garwood, J Sutherland, N Rolfs and R Cardigan
Components Development, National Blood Service, Brentwood, UK

Background: Many institutions have used the Sigma assay to measure 2,3-diphosphoglycerate (2,3-DPG) levels in red cell concentrates (RCC) for assessing their quality. As the Sigma kit is now obsolete we have evaluated an alternative enzymatic method (Roche Diagnostics).

Methods and results: As the assay is designed for use with heparinized whole blood (WB) the use of CPD as an anticoagulant, and the effects of SAG-M and increased haematocrit (Hct) were studied. Linearity was demonstrated using purified 2,3-DPG to allow maintenance of high 2,3-DPG levels, without concurrent ATP decline.

P.8.4 Storage of RCC with maintenance of both 2,3-DPG and ATP during at least 42 days
D de Korte*, M Kleine and AJ Verhoven
Sanquin Research at CLB, Amsterdam, The Netherlands

Background: During preparation and storage at 4°C of RCC, 2,3-DPG levels fall rapidly. We developed an additive solution based on the ‘chloride-shift’ principle, allowing maintenance of high 2,3-DPG levels, without concurrent ATP decline.

Methods: RCC were washed with PAGGS-M or experimental solution (PAGGS-M containing gluconate instead of saline; pH 8.2), using a Haemonetics ACP215. RCC were stored at 2–6°C and weekly sampled.

Results: 2,3-DPG content of RCC washed with experimental solution increased during storage, up to 350% of initial value (7–25 μmol/g Hb) and started decrease after 42 days. ATP content at day 1 was 15% lower than in PAGGS-M, but at day 42 ATP was still at starting value (4.2 μmol/g Hb). In experimental solution, the intracellular pH starts at 7.4 vs. 6.4 in PAGGS-M and gradually decreased to 6.2 at day 42. At day 42, hemolysis was 0.4% vs. 0.5% in PAGGS-M and 1% of cells exposed PS (Annexin-V binding), in contrast to 3% for PAGGS-M. Glucose consumption and lactate production was increased in our experimental solution, but this was not reflected in a lower pH. If directly added to RCC (no wash), the experimental solution showed similar, but less pronounced and less durable effects (not shown).

Conclusions: Using a modified PAGGS-M, we were able to store RCC for at least 42 days with a 2,3-DPG content similar to that in fresh whole blood, without compromising other quality parameters such as haemolysis, plasma stability (not shown) and ATP content.

P.8.5 The effect of storage times on the cellular composition in blood products
J de Wildt-Eggen*, A Heethuis and PF van der Meer
Sanquin Blood Bank North East Region, Groningen, and 7 North West Region, Amsterdam

Background: In the Netherlands whole blood units (WB) can be stored overnight and components must be prepared within 24 h after collection. The effect of storage time on the cellular composition of WB and filtered red cell concentrates (RCCs) was studied.

Methods: Three inline filter systems were used, Baxter [A; n = 250], Fresenius [B; n = 251] and MacoPharma [C; n = 252] and divided in five groups; with WB storage times at 4–8, 8–13, 12–16, 16–20 and 20–24 h. Samples were taken for counting WBCCs and platelets. WBCCs were counted with a flow cytometer.

Results: Platelet counts in WB collected with system A and C prepared within 8 h showed significantly lower platelet counts (ANOVA; P = 0.001) compared with the other storage times [A; 4–8 h: 36 ± 54 h vs. 8–12 h 37 ± 53 ± 101; C; 4–8 h 127 ± 81 h vs. 8–12 h 211 ± 57 ± 101]. The overall results of the filtered RCCs (n = 743) met the European requirements of >90% containing <1×10^8 WBCCs/U. At the storage time <12 h, all filtered RCCs contained <1×10^6 WBCCs/U. At the storage time >20 h some filtered RCCs units contained >1×10^6 WBCCs/U (A; 3, 50; B; 2,51 and C, 5,50). The WBCC count increased during storage. These counts were lower at storage <12 h, compared with storage >12 h (ANOVA; P < 0.01).

Conclusion: This study showed that when all filtered RCCs are prepared within 12 h of storage no unit will contain >1×10^6 WBCCs/U. The platelet counts of WB prepared at the storage time <8 h are significantly lower compared with these prepared >8 h.

P.8.6 The effect of whole blood storage on the presence of weak propidium iodide positive events
J de Wildt-Eggen*, A Heethuis and PF van der Meer
Sanquin Blood Bank North East Region, Groningen, and 7 North West Region, Amsterdam

Background: White blood cells (WBCs) in filtered red cell concentrates (RCCs) are counted in a flow cytometer using nuclear staining with Propidium Iodide (PI). In the resulting scatter plots weak-positive PI-events (wPI+ events) potentially WBCs, were observed outside the WBC-gate. Our aim was to evaluate the effect of varying whole blood storage time and of the use of different filter systems on the presence of these wPI+ events and their relation to the number of residual WBCs.

Methods: Three inline filter systems were used, Baxter [A; n = 250], Fresenius [B; n = 251] and MacoPharma [C; n = 252], and were divided in five groups; with WB storage times between 4–8, 8–12, 12–16, 16–20 and 20–24 h. WBCs (inclusive and exclusive wPI+) were counted with two flow cytometers [Beckman Coulter (BC) and BD Biosciences (BD)].

Results: Residual WBCs were higher using BC compared with BD method, 0.62 ± 0.96 vs. 0.48 ± 0.76 WBCs/ml (ANOVA; P < 0.01). The WBC count were lower at storage <12 h compared with storage >12 h [ANOVA; P < 0.01]. The wPI+ events appeared after 12 h of storage and were seen predominantly using BD method [BC: 34/452 (8%) vs. BD: 190/452 [27% of the units with wPI+]]. Residual WBCs were significantly higher when filter C were used, when compared with systems A and B [ANOVA; P < 0.01], and was associated with significantly more wPI+ events when compared with system A or B.

Conclusion: The presence of wPI+ is related to the residual WBCs and varies depending on the filters used and on storage time.
Leukoreduction filters – QC of each LOT, must or too-much?
S Edlin*, G Prober, O Frenkel, S Gelman, V Yahalom and E Shinar
Magen David Adom National Blood Services, Israel
Objective: To investigate the possible variance in efficiency of filtration, using different lots of a filter
Materials and methods: Packed RBCs were stored at 4 ± 2°C and filtered within 48 h using Immugard III-RC WBC - reduction filters (Terumo). For each lot 5–10 U were tested [total 76 U]. Each unit was analyzed prefiltration for Hct, RBC, WBC (by Cell-Dyn 1600, ABOTT) and postfiltration (WBC after filtration by FACSCalibur, Leuco-COUNT Kit, Becton Dickinson), Mean, SD, t-test and coefficient correlation were calculated.
Results: Mean RBC recovery of all lots met the AABB standards (retain 85% of original red cells and contain <5 x 10⁶ residual WBC). However, 14 U threw eight different lots did not meet the 1st criteria [total 18% units] and 2 U (13%) from one lot did not meet the 2nd. Statistically significant variation following filtration was found in unit volume reduction (P < 0.05), RBC recovery and WBC content in the final product (P < 0.01). No correlation was detected between RBC volume reduction and RBC recovery (r² = -0.16).

Results
|                | Volume reduction (ml) | RBC recovery [%]
|----------------|-----------------------|--------------------|
|                |                       | Prefilter [x=10⁶/μl] | Postfilter [x=10⁶/unit] |
| Maximum        | 42 ± 10               | 90.9 ± 4.6          | 3.5 ± 1.3              | 3.2 ± 2.2              |
| Minimum        | 30 ± 8                | 84.9 ± 5.5          | 2.5 ± 0.3              | 1.0 ± 0.6              |
| Mean           | 37 ± 13               | 87.3 ± 4.0          | 3.1 ± 0.8              | 1.8 ± 1.5              |

Conclusion: Lot to lot variability must be taken into account when validating filters and quality control organization.

P 8.8

Packed RBCs can be stored for up to 42 days. Do these filters meet these standards?
J Georgsen*
Department of Clinical Immunology, Odense University Hospitals, Denmark
Background: Process control is an element of AABB's quality system, of ISO 9000 and of GMP. ISBT 128 - the international standard for labelling of blood components - comprises features to control critical points in labelling.
Materials and methods: Flag digits allocated by ICCBBA and flag digits defined in the international standard for labelling of blood components – comprises features to control critical points in labelling.
Results: Examples of critical points that can be controlled are: labelling at collection, after separation, after change of product code [same bag [e.g. irradiation, thawing] or new bag [e.g. filtration, washing]], pooling, splitting, and issuing of component. Examples will be provided. During 2003 16 incidents of relabelling were registered in the deviation database. All 16 were at collection and discovered by the automated process control.
Conclusion: The automated procedure to control critical points during labelling is a useful tool to discover and correct labelling errors.

P 8.9

Testing variables that affect quality of blood salvage
E Hansen*, V Bechmann and G Roth
Department Anaesthesiology, University Regensburg, Germany
A number of variables are thought to affect the process quality of blood salvage. Actually, such effects rarely have been tested, and usually were tested with outdated banked RBC hardly representative for the sedimentation characteristics of fresh blood from blood salvage.

Method: Matched blood from fresh donations were combined, diluted after introduction of 5% hemolysis, and processed in an autotransfusion device (ELECTA, Dideco) using a 175 ml bowl under varying settings. RBC recovery and plasma elimination as calculated from measurements of volumes, hematoctrits and concentrations of protein or free hemoglobin in reservoir and transfusion bag were used for quality parameters. Results: Hemolysis induced by the negative pressure during blood collection is negligible, but more pronounced after air mixing during suction. Fast filling or washing reduces both recovery and elimination, while an increased wash volume results in low improvement in elimination rate but in a higher loss of RBC. Half-full bowls should be avoided because of a lower wash-out efficacy. Elimination rates calculated from free hemoglobin always are lower than derived from protein determinations.

Conclusions: Compared with a loss of about 10% in RBC by the washing process hemolysis due to suction during collection is lacking clinical significance. Protein determination is preferred to free hemoglobin as it reflects plasma elimination and is not obscured by additional hemolysis during the process.

Leukoreduction filters – QC of each LOT, must or too-much?
S Edlin*, G Prober, O Frenkel, S Gelman, V Yahalom and E Shinar
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Objective: To investigate the possible variance in efficiency of filtration, using different lots of a filter
Materials and methods: Packed RBCs were stored at 4 ± 2°C and filtered within 48 h using Immugard III-RC WBC - reduction filters (Terumo). For each lot 5–10 U were tested [total 76 U]. Each unit was analyzed prefiltration for Hct, RBC, WBC (by Cell-Dyn 1600, ABOTT) and postfiltration (WBC after filtration by FACSCalibur, Leuco-COUNT Kit, Becton Dickinson), Mean, SD, t-test and coefficient correlation were calculated.
Results: Mean RBC recovery of all lots met the AABB standards (retain 85% of original red cells and contain <5 x 10⁶ residual WBC). However, 14 U threw eight different lots did not meet the 1st criteria [total 18% units] and 2 U (13%) from one lot did not meet the 2nd. Statistically significant variation following filtration was found in unit volume reduction (P < 0.05), RBC recovery and WBC content in the final product (P < 0.01). No correlation was detected between RBC volume reduction and RBC recovery (r² = -0.16).

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| Mean           | 37 ± 13               | 87.3 ± 4.0          | 3.1 ± 0.8              | 1.8 ± 1.5              |

Conclusion: Lot to lot variability must be taken into account when validating filters and quality control organization.

P 8.8

ISBT 128: use of flags in the donation number for control of critical points
J Georgsen*
Department of Clinical Immunology, Odense University Hospitals, Denmark
Background: Process control is an element of AABB’s quality system, of ISO 9000 and of GMP. ISBT 128 - the international standard for labelling of blood components - comprises features to control critical points in labelling.
Materials and methods: Flag digits allocated by ICCBBA and flag digits defined in the international standard for labelling of blood components – comprises features to control critical points in labelling.
Results: Examples of critical points that can be controlled are: labelling at collection, after separation, after change of product code [same bag [e.g. irradiation, thawing] or new bag [e.g. filtration, washing]], pooling, splitting, and issuing of component. Examples will be provided. During 2003 16 incidents of relabelling were registered in the deviation database. All 16 were at collection and discovered by the automated process control.
Conclusion: The automated procedure to control critical points during labelling is a useful tool to discover and correct labelling errors.

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Conclusions: Compared with a loss of about 10% in RBC by the washing process hemolysis due to suction during collection is lacking clinical significance. Protein determination is preferred to free hemoglobin as it reflects plasma elimination and is not obscured by additional hemolysis during the process.

P 8.10

Performance of the laboratory leukoreduction filters, Leucolab LCG and Leucoflex LSV1
K Hiruma*, Y Okuyama, Y Kunitomo, K Ishii, M Yazawa, K Sakuma, T Takeda, T Takagi, S Fujimoto, Y Nakagawa and N Ozawa
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Aim: Leucolab LCG and Leucoflex LSV1 filters (MacoPharma) have been widely used in European countries for universal leukoreduction, but not been used to date in Japan. Here, we report on the performance of these leukoreduction systems for filtration of red blood cells with mannitol-adeno-phosphate solution (RC-MAP).
Materials and methods: RC-MAPs were kindly provided by the Japanese Red Cross, Tokyo Blood Center. Single- or double-unit RC-MAPs were filtered with Leucol LCG2/4 or Leucoflex LSV1, respectively. We assessed leukoreduction performance and red cell recovery, amongst other criteria. The number of residual leukocytes after filtration was determined by LeucoCOUNT Kit (Becton Dickinson).
Results: Ten RC-MAPs were filtered with each leukoreduction filter, Leucol LCG2, Leucol LCG4, or Leucoflex LSV1. The shelf lives of RC-MAPs were 6.6 ± 0.8, 7.9 ± 1.9 and 4.2 ± 1.5 (days), respectively. After filtration, the number of leukocytes was 2.42 ± 2.15, 8.35 ± 16.35 and 1.86 ± 1.88 (x10⁷/bag), and log leukocyte reduction rate was 4.56 ± 0.34, 4.47 ± 0.6 and 4.52 ± 0.48, respectively. The red cell recovery rates were 86.6 ± 1.7, 89.0 ± 2.7 and 85.5 ± 2.2 (%), respectively.
Discussion: Universal leukoreduction is scheduled for implementation within a few years in Japan. The requirement for the number of residual leukocytes has been determined as <1 x 10⁷/bag. This study shows that these filters comply with these requirements and are useful for universal leukoreduction.
Quality of Apheresis-Double-dose-RBC (ALYX component collection system) compared with standard RBC

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Background and purpose: Donor pool utilization, product standardization and quality of blood components are sought to be optimized by collection through apheresis. Here, performance of the new mobile apheresis device ALYX with respect to RBC quality and variability is compared with conventionally prepared RBC.

Material and methods: A total of 32 male blood donors were enrolled for two subsequent blood donations: First, conventional RBC were prepared; 10 weeks later a double-dose RBC donation was collected with ALYX. One bag of each ALYX donation was irradiated. A paired comparison for various parameters was performed over 49 days.

Results: ALYX RBC fulfilled all European quality requirements. As regards volume and hematocrit, ALYX RBC were less variable than conventional RBC. Standard RBC had a lower hematocrit, higher pH and higher intracellular ATP concentration throughout storage. The damage of irradiated ALYX RBC was reflected in a higher hemolysis rate, higher extracellular potassium concentration, higher MCV increase and faster intracellular ATP decrease compared with nonirradiated units just as in conventional RBC.

Conclusion: Superior standardization was the major argument in favor of ALYX RBC. Some parameters showed no difference, some, like pH and intracellular ATP concentration were advantageous for conventionally prepared RBC. The decision whether or not to introduce the ALYX component collection system should focus on other issues than RBC quality.

Two cases of adverse reaction due to leukocyte-reduction filter

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Aims: Most adverse reactions associated with transfusion are nonhemolytic transfusion reactions (NHTR) with allergic symptoms such as rash, itching and urticaria. The causes of the reaction are difficult to be determined in most cases. We examined the cause of NHTR in association with leukocyte-reduction filters (L-R filters) that are widely used to prevent allogeneic transfusion.

Materials and methods: NHTR occurred in two patients: a 13-year-old female with aplastic anemia (case 1) and a 69-year-old male with myelodysplastic syndrome (case 2). To investigate the cause of NHTR in them, several antibodies such as anti-platelet, anti-hemocytoblast, anti-erythrophagocytic and anti-IgG were measured. We tried to use L-R filters from different manufacturers and transfuse the products of washed red blood cells and washed platelets.

Results: In case 1, she showed rash, cough, and dyspnea immediately after transfusion of RBC-MAP with L-R filter from T company at her sixth transfusion. In case 2, she showed rash, cough, and dyspnea immediately after transfusion of RBC with L-R filter from T company. In each case, the products were washed and tested.

Conclusions: The L-R filters from T company were suspected as the cause of NHTR in association with leukocyte-reduction filters (L-R filters).

No detectable riboflavin or photoproduct association was observed in platelets or TCA-precipitated proteins obtained from platelets. The amount of RB and photoproducts bound to TCA precipitated plasma proteins varied from 1.26–4.26%. Large variations were observed from sample to sample. Only one of the six samples preillumination and four of the six samples postillumination demonstrated measurable levels of binding.

Conclusions: Most of the 14C-RB and 14C-photoproducts were associated with plasma in a free, unbound form. No detectable amount of 14C-RB or its photoproducts were covalently bound to platelets. Consistent with prior literature reports, insignificant amounts of RB or its photoproducts were associated with plasma proteins.

Effect of prestorage irradiation and storage time on content of 2,3 DPG and ATP in RBCs

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Background: Irradiation of cellular components is currently the only accepted method of preventing transfusion-associated graft vs. host disease (TA-GvHD). It may however damage the cellular elements, particularly if irradiated cells are stored for prolonged periods. The aim of this study was to determine the levels of 2,3 DPG, ATP, pH and potassium in irradiated RBCs with 25 or 50 Gy and stored for 42 days.

Materials and methods: The ATP, 2,3 DPG, pH and potassium measurements estimated the metabolic activity of the red cells. Measurements were performed on days: 3, 14, 28, 35 and 42. The ATP level is an imperfect correlate to red cell viability.

Results: The 2,3 DPG concentration correlates highly with red cell hemoglobin function. In irradiated units ATP levels of RBCs gradually decreased from 4.8 μmol/g Hb (on third day) to 2.27 μmol/g Hb (on 42 day). There were significant differences in storage time for units irradiated with 50 Gy. Significant decreases in 2,3 DPG have been reported for RBCs irradiated with 50 Gy after 14 days of storage. Extracellular pH decreased in all units during storage. After 28-day storage and irradiation with 25 Gy potassium level increased significantly when compared with nonirradiated units.

Conclusions: Units irradiated with 50 Gy cannot be stored longer than 1 week after collection and irradiation.

Erythropagocytosis of stored RBCs

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Background: During physiological ageing, and upon binding of autologous, senescent cell-specific IgG, old RBCs are recognized and subsequently phagocytosed. We recently determined the quantity of bound autologous IgG on stored RBCs, the capacity of stored RBCs to bind autologous IgG upon recombination with plasma, and whether these characteristics changed with the storage time. The analyses did not indicate a storage-related change in the presence of or binding capacity for autologous IgG during 15 days of storage. However, there is a considerable difference in the amount of cell-bound IgG before and after incubation with PBS-BSA or plasma. It seems that cell-bound IgG present before incubation with PBS-BSA or plasma has a low avidity, which can be removed by incubation.

Aim: The aim of this study is to determine the physiological consequences of these amounts of bound autologous IgG by measuring the phagocytosis of stored RBCs.
Method: The phagocytosis of stored RBCs is measured flowcytometric by labelling RBCs with CMFDA and monocytes with CD14-PE. RBC concentrations (n = 6) are measured weekly for storage up to 49 days under blood bank conditions.

Results: This will lead to a correlation between cell-bound IgG (high and low avidity) and phagocytosis, and can be used as a possible parameter forecasting the time of survival of stored RBCs after transfusion into the recipient. Data of phagocytosis of stored RBCs are currently collected (February–April 2004) and will be presented.

P 8.18

Donor safety and in vivo regeneration after 2RBC collection

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Background: There is an increased demand for an efficient and safe RBC component production with fully automated data management.

Materials and methods: Performed were 23 RCC double unit procedures by ALYX® (Baxter). Eligibility criteria for donor safety: Hb > 135 g/l, Hct > 40%; ferritin > 13 ng/ml; male >60 kg, >170 cm; female >70 kg, >168 cm. Two units of leukodepleted RCC were collected within 27 ± 5 min, resuspended in SAG-M (RCC volume: 273 ± 10 ml, Hb 51.2 ± 1.4 g/μl), and stored for 42 days. Donor safety and volume balance was maintained during all 2RBC procedures (during each return phase, plasma and saline are returned to the donor). Donors were analyzed for regeneration in frequent intervals for 120 days.

Results: Immediately after donation donor Hb was 119 ± 12 g/l. RCC and Hb level slowly increased to their initial values between days 70 and 120 p.d. Reticulocytes peak at day 5, PLT and WBC show relatively stable figures over 120 days. Ferritin decreased to day 42 after 2RBC collection and recovered continuously to day 120 at 66% of initial values. sTfR decreased to day 3, reached initial values at day 120. Both ferritin and sTfR are donor gender dependent as regeneration slopes were different.

Conclusions: Collection of two RCCs from one donor is a safe procedure. 70 days can be recommended as an interval between double RCC procedures if donor ferritin is >20 ng/ml.

P 8.19

Evaluation of a new in-process leucocyte-depleting filter for red cell units

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Background: This evaluation assessed the handling and performance of the Pall Leukotrap RCPL filter system containing a new leucocyte depleting filter for red cells (RC2D) at Centro Hospitalar Vila Nova de Gaia.

Materials and methods: Whole blood (WB) units were collected using routine procedures and were held in a temperature-controlled room (21°C) for either <4 h (n = 6) or 18–20 h (n = 8). The WB units were then sterile connected onto Pall Leukotrap RCPL systems containing RC2D leucocyte depleting filter for red cells. Primary centrifugation was performed using Mistral 6000 centrifuge – total centrifugal force 46 326 g. Platelet rich plasma (PRP) expression and filtration was performed using manual expressors. Red cell unit filtration through RC2D was performed as per manufacturers instructions for use.

Results: Mean leucocyte residuals of the depleted red cell units were 0.045 ± 10^6/unit (0.019 ± 0.1 × 10^6) and 0.134 ± 10^6/unit (0.038 ± 0.28 × 10^6) for WB held <4 h and 18–20 h, respectively. Results for volume (ml), haemoglobin (g/unit) and haematocrit (%) for all units met both local bloodcentre objectives and Council of Europe (CE) Guidelines.

Conclusion: There was little difference in the handling of the Pall RCPL system containing the RC2D filter compared with red cell filter ‘RCM1’ in use at Centro Hospitalar Vila Nova de Gaia. The residual leucocyte level and other red cell unit parameters met CE Guidelines for all red cell units produced.

P 8.20

Washed apheresis and whole blood derived red cell units: protein and poststorage potassium content

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Purpose: Residual proteins and accumulating metabolites in red cell (RC) units may cause unwanted complications following transfusions. Washing the RBCs can prevent such events. We analyzed differences between apheresis derived (AP) and whole blood derived (WB) RC units on protein depletion and metabolite content after 14 day postwash storage using potassium as the marker.

Methods: Thirty WB and 30 AP-derived RC units were subdivided into three groups (10 U each) depending on the age before washing (1 week vs. 2 weeks vs. 3 weeks, group 1 vs. group 2 vs. group 3, respectively). All units were washed with saline and resuspended in SAG-M in an automated closed system (ACP 215, Haemonetics, USA), measuring lactate, pH, potassium, free and total haemoglobin after 14 days while total protein and IgA depletion were measured immediately after washing.

Results: Removal of protein and IgA was more efficient for AP units, although differences reached significance only in group 3 for protein and group 1 for IgA. After 2 weeks of storage potassium was significantly lower in all AP groups (P < 0.01). Haemolysis after 2 weeks of postwash storage was lower in all AP groups. Differences reached significance in group 1 and 2.

Conclusion: Automated washing in a closed system assures effective removal of proteins and metabolites with postwash storage for up to 2 weeks regardless of the production method. Better washing-efficacy and less haemolysis was observed for all AP groups.

P 8.21

Decreased iron stores in two European regular whole blood or aphaeretic donor populations

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Purpose: Double red blood cell collection can help to overcome availability shrinkage to support increasing demand, but could increase donor iron loss. This work intended to search on the iron status of current donors from two European regions.

Methods: Donors from Tyrol (n = 387; >1000 m altitude; T) and from Mallorca (n = 334; sea level; M) were tested for location, sex, age, diet, iron intake, smoking, donation history, Hb, Hct, red cells and ferritin (Chemiluminescence-microparticle immunoassay). Iron deficiency threshold was established at ferritin <20 μg/ml for all donors (<12.5 mg/l for women and <30 mg/l for men). Data in access database was analysed using Pearson correlation and multivariate linear regression with SPSS.

Results: Donors (47%) were women in T and 29% in M. Good correlation between Hb and Hct (coefficient = 0.872), but poor between Ferritin and Hb (0.047) or Hct (0.395). Women show lower ferritin values (38 μg/ml vs. 90 μg/ml in men), but men show a higher iron deficiency rate (28% vs. 22%). M donors show less iron (30 mg/l) than T donors (74 mg/l). Ferritin-deficient donors gave significant more previous donations (13) than nondeficient ones (10), independently of donation type (WB = 75% or aphaeresis = 10%) or time period considered (2002 or 2003). Effect was maintained after adjustment for sex, age and location.

Conclusion: Donor iron stores are strongly affected by regular donation and region. Donor iron stores should be measured in regular donors once per year.

P 8.22

Preparation of leuko-reduced red blood cell (RBCC) and platelet (PC) concentrates on Haemonetics MCS+5

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Aim: To compare the quality of SAGM RBCC produced by apheresis on MCS+ and filtered at 4°C or at room temperature (RT).

Methods: Forty-five RBCC were collected using the LDP-RBC protocol, AB16 anticoagulant and LDH46FF sets [with integrated RBC filter RC21 Pall]. All subjects (nine female, 36 male) met the French criteria for combined RBCC and PC donors. Group 1: 23 RBCC filtered at RT between 2 and 10 h after collection. Group 2: 22 RBCC stored at 4°C within 1 h after collection and filtered at 4°C within 24 h after collection. RBCC quality was evaluated in vitro before/after filtration and on day 14, 28 and 42 of storage.

Results: All parameter results complied with the French regulatory characteristics for apheresis leukoreduced RBCC, with addition of a preservative solution: volume (without SAGM) 25 ml, haemoglobin content >40 g, hematocrit between 50 and 70%, and maximum leukocyte level of 1 × 10^6/unit. The only significant difference found between the groups concerned the leukocyte log reduction performance (P = 0.016): 4.4 ± 0.3 for group 1 (RT), 4.7 ± 0.3 for group 2 (4°C). Hemolysis was minimal in both groups, 0.09 ± 0.02 % and 0.13 ± 0.04%, respectively, at day 42 (10 RBCC tested).

Conclusions: RBCC produced by apheresis using the LDP-RBC protocol on MCS+ and AB16 anticoagulant, suspended in SAGM and filtered at RT or at 4°C meet the French requirements for such product. Filtration at RT or 4°C provides equivalent RBCC quality. This will simplify the preparation process of these RBCC.
**P.8.2.3**

*In vitro and in vivo evaluation of the freezing of leukoreduced Trima accuses RBC units*

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FDA regulation allows for freezing of red cells (pRBC) from whole blood (WB) on day 0, and 24 h storage of pRBC after thawing and deglycerolization. However, the freezing, thawing, and 24 h post-storage of pRBC collected on the Trima blood collection system have not been validated. This study compares freezing of leukoreduced pRBC units collected using the Trima system in ACD-A/AS-3 (test) to WB derived AS-5 pRBC (control) in a paired design.

**Methods:** Test and control (n = 12 each) pRBCs were leukoreduced on day 0 and refrigerated for 6 days before glycerolization and freezing at −70 °C. After at least 28 days frozen, the units were thawed and deglycerolized (Merrymand method) and refrigerated for 24 h. Samples were taken day 0, day 6, and 24 h post-thaw for *in vitro* measurement of CBC, plasma hemoglobin, pH, pO2, pCO2, Na+, K+, glucose, ATP and osmotic fragility. Within 20–24 h after deglycerolization, in vitro single and double label 24 h percent recovery was measured.

**Results:** *In vitro* comparison of test and control pRBC on days 0 and 6 showed some statistical differences that were not clinically significant. At 24 h post-thaw, test and control pRBC showed similar results except for slightly higher ATP and slightly lower 24 h double label percent recovery in Trima pRBCs, but well above the FDA’s recommendation.

**Conclusion:** Results indicate that Trima pRBCs are acceptable for transfusion after freezing on day 6, frozen storage for 28 days, and 24 h post-thaw.

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**P.8.2.4**

Characterization of the effects of leukocyte-filtered red blood cell transfusions in major surgery

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By a multi-centre randomized controlled trial (RCT) in major surgery, the effects of leukocyte filtration of red blood cells (RBC) on postoperative complications such as mortality, ICU-stay, length of hospital stay (LOHs), multi-organ failure (MOF) and infection have been investigated. Patients in the leukocyte filtered (LD) arm show reduced LOHs (1.8 ± 1.8 day) in a study population of 1403 aneurysm-, oncological gastro-intestinal (GI) and orthopaedic surgery patients when compared with the patients randomized for buffy coat depleted RBC. Beside 30% reduction of MOF incidence (95% CI: 0.49–1.00) in elective aneurysm- and GI surgery patients and a 53% reduced mortality (95% CI: 0.23–0.39) in GI surgery patients randomised for LD, no other significant endpoints are found. Although the reduced LOHs by LD is significant, this does not significantly compensate for the costs of universal LD-transfusions in the Dutch Health Care system. The 95% CI of costs could imply saving of 93 Mi Euro, but also an increase of costs of 30 Mi Euro. Reduced LOHs by LD is determined by a few (1%) patients who LOHs was >90 days, the transfused elective aneurysm patients (P = 0.0027) and the nonsurvivors randomized for LD. Therefore, the universal LD program in the Netherlands may be more of interest for only a few specific patients groups. A more precise characterization of favorable and unintentional effects on patient’s health and LOHS by LD will specify the cost-effectiveness of LD-programs.

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**P.8.2.5**

Combined analyses of patient data from three cardiac surgery studies

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**Background:** We performed several clinical trials comparing by-filtration leukocyte reduced RBC transfusions with Buffy-coat depleted RBC transfusions. Three of these included patients undergoing open-heart surgery. Combining the raw data from these patients in a single database, we performed a combined analyses stronger then a met-analysis would be.

**Materials and methods:** In the three studies, variables had been scored in a similar way (e.g. identical CDC criteria to define infections). Endpoints in these analyses are: mortality (in-hospital, 30 days), postoperative infections, length of hospital stay (LOS), ICU-stay.

**Results:** A total of 1582 cardiac surgery patients had participated, in three hospitals. The variables randomization, hospital, gender, age, type of surgery, duration of surgery, aorta-clamping time, and the number of RBC, plasma and platelets transfusions were all, univariate, significantly correlated to one or more of the endpoints and were therefore entered in the multivariate (MV) analyses. In the MV analyses, infections were correlated to number of RBC (P < 0.001), randomization (P = 0.001), age (P = 0.004), and number of platelet transfusions (P < 0.009). In-hospital mortality was correlated to the same four factors and 30-day mortality additionally to hospital. LOS and ICU-stay were not correlated to randomization.

**Conclusion:** In cardiac surgery patients, transfusion of filtered RBC is associated with a reduction in mortality and infection, not with a reduction in LOS or ICU-stay.

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**P.8.2.6**

Blood product thermodynamic understanding – impact in blood banking

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**Purpose:** Understanding thermodynamic should permit a comprehensive handling of blood products in different environments. This study shows the T° variations of red cells and plasma during the blood banking process.

**Method:** The core of the study was to measure the T° of blood products in various storage devices, ambient environments and during transports as well as the time that a blood product can stay at ambient T° [c. 20 °C] according to the specifications.

**Results:** T° decreases following T°final = T°initial + (T°final − T°initial)e−c, and increases following T°final = T°initial + (T°final − T°initial)e−k. k is the variable that influences the time for getting the desired blood product T°: k = 0/0.1, where 0/0 is the energy from the environment and t is the time value established by the heat capacity and the thermal conductance of blood products.

**Examples:** (1) Plasma [>2 h] are needed to bring the plasma T° from −23 °C (retective T°) to −30 °C in a −30 °C storage device, while it takes c. 20 min in a −70 °C storage device. At ambient T°, it takes 25 min for increasing the plasma T° from −65 to −30 °C, while it takes c. 10 min from −30 to −23 °C. (2) Red cells [c. 2.5 h] are needed to decrease the red cell T° from +10 to +6 °C in a storage device at +4 °C. At ambient T°, it takes 30 min for increasing the red cell T° from +6 to +10 °C.

**Conclusions:** Based on these results, comprehensive proposals for handling blood products in the blood bank as well as for the organization of the transports can be proposed.

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**P11 Plasma-derived coagulation proteins**

**P 11.1**

Characteristics of von Willebrand factor in plasma from five different apheresis procedures

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**Background:** We studied the impact of apheresis on plasma VWF content, functional activity, and HMW multimers.

**Material and methods:** Five groups of 30 plasma donations were collected with Haemonetics PCS2 (Rev F, Rev G, HSC, or FC procedures), or with Baxter Auto-C. VWF/Ag, RCo, CB and HMW multimers were determined in 10 individual plasma donations and on 30 donations pools. VWF:Ag, RCo, CB and HMW multimers were determined in 10 individual plasma donations and on 30 donations pools.

**Results:** Mean VWF:Ag was >100 U/dl, RCo >90 U/dl, and RCo/Ag ratio at the rate of one for all procedures. Mean percentage of multimers of 10 to 15-mers was normal, percent of >15-mers was significantly less in Rev G (5.2 ± 1.8 %; range 3.5–9.8) than in Auto-C, HSC, and FC (P = 0.0211, 0.0257, and 0.0376, respectively). VWF:CB was 61 in Auto C and 60 U/dl in HSC pools, 50 U/dl in Rev F and FC pools, and 43 U/dl in Rev G pool. The CB/Ag ratio of the pools was 0.54 (Auto-C), 0.49 (HSC), 0.46 (Rev F), 0.45 (FC), 0.37 (Rev G), and 0.88 in ref plasma. Percent of HMW >15-mers was 7–8% in FC, HSC, and Auto-C pools, 4.8 and 5% in Rev F and Rev G pools, and 12.6% in ref plasma.

**Conclusion:** VWF:Ag, RCo and multimers of 10 to 15-mers were well preserved in plasma from all procedures. VWF:CB and content of the highest MW multimers (>15-mers) were consistently less than in ref plasma, and differed slightly among procedures, possibly influenced by the residual cell content of plasma. Impact, if any, on the clinical outcome of transfusion plasma in TIP treatment or on the production of vWF concentrates would be interesting to evaluate.
P 11.2
Characteristic of human parvovirus B19 in blood coagulation factor VIII manufacturing process
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Objectives: Human parvovirus B19 (B19) is a kind of small nonenvelopedivirus, resistant to solvent-detergent (SD) treatment, and it is difficult to remove it from plasma-derived products. In order to develop an effective method to remove B19, B19 was characterized by spiking it into the blood coagulation factor-VIII (FVIII) production process.

Methods: B19 spiked into a manufacturing step was analyzed by anion exchange chromatography. After a B19-spiked solution was applied to Q-Sephrose, the column was washed with adequate buffer, and B19 was eluted with linear gradients both of NaCl and pH. Each eluted fraction was subjected to B19 DNA testing, DNase treatment and B19 capsid protein detection.

Results: There were at least three types of B19 in the manufacturing process after elution from Q-Sephrose. One of these was the DNase-resistant virus virion itself, including capsid protein, second type was a disrupted DNase-sensitive virion that contained capsid protein. The third type was a DNase-sensitive DNA fragment that did not contain capsid protein. When B19 spiked into the process solution was filtered with a 35-nm pore-size filter, all three types of B19 were detected, but only B19 DNA fragment was detected in the solution when filtered with a 20-nm pore size filter. The B19 DNA fragment found in the filtrate with the 20-nm pore size filter could be separated from FVIII with anion exchange chromatography, because their degrees of negative charge differ.

P 11.3
Stability of Octaplast, a solvent/detergent (SD) treated plasma, during 3 days after thawing
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Aim: Investigation of the stability of coagulation factors and protease inhibitors in thawed plasma bags of Octaplast SD treated plasma.

Materials and methods: Four frozen bags derived from Octaplast lots of different blood groups were thawed and stored thereafter at +4°C and RT for 3 days. Samples drawn during the observation period were investigated in particular on activities of coagulation factors V, VII, VIII, IX and XI, and the protease inhibitors protein C, protein S and antithrombin by standard coagulation and chromogenic assays. The generation of FVIIa was followed as an indicator of activation.

Results: At +4°C, all coagulation factors and protease inhibitors were stable for 8 h after thawing. At this time, FVIII and protein S activities decreased significantly. At the same time, an activation of FVII to FVIIa was observed. During storage at RT, activities of both lhale coagulation factors, V and VIII, decreased with time, leading to a concomitant prolongation of the partial thromboplastin time. Protein S levels started to decline earlier as during storage at +4°C, 4 h after thawing activity loss was 15%.

Conclusion: The above stability study has demonstrated that Octaplast can be thawed and stored at +4°C for up to 8 h without any significant loss of coagulation and inhibitor activity, and without activation of FVIIa. Alternatively, to store at +4°C, Octaplast can be stored at RT for 4 h maintaining its clinical efficacy and safety margin.

P 11.4
Autologous fibrin glue (AFG) reduces blood loss in patients submitted to total hip replacement (THR)
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Introduction: Total hip replacement is associated with high perioperative blood loss. Fibrin has been shown to be effective in reducing bleeding in different type of surgery. Aim: To verify the efficacy in reducing perioperative blood loss of AFG produced through an automated system.

Methods: Up to January 04, 11 patients candidate to THR and eligible to autologous blood predecessor were enrolled into the study. Five patients received AFG as a local hemostatic agent during surgery. Age, sex, weight and peripheral blood volume (PBV) were similar in the two groups. ‘Home made’ AFG was produced from autologous plasma by Cryoseal Thermogenesis DIDEKO system. All the patients were operated by the same surgeon with the same technique and received standard surgical haemostatic procedures. Blood loss occurring from presurgery to fith day was evaluated in terms of RBCs loss according to the formula: Blood loss = PBV × (Basal Hct – day 5 Hct) + RBC transfused.

Results:

| Parameters                          | AFG (n = 5) | Controls (n = 6) | Student’s t-test |
|------------------------------------|------------|-----------------|-----------------|
| Basal Hct%                         | 38.8 ± 2.8 | 40.4 ± 3.9      | NS              |
| Patients transfused (%)            | 3.6[60%]   | 5.8 [83%]       | –               |
| B19 transfused/patient (ml)        | 182 ± 178  | 330 ± 46        | P < 0.05        |
| Hct Day 5%                         | 32.3 ± 5.3 | 30.7 ± 1.5      | NS              |
| RBC loss ml (±SD)                  | 613 ± 223.9| 899 ± 248       | P < 0.05        |

Conclusions: AFG significantly reduced perioperative blood loss and transfusional requeiment in the patients studied.

P 11.5
Preparation of an autologous thrombin for use in a fibrin glue
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Introduction: Fibrin glue is used to enhance hemostasis during surgical procedures. Commercial preparations (known fibrinogen level) are available but expensive and in small volumes. We developed a computerized device for cryoprecipitate (1). This was employed for simultaneous production of an autologous thrombin to produce an entirely autologous fibrin glue.

Methods: Plasma (250 ml) was processed in the Thermogenesis Cryosealreg device with cryoprecipitate made in 50 min. The thrombin was generated using the thrombin activation device. Factor VIII, fibrinogen and von Willebrand Factor was done on cryoprecipitate and thrombin activity was measured. For combined product rate of clot initiation (R) and strength was measured by thromboelastogram (TEG).

Results: The total fibrinogen was 122 ± 13 mg (20% yield). Total FVIII was 91.2 ± 48 IU, vWF was 123.4 ± 53 IU, and total protein was 0.44 gm. A total of 447 U or 45.8 ± 7.8 U/ml of thrombin was generated. TEG for a combined product was similar to that seen with cryoprecipitate and bovine thrombin (R = 18.7 ± 7.1).

Conclusion: This process provides autologous human thrombin in sufficient concentration to initiate clotting and crosslinking of cryoprecipitate for use as autologous fibrin glue (Rock G et al. A novel, automated method of temperature cycling to produce cryoprecipitate. Transfusion 2003; 41: 232–235).

P 11.6
Removal of small nonenveloped viruses with a larger-sized nanofilter
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Objectives: Nanofiltration is one of the most effective virus reduction methods in the manufacturing process of plasma products. However, it is difficult to separate small viruses from high molecular weight protein solutions such as immunoglobulin G by nanofiltration, because the size of the protein is similar to that of viruses. In order to separate the viruses from these proteins by nanofiltration, it is necessary to change the size of either one. In this study, we report that such nonenveloped viruses as human parvovirus B19 (B19), EMC, PPV and HAV aggregate in the presence of certain kinds of amino acids. When 5% globulin or 5% albumin was added to a 0.3 M glycine solution was reduced to 1:107.5 by nanofiltration with a 35 nm pore-sized filter. Virus removal by nanofiltration was either evaluated by PCR or by infectivity assay.

Results: B19 in a 0.3 M glycine solution was reduced to 1:107.5 by nanofiltration with a 35 nm pore-sized filter, whereas in PBS it was not reduced. Similarly, B19 was also reduced when suspended in other amino acid solutions. This effect was also confirmed with EMC, PPV or HAV. When 5% globulin or 5% albumin was added to a 0.3 M glycine solution, the removal rate was decreased.

Conclusions: These data suggest that viruses in the presence of certain kinds of amino acids could aggregate and effectively be removed with a filter, the pore size of which is larger than the size of the viruses.

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P12 Immunoglobulin therapies

A possible role for IVIG in treatment of relapsed TTP
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Background: Standard therapy for thrombotic thrombocytopenic purpura (TTP) is plasma exchange, often with corticosteroids. This report describes a patient with relapsed TTP that responded to intravenous immunoglobulin (IVIG).

Case report: A 10-year-old girl with recent history of upper respiratory infection was admitted with jaundice (bilirubin 2.6 mg/dl), anemia with schistocytes (Hgb = 5.9 g/dl) and thrombocytopenia (Plts = 14K/mm^3). ADAMTS-13 activity was reduced at <6% (Ref. 67-177). She had high plasma inhibitor levels >10.0 U (Ref. <0.4). TTP was suspected. Her father died from TTP 9 years before. Daily plasma volume exchanges (PE) and prednisone (1 mg/kg/day) were initiated but she did not respond as quickly as expected. On hospital day 13, weekly vincristine was added. She was discharged on prednisone (40 mg/day) after 5 weeks. During the last week of hospitalization her platelets were >300K/mm^3 but within 10 days decreased to 79K/mm^3. She was readmitted and her prednisone increased to 80 mg/day. She was transfused with 2 U of cryoprecipitate and given a 3-day course of IVIG at 1 gm/kg/day. On day 2 after IVIG, her platelet count was 19K/mm^3. Her latest platelet count 3 months from IVIG and off of steroids is 259K/mm^3.

Conclusion: The response of this patient with relapsed TTP to IVIG suggests that its use may be an effective supplement to current, more standard therapies of PE and corticosteroids for some patients.

P12.2 Intravenous immunoglobulin (IVIG) utilization in tertiary care hospital
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IVIG utilization should be monitored because of increasing IVIG consumption and cost, as well as evidence of inappropriate use and risks to patients.

Aim: To evaluate utilization of IVIG in tertiary care hospital and estimate whether IVIG were used for approved clinical indications.

Material and methods: We retrospectively analyzed use of IVIG in Clinical Hospital Center Zagreb from 1997 to 2002. Medical conditions treated with IVIG were categorized into three categories: i) convincing evidence of benefit, ii) inconclusive evidence of benefit and iii) no evidence of benefit.

Results: Annual use of IVIG increased from 5772 gr in 1997 to 12801 gr in 2002, although number of patients treated with IVIG remained the same (173 patients in 1997 vs. 173 patients in 2002). Significantly higher number of neonates were treated with IVIG in 1997 than in 2002 (36% vs. 4%). In 1997 the most common medical condition treated with IVIG was sepsis (54%), but in 2002 that were neurological diseases (32%). Although IVIG use for approved medical conditions was higher in 2002 than in 1997 (17% vs. 41%), there was still high proportion (39%) of patients treated for medical conditions without evidence-based benefit of IVIG therapy.

Conclusion: The optimum IVIG utilization management require publishing of guidelines and media containing IVIG can produce purest DCs. According to CD1a expression, AP1 produces most DCs.

P15 Extracorporeal photochemotherapy in graft vs. host disease
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Extracorporeal photochemotherapy (ECP) seems to be an effective treatment for some life-threatening T-cell disorders. Nevertheless, its mechanism is still uncertain and the optimal procedure has not been determined yet. We reviewed the records of 155 ECPs performed on seven patients with acute (four patients) and chronic (three patients) GVHD. We used a two-step protocol: mononuclear cell (MNC) concentrate by Cobe Spectra and Haemospectra MCS+, plus ex vivo 8-MOP / 1 J/cm² UV irradiation by PUVA COMBI LIGHT device hand irradiator. The mean procedure time was 3 h, and two blood volumes were processed. The table shows the number of cell collected (mean) and their ratio to the pts whole blood (WB):

| Tot 8 | Tot 9 | Tot 10 | WBC | Mono | Lymph | % WB | % WB |
|-------|-------|--------|-----|------|-------|------|------|
| 1 aGVHD | 2.15 | 0.5 | 1.3 | 8 | 68 | 45 |
| 2 aGVHD | 4.2 | 0.6 | 3.2 | 38 | 131 | 84 |
| 3 aGVHD | 2.5 | 0.6 | 1.5 | 13 | 88 | 83 |
| 4 aGVHD | 1.9 | 0.7 | 0.8 | 12 | 87 | 85 |
| 1 cGVHD | 9.6 | 0.8 | 8.2 | 48 | 97 | 60 |
| 2 cGVHD | 18 | 5.5 | 12 | 39 | 109 | 133 |
| 3 cGVHD | 6.7 | 1.1 | 4.7 | 34 | 102 | 92 |

All aGVHD pts had a good response, cGVHD patients showed either partial (two patients) or no response (one patient). We compared our data with those of others who performed ECP with different devices treating 5–10% of whole blood lymphocytes or even less. Since such differences have no impact on the clinical outcome, we conclude that under these conditions there is no evidence of a dose-response effect in ECP. Thus, the following questions should be addressed: (i) What is the cell-number threshold to get clinical efficacy? (ii) Could a less-challenging procedure render more patients eligible for ECP?
Results: No relevant side-effects, no increase in infection rate and transfusion demand were observed.

| Patients          | Adult patients | Pediatric patients |
|------------------|----------------|-------------------|
|                  | cGVHD          | atGVHD            | eGVHD          |
| Patients         | 51             | 22                | 20             |
| Median age (years) | 39 (18–58) | 85 (3–17)         | 12.8 (6–19)    |
| Median weight (kg) | 68 (48–98) | 28 (9–55)         | 42 (16–94)     |
| Sex              | 36 M; 15 F  | 13 M; 9 F         | 12 M; 8 F      |
| Overall GVHD     | 25 extensive  | 6 (IV)            | 12 extensive   |
| grade            | 26 limited    | 14 (III)          | 8 limited      |

Conclusions: ECP confirmed to be a valid second line treatment in drug resistant GvHD, permitting at the same time to reduce or suspend the IST with a real impact even on quality of life. The “off line” treatment we adopted permits to treat highly com-

P 15.4

Prenatal high-dose intravenous immunoglobulin (HDIVIG) in severe Rh D alloimmunization

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Rh immunization is the most common form of severe haemolytic disease of the newborn (HDN). The introduction of prophylaxis with anti-D Rh immunoglobulin has resulted in a marked reduction in the sensitization of Rh-negative women and deaths attributable to Rh HDN. Intravenous immunoglobulin given to alloimmunized pregnant women may decrease the severity of fetal haemolysis and maternal antibody titters. We present three severe Rh D sensitized pregnant women treated with HDIVIG. All patients presents multiple obstetric history, rising in severity with time, of previous Rh isoimmunization, fetal deaths and miscarriages. Their anti-D titers at the beginning of treatment were 64, 1034 and 2048. The IgG1 subclass was predominant in all cases, but in one a minor contribution of IgG3 subclass was found. These women were treated with HDIVIG (2 g/kg/day for two consecutive days) every 3 weeks from 13th week of gestation. The course of the disease in two of three patients was less severe than anticipated, reverting the tendency of previous pregnancies. The babies were born with 33.5 and 36 weeks’ gestations, requiring exchange transfusion and neonatal care with good evolution. Transfusion in utero was indicated in the refractory case and the fetus died as a result of the process with 25.5 weeks’ gestation. We thought that the HDIVIG treatment may have modified the severity of the disease and appear to be useful in treatment of prenatal severe Rh disease.

P 17.1

In vitro materno–fetal transfer of native and Fc-mutated recombinant RhD antibodies

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Several therapeutic approaches have been investigated to ameliorate hemolytic disease of the newborn. Ifc-mediated effector cell destruction of fetal red cells is central to the process. Through site-directed mutagenesis, the Fc region of monoclonal anti-D (Fog1G1) has been modified (Fog1G1 A nab) to abrogate FcRI, II and III binding, and leaving binding to the placental transport receptor FeRn intact. Placental transfer capability of both wild type and the Fc-mutant anti-D was investigated using a dually perfused, isolated human placental model. After 1 h of endogenous IgG washout, 5 mg of antibody was added to the maternal circuit. Hourly samples from fetal and maternal circuits over 5 h of closed-circuit perfusion were quantified for anti-D concentration by indirect flow cytometry. Transport fractions were calculated from the ratio of fetal to maternal anti-D concentration, expressed as a percentage (%Tf). Both native and wild type anti-D crossed the placenta. After 5 h, the %Tf for Fog1G1 was 0.16 ± 0.17 and for Fog1G1 A nab was 0.06 ± 0.02 (n = 8, mean ± SEM, respectively). The hourly increase in Tf was significantly lower for Fog1G1 A nab (P < 0.005, two-way ANOVA). The Fc-
mutated anti-D retains the ability to cross the placenta but with slower kinetics than the wild type anti-D, suggesting that transport mechanisms in addition to FeRn binding are involved. Duncan B et al. (1996) Reproduction, Fertility & Development, 7, 1547–1550.

P 17.2

Integrin receptors specificity of red cell ICAM-4. Critical residues for α1β3/3 and αβ3/3 binding

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The ICAM-4 protein binds different members of the β2 integrin family, but binding to α2β1 has been controversial. To better define the receptors specificity of ICAM-4, adhesion assays were carried out with L or CHO transfectants expressing different integrins individually, and with a leucocyte adhesion deficient (LAD) cells. The results confirmed that α5β1, α5β2, α4β1 and α4β2 (activated) transfectants bind specifically and dose dependently to ICAM-4. Interestingly, ICAM-4 also specifically interacted with L cells expressing α4β1 (vitronectin receptor). However, α2β1, L cells and β1, β2-defective LAD cells did not bind to ICAM-4 even after phorbol ester activation or stimulation by mAb Ts2/16 under which conditions they bind to VCAM-1 and fibronectin. ICAM-4 residues critical for interaction with α5β2 and α5β2 were identified. Domain deletion, mutagenesis and peptide inhibition indicated that β1 binding sites encompassed the D1 and D2 domains. The domains-binding site was located on the ABED face of D1 with an extension in the C’E loop of D2, whereas the distinct but adjacent α2β1 binding site spanned the two faces ABED and C’F of D1, including an extension in the CE loop of D2. Comparative analysis indicated that different integrins bind to different but partly overlapping sites on ICAM-4. Thus, ICAM-4 may accommodate multiple integrin receptors present on leukocytes, platelets and endothelial cells, with biological relevance in situations where such interactions occur.

P 17.3

Transient loss of Cromer antigens and anti-IFC in a patient with chronic lymphatic leukaemia

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Transient loss of Cromer antigens and anti-IFC in a patient with chronic lymphatic leukaemia

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The Cromer (Cra) system is a group system. Cromer antigens reside on the CD55 glycoprotein, decay accelerating factor (DAF). Five IFC-propositi are described, three of which have a single-nucleotide
substitution in the CD55 gene. We describe a case of transient loss of Cramer antigens and anti-IFC.

Case study: A 78-year-old untransfused female (MJ) with chronic lymphatic leukaem-ia had a history of three pregnancies. She was hospitalized 2 weeks postcesation of treatment with the cytotoxic drug fludarabin. Over a 4-week period her white cell count rose from 4.2 to 17.5 x 10^9 and an antibody to a high incidence antigen was detected in her serum.

Results: MJ cells were negative for the Cramer antigens Cr^a, Dr^a, Te^a, IFC and with monoclonal anti-DAF and anti-IFC was identified in her serum. One month later (white cell count 11.4 x 10^9) the patient’s antibody had diminished in strength, however her cells were still negative for Cr^a, Dr^a, Te^a and IFC. Three months later the antibody was no longer detectable and her cells expressed Cramer antigens.

Discussion: Weakening or loss of red cell antigens and concomitant antibody produc-
tion is described in several blood group systems. However, only one previous case of loss of Cramer antigens and anti-IFC is known; the patient had splenic infarctions. It is not known if the transient loss in the case we describe here is associated with her leukaemia.

P 17.4

Two new cases of anti-ABTI showing an association between ABTI and Vel

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Background: ABTI is a high frequency antigen reported in one inbred Israeli Arab family, three members of which have anti-ABTI. We describe the fourth and fifth examples of anti-ABTI, found in unrelated German females. A serological association between ABTI and the high incidence Vel antigen is known. Neither ABTI or Vel are associated to a red cell membrane protein and the molecular bases are unknown.

Case studies/results: Case 1: a previously transfused 69-year-old Bavarian female (MH) was found to be ABTI negative with anti-ABTI in her serum reacting by IAT. MH red cells had weak expression of Vel and eight examples of Vel-negative cells were strongly positive with her serum. Four family members were positive for ABTI and VelCase 2: A 74-year-old female from Berlin (AH) with carcinoma and unknown transfusion history, was found to be ABTI-negative with anti-ABTI in her serum reacting strongly by IAT. Five examples of Vel-negative cells were only weakly posi-
tive with her serum.

Discussion: We describe two new examples of the ABTI-negative phenotype and anti-
ABTI in unrelated females of German origin, the only ABTI negatives to be found outside the original Israeli Arab family. Both cases showed an association between ABTI and Vel. ABTI and Vel have similar serological characteristics in being resistant to proteolytic enzyme treatment. It remains to be seen whether the antigens are found to be on the same membrane protein and become part of the same blood group system.

P 17.5

The band 3 macro-complex, a role in red cell CO2/O2 gas exchange?

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Background: Proteins of the band 3 anion exchanger (AE1) complex (band 3, ankyrin, protein 4.2, glycoporphin A) are reduced or absent in band 3-deficient red cells. Previous studies suggest that the band 3 complex associates with the Rh complex [Rh-associated glycoprotein (RhAG), Rh polypeptides, CD47, glycoporphin B, LW]. We studied protein 4.2 deficient human red cells and band 3-deficient mouse and human red cells to further define this association.

Methods: The relative amount of red cell membrane proteins in the variant red cells was assessed by SDS-PAGE, Western blotting and flow cytometric measurement. Proteins were co-immunoprecipitated with band 3 from deoxycholate-solubilized membranes.

Results: CD47 was markedly reduced in protein 4.2 deficient red cells and RhAG migrated with a higher apparent MW on SDS-PAGE. All the proteins of the Rh complex were reduced or absent in band 3-deficient red cells. Mouse band 3 (−/−) red cells differed from human band 3-deficient red cells in that they retained CD47. RhAG and Rh were efficiently co-immunoprecipitated with band 3 from deoxycholate-solubilized membranes.

Discussion: The results suggest that band 3 forms the core of a macrocomplex of integral and peripheral red cell membrane proteins. The presence of these proteins in a single structural macrocomplex suggests that they have linked functional or regulatory roles. We speculate that this macrocomplex may function as an integrated CO2/O2 gas exchange unit in the erythrocyte.

P 17.6

Comparison of antibody identification results by resolven 3 AbID software and manual methodology

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Introduction: The most critical aspect of antibody identification (AbID) is the evalu-
ation and interpretation of data to correctly identify blood group antibodies. The process is complex, requiring application of multiple processes for a conclusive identification. Automated testing and electronic evaluation can achieve enhanced safety of AbID. A computer-assisted (CA) AbID software program, a decision-making tool, was compared with manual (M) AbID.

Method: A total of 94 samples with blood group antibodies of single and multiple specificities of varying reactivity were evaluated comparing the MAAbD process to a CAAbID software [Resolven 3 (R3)]. All samples were tested at AHB using the Ortho BioVue System and AbID panels (0.8% Resolven Panel A and Panel B). Both processes were used to evaluate each sample’s AbID test results.

Results: Of the 94 samples evaluated 77 had a single Ab specificity and 17 more than one. Ninety-three demonstrated agreement with both processes. Of 11 with inconsistent reactivity, the CAAbID provided a suggested probable specificity matching the MAAbD process. The interpretation suggested by R3 and by MAAbD in the one discrepant sample was resolved by antigen typing.

Conclusion: This study shows the high correlation of R3 with MAAbD. The routine use of R3 with a validation process provides an improved process for AbID, in compliance with local regulations. CAAbID interpretation of results for AbID represents an important tool for decision making and enhancing safety.

P 17.7

Using six Sigma tools to evaluate an automated system for IH testing for error reduction potential

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Introduction: Enhanced safety in transfusion service operations means reducing errors or the potential for errors. Test standardization and process controls through auto-
mation can help decrease the potential for error. This study compared a fully auto-
mated system [AutoVue® Innovia] (AVIN) to both manual BioVue® [MB] and tube (MT) tests, for a commonly used group and screen (G&S) European profile. Six Sigma tools, notably process mapping (PM) and failure modes and effects analysis (FMEA), were incorporated in the evaluation.

Methods: PMs with process inputs, steps and outputs were documented. Using the PM, a FMEA was developed for each test process. FMEAs identified potential failure modes and defect opportunities at each step. Potential failure modes were rated by assigning a numerical value for severity of the failure effect, frequency of occurrence, and po-
tential for detection. These three values were multiplied to produce a risk priority number (RPN) for each process step. A total RPN value was calculated for each test method. A critical RPN was determined to be any RPN value >200.

Results:

Table 1. Comparison of MT, MB and AVIN for G and S

| Method     | No. of process steps | No. of defect opport. | Total RPN | Critical RPN |
|------------|----------------------|-----------------------|-----------|--------------|
| MT         | 42                   | 317                   | 15 072    | 14 372       |
| MB         | 10                   | 89                    | 3670      | 3470         |
| AVIN       | 2                    | 16                    | 26        | 0            |

Conclusion: Based on the quality tools used in the analysis, the AutoVue Innova substantially reduces operator intervention and process steps, thereby helping to eliminate more defect opportunities and error potential.

P 17.8

New reagents for red cell antibody identification: transfected cells expressing blood group antigens

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Up to date, red cell antibody identification requires reagent cell panels of human RBCs. These cells have limited viability and may carry potential biohazard risks.

Objective: To obtain cell lines stably expressing high levels of the blood group anti-
gens Dia or Jka and to evaluate their use as reagents for the identification of these specificities in human sera.

Methods: The Jka cDNA was obtained by RT-PCR and the Band 3 cDNA was modified by site directed mutagenesis to obtain the low incidence Dia coding sequence. cDNAs
were subcloned into the pSF91retroviral vector and introduced in two erythroleukemia cell lines (K562 and MEI). Dia and Jka expression was analysed by flow cytometry using MoAbs. Clones of stably transfected cells were also tested with known human sera.

Results: We have obtained transfected cell lines expressing high levels of Dia or Jka blood group antigens. Antigen expression measured as the MFI compared with the wild type negative control was significantly higher than the MEI obtained with RBCs. The clones cultured for several months maintain the expression levels, and so they do after freezing/thawing. We have also demonstrated that these transfected cells are capable of detecting the corresponding antibody specificities in human sera.

Conclusions: Expression of antigens in transfected cells may solve the limited availability of human RBCs expressing low-incidence antigens as well as other drawbacks of using red cell panels.

P 17.9
Quantification of monoclonal anti-D in placental perfusate using flow cytometry and autoanalyser
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To allow calculation of materno–fetal transfer of wild type and Fc-mutated human monoclonal RhD antibodies (Fog:1G1 and Fog:1G1.4 nab, respectively), we determined if flow cytometry (FC) or Autoanalyser could be used for anti-D quantification in perfusates from the human placental perfusion model. Serial dilutions in Isoton (1–500 ng/ml) of both antibodies provided calibration curves for FC anti-D measurement. These were used to calculate antibody concentrations in maternal and fetal perfusate samples and compared against data generated by Autoanalyser. FC calibration curves were almost identical for both wild type and mutated versions of the anti-Ds. Fresh batches of either antibody produced hyperbolic curves (r² = 0.99) but on storage at −40 °C (>1 year) these changed to sigmoidal curves (r² < 0.99), indicating reduced sensitivity of detection at lower concentrations. The lower limit of detection for flow cytometry was 1.0 ng/ml using fresh antibodies, but only 4.5 ng/ml by Autoanalyser. For maternal samples with anti-D concentrations between 15 and 30 mg/ml, Autoanalyser results more closely reflected the IgG concentrations added, while flow cytometry values were three to four times higher than expected. We conclude that flow cytometry can be employed in the quantitation of low anti-D concentrations in fetal perfusate samples. Furthermore quantification by FC of the Fog:1G1.4 nab antibody was the same as the native determinant despite modification of the Fc region.

P 17.10
Recessive Lunull phenotype: a new example and a new mutation
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The Lutheran null phenotype, Lu null or Lu(a−/−), can arise from three genetic backgrounds: autosomal recessive (Lu/a−); dominant suppressor gene [Lu(Lu)]; recessive X-linked suppressor gene (Lu/-). We studied an example of rare recessive Lu(a−) phenotype in an untransfused female of Caucasian origin who had been pregnant and was admitted to hospital for cholecystectomy. In serological tests the patient’s red cells were Lu(a−/−) and negative for Lu1, Lu4, Lu6 and were Anw+V. Her serum contained anti-Lu3 reacting by the anti-globulin technique. All examples of Lu(a−/−) cells of dominant or recessive type were negative with her serum. DNA was isolated from the blood of the patient. PCR amplification and direct sequencing of all 15 exons of LU revealed a homozygous C161T mutation in exon 3, which introduced a premature stop codon 361TGA, causing an Arg121STOP change in the first immunoglobulin superfamily extracellular domain of the Lu-glycoprotein (Lu-gg). This mutation differs from two others previously found to be responsible for recessive Lu(a−) phenotype. Lu-gg binds the extracellular matrix glycoprotein laminin, though its precise function is not known. Homozygosity for a nonsense mutation in LU suggests that this patient has Lu−/− in none of her tissues and at no stages of development.

P 17.11
The lectin from Glechoma hederacea is Tn-antigen specific
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Leaves of ground ivy (Glechoma hederacea) contain a lectin (called Gleheld) that is structurally and evolutionarily related to classical legume lectins. It is found mainly in the leaves, where it acts as a potent insecticidal protein for larvae of the Colorado potato beetle (Leptinotarsa decemlineata). This activity may be associated with the carbohydrate-binding specificity of the lectin. The aim of the work was to investigate whether this activity was also demonstrated against human red blood cells. Standard tube haemagglutination tests with both normal and variant cells were used. Serial dilutions of the lectin exhibited titres of 1:1000 against normal group O, A1, A2 and B red cells, 1:40 000–1:155 000 against four different examples of Tn polyagglutinatable red cells, 1:600–1:40 000 against CaeD cells (three examples), 0–1:600 against Sda+++ cells (eight examples), 1:2000 against a single T + Tk cell, 1:400 against a T cell and 1:5000 against a Tb cell. These reactions were compared with those of a monoclonal anti-Tn antibody, which showed no cross reactivity. Papain modification of Tn cells resulted in reduction from a titre of 1:40 000 to 1:8 in one case and to 1:4000 in another. Neuraminidase treatment of one example of CaeD resulted in an increase in titre from 1:600 to 1:5000. The results of haemagglutination studies with variant red blood cells suggest that the lectin has a preference for reaction with internal GalNAc alpha-0-linked to ser/thr.

P 17.12
Autoimmune hemolytic anemia after nonidentical T cell-depleted allogeneic BMT in patients with SCID
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Immune-mediated hemolytic anemia is a well-recognized complication after allogeneic stem-cell transplantation (SCT). Autoimmune hemolytic anemia (AIHA) has been described with increased frequency in patients (pts) undergoing SCT, particularly in those undergoing non identical T cell-depleted (NI-TCD) SCT, presumably due to immune dysregulation. We report three pediatric pts who developed AIHA following NI-TCD allogeneic BMT. These pts were identified from a total of 13 with severe combined immunodeficiency disease (SCID) who received TCD grafts between 1996 and 2003. They were classified as having AIHA if they met the following criteria: positive DAT and clinical evidence of significant hemolytic anemia. Red cell alloimmunization was previously excluded.

Results: Three of 13 pts (23%) transplanted with NI-TCD marrow grafts developed AIHA. The onset of AIHA was between 6 and 9 months post-SCT. Two pts had warm reacting autoantibodies and one had concurrent cold and warm antibodies, with no apparent specificity. Two pts had complete remission from AIHA (they were treated with RBC Tx, steroids, IVIG and/or monoclonal anti-CD59). One died for causes other than AIHA (multorgan failure).

Conclusion: We found a high incidence (23%) of AIHA in 13 pts with SCID who underwent a NI-TCD allogeneic SCT. Early antibody classification may improve clinical treatment of the AIHA.

P 17.13
Use of hydroxyethylamide for K1 phenotyping in a PK 7200 Olympus Equipment
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Background: K1 is one of the most immunogenic red blood cell antigens. The selection of negative K1 red blood cell units is indicated for sensitized patients with anti-K1 and for those in chronic transfusion regimen, among others. The objective of this study was to evaluate the use of hydroxyethylamide for K1 phenotyping in automated equipment.

Methods: A total of 5013 samples of blood donors collected in EDTA were phenotyped for K1 antigen using a PK7200 Olympus Equipment (Olympus America Inc, USA). Twenty-five microliters of 1.7% red blood cell suspension in bromelain solution of each blood sample was dispensed on special microplates to which was added 25 µl of commercial anti-K1 diluted in hydroxyethylamide 1:64. After homogenization and 1 h incubation at 30 °C the reading was performed automatically by a CCD camera. As control, it was used 20 samples obtained from blood donors with positive and negative K1 phenotyping in each batch. All positive and inconclusive results were tested for K1 phenotyping using conventional tube test technique (CTT).

Results: We found only 4 (0.08%) inconclusive results hydroxyethylamide. All of them showed to be negative when tested by CTT. A total of 216 samples (4.3%) showed positive results for K1 phenotyping by both techniques. We found no false-negative results.

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P 17.14

Immunogenicity of D VI: less immunogenic than C and G in a case of fetomaternal immunization

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Objective: There is little information on the immunogenicity of D variants.

Case report: A 30-year-old caucasian woman, AB RhD (-ccdeee) had three pregnancies by the same caucasian husband. She received one TU of AB RhD- (-ccdee); no other immunization events or autoimmunity diseases. In the first pregnancy she was given anti-D after amnioncensis. Husband at first grouped as 0 RhD-was found to carry D VI type 2 with low number of D sites (C in trans- CCFvle). First babies were typed RhD-, later testing confirmed presence of weak RhD. In the third pregnancy (10th week) a suspect weak anti-D and in 16th week anti-C were identified. The titers were rising to 1:32 after delivery. A cordocentesis (37th week): 3+ DAT (titre 1:30 1+ and no IgG1 nor IgG3 (DiaMed DAT Cards)). The fetus was not anemic and had no other pathology. A healthy child was delivered in 41st week with a 3+ DAT (titre 1:100 1+ and weak IgG1 (1:1 1+) and no IgG3), there was a borderline bilirubin level but no need for therapy.

Serologic testing: The apparent anti-D anti-C reactions were in fact due to presence of anti-G and anti-C as confirmed by absorption and elution study with D-C+ and D-C- RBCs. No anti-D reactivity remained after absorption with D-C+ RBCs.

Conclusion: This case and lack of any report of maternal anti-D immunization by D VI suggest that D VI is less immunogenic than C and G antigens, probably due to lacking epD6/7 and other parts of D protein with higher immunogenic potential.

P 17.15

A novel high prevalence antigen in the Scania blood group system

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Background: Over 20 years ago, a proband was described whose RBCs typed Sc1,-,2,3. His serum contained an IgG alloantibody that reacted by the indirect antiglobulin test with all red blood cells tested except those with the Sc1,-,2 phenotype, his own, and those from his brother (Skradski, et al., Transfusion 1982; 22: 406; Devine, et al., Transfusion 1988; 28: 346). The purpose of this study was to determine the molecular basis associated with this novel high prevalence antigen.

Methods: Samples from frozen storage were obtained from the antigen-negative proband and his brother. DNA was extracted using a QIAmp Blood kit. Primers were designed to amplify SC ERMAP exons 2, 3, 4 and their flanking regions. The amplified products were sequenced in both directions using an ABI373XL sequencer.

Results: A single nucleotide mutation was detected (119G>A) in exon 3 of SC (ERMAP) that is predicted to encode a change of amino acid 47 from glutamic acid to lysine. This mutation did not introduce a restriction enzyme site.

Conclusion: It is likely that lytic in position 47 of the Sc glycoprotein is associated with the absence of the high prevalence antigen detected by the proband’s antibody. This amino acid change is located on the extracellular portion of HERMAP, 10 residues from the polymorphism associated with Sc1 and Sc2 [Gly57Arg]. This novel high prevalence antigen expands the Sc blood group system to five antigens.

P 17.16

Capillary centrifugation for blood grouping with distinct areas for positive and negative reactions

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Background: Gel techniques are well established diagnostics in blood group serology. Ease of reading of the results is one of their key strengths. However, weak-positive results are sometimes difficult to discern from negatives.

Aim: To develop an agglutination technique with distinct areas for positive and negative reactions.

Material: A plastic chip, similar in size with an ID-Card (DiaMed), containing an intrinsic micropapillary system instead of the gel matrix, was constructed with the following top to bottom design: (i) reaction chamber; (ii) reagent chamber with profiled reagents; (iii) capillary system; (iv) flash-sized chamber. The capillary zone and the flash-sized chamber are the areas of positive and negative reactions, respectively.

Method: Diluted whole blood (10 µl) are pipetted into the reaction chamber of a chip carrying reagent channels filled with anti-A or anti-B. The chip is centrifuged in an ID-CentriFlo. Positive and negative results are recognized as haemagglutinates that are retained within the capillaries or as a button of red cells in the negative chamber, respectively.

Results: A total of 100 patient bloods have been tested in a prototype card. All results were in agreement with the results received with a similar ID-Card (DiaClon ABD, DiaMed).

Discussion: This technique may overcome disadvantages of gel techniques i.e. interpretation of very weak-positive reactions and lot-to-lot variances. Further investigations including reverse typing and IAT are underway.

P 17.17

Lateral flow assay for simultaneous typing of ABO, Rhesus subgroups and Kell

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Background: Current rapid methods for blood typing do not have stable end-points. Sophisticated techniques, which provide more objective and stable results, are rather slow and need a centrifugation step. Both have in common that only one parameter at once can be determined.

Aim: To develop a blood grouping format with multi-parameter testing and stable end-point, but without centrifugation.

Material: A lateral flow device was constructed with a separation membrane equipped in a cassette housing having a central application zone and two equidistant detection areas printed with parallel lines of antibody reagents directed against blood groups A, B, AB, D and C, Cw, c, E, e, K, respectively.

Method: Diluted blood (200 µl) are pipetted into the application zone, followed of 200 µl of a washing buffer. Results can be read after 5 min. Positive results are recognized as distinct red bands, negative results are monitored by the absence of the respective bands.

Results: The bloods of 865 donors, previously typed for the respective blood groups with the Olympus PK-80, have been tested with the new lateral flow test. The results for all antigens were in agreement with those of PK-80.

Discussion: A simple, rapid and flexible method for blood typing with stable end-point is presented, allowing for: (i) miniaturization; (ii) parallel testing of multiple blood groups in the same assay and (iii) use in nonlaboratory situations. Further evaluations are currently underway.
Results: BFU-E and CFU-E colonies were inhibited significantly by polyclonal IgG anti-K and anti-k, and by monoclonal IgM anti-K relative to control cultures containing conditioned media only (Student's t-test, P < 0.005). Also, the suppressive effect was observed in anti-K and anti-k cultures containing excess soluble IgG (5 mg/ml final) when compared with control cultures with and without soluble IgG (P < 0.005).

Conclusion: Antibodies against the Kell glycoprotein suppress the proliferation of BFU-E and CFU-E colonies. Furthermore, suppression by IgM anti-K and in the presence of soluble IgG suggests that Fc-

P 17.19
A case of DAT-negative haemolysis and transient loss of an Rh-related antigen
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A 9-month-old male presented with a 2-week history of jaundice and acute onset of dark red urine. There was no history of infection. He had mild developmental delay, dysmorphic changes of the head and a 2 cm splenomegaly. HB was 60 g/l, reticuloocytes raised at 422·10^11/l and raised LDH. WBC and platelet count was normal. There was no history of transfusion. A strong antibody was detected in the patient's serum reacting with all untreated and papain treated panel cells. The DAT was normal. Four examples of -e/-e (Rh-17) cells were compatible with his serum but his cells typed normally for Rh 17. A panel of anti-e sera revealed aberrant expression and he typed as hr^-. The presence of anti-hr^- was not confirmed as two examples of hr^- cells were incompatible. Absorption studies suggested there were no additional antibodies. The patient received folic acid but was not transfused. His HB gradually rose with persisting haemolysis but this ceased after 7 months and his HB became normal. The patient was investigated 2 years later with no evidence of further haemolysis. At this time he had normal expression of hr^-, Rh 17 and e with no atypical antibodies. Both parents who were not consanguineous had normal expression of these antigens. Transient suppression of hr^- has been previously described [1]. Whether antigen loss occurs initially with later emergence of apparently alloreactive antibodies or is due to autoantibody action is unknown. [1] Transfusion 1991; 31: 254–256.

P 17.20
Serum or plasma: the debate continues!
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We report on the antenatal management of a patient with anti-Vel. The patient had two previous uncomplicated pregnancies. Samples referred to the WBS during the third pregnancy were found to be antibody screen positive. Manual testing using serum samples revealed an antibody to a high frequency antigen detectable by papain and LISS AGT tests. The antibody was subsequently confirmed as anti-Vel with no further red cell antibodies detectable. When using the plasma sample the anti-Vel was not detectable in a LISS AGT, but was detected by a two-stage EDTA method. Titration of the serum sample was performed, the anti-Vel had a LISS titre of 1:4, DTT treatment of the patient's serum resulted in the destruction of the anti-Vel activity. A chemiluminescence test performed using the patient's serum sample was negative (Opsonic Index < 1.2). Our findings confirmed the presence of an IgM anti-Vel, which as was anticipated, did not cause complications during the pregnancy. The mother delivered at 40 weeks gestation, the cord haemoglobin was 21.9 g dl^-1 and the DAT was negative. IgM anti-Vel, although not associated with HDN is implicated in haemolytic transfusion reactions and would have caused problems if the antibody had remained undetected and postdelivery transfusions were required. The case serves to demonstrate the advantages of using clotted samples particularly for the identification of antibodies whose detection relies on their ability to activate complement.

P 17.21
A novel XK mutation in McLeod syndrome associated with severe psychiatric symptoms
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McLeod syndrome (MLS) is characterised by: weakened antigens of the Kell blood group system and absence of red cell Kx; acanthocytic red cells; muscular, neurological and psychiatric symptoms in middle age. It results from hemizygosity for deletion of, or inactivating mutations in, the X-linked gene XK, which encodes the Kx antigen. A 50-year-old man was referred to psychiatric services because of impulsive and disinhibited behaviour. Further examination revealed an inappropriate jocular manner, choreothetosis, borderline wasting of lower legs and areflexia. CT scan of the brain showed caudate atrophy. Creatine kinase was elevated at 1130 U/ml and acanthocytosis were present. His behavioural disturbance progressed, requiring compulsory detention under the mental health act. The red cells of the patient were K^- k^-w Kp(a^- b^+) and Kx^--. The degree of weakening of k and Kp^- was comparable with McLeod phenotype control cells. Amplification and direct sequencing of the 3 exons and flanking intronic sequence of XK revealed one mutation, deletion of 172G within exon 1. This unique mutation among the plethora associated with McLeod syndrome introduces a reading frameshift after the codon for Phe57 (GTA>TAC) and subsequent premature termination of translation at codon 129 (exon 2). This predicts a very truncated protein, which would probably not be inserted into the membrane and could not be linked to the Kell glycoprotein, causing weakened expression of Kell antigens.

P 17.22
Another example of Inab phenotype in Japanese and production of human monoclonal anti-DAF
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Background: Antigens of the Cromer blood group system reside on the decay-accelerating factor (DAF). The Inab phenotype is the null type of this system. So far, only five propositi have been reported who exhibit this phenotype. This report describes another example of Inab phenotype in Japanese and production of monoclonal anti-DAF by EB (Epstein-Barr) virus infection.
Methods: Sequence analysis were performed on cDNA prepared from the patient's blood. In order to epitope map, Chinese hamster ovary stable transfecants expressing DAF or mutants lacking specific DAF SCR's (short consensus repeats) were screened by flow cytomtery with the human monoclonal anti-DAF produced by EB virus transfection.
Results: Sequence analysis revealed a point mutation of 263C<A, which creates a cryptic splice site in exon 2. This results in a 26 bp deletion and alters the open reading frame. As a consequence, Ser54 is changed to a stop codon. The monoclonal anti-DAF (IgG4) was detected by antiglobulin methods with a titer of 4096, and reacted all red cells tested except for Inab red cells. Screening of DAF deletion mutants without the monoclonal antibody verified that the antigenic determinant was located within SCR1. Conclusion: In molecular basis of Inab, two different types, proband Inaba and proband HA, have been identified. The Inab phenotype propositus described here, who has the same mutation as HA type. The antigen recognized by the human monoclonal anti-DAF was identified in SCR1 of DAF.

P 17.23
A separate genetic origin of the partial D antigen of category DiVs in Japanese
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Background: In Blacks, the partial D antigen of category DiVs is encoded by an RH-CE-D-CE-D gene in which part of exon 3 and part of exon 7 of RH are replaced by the equivalent portion RHCE. In this study, the molecular basis of DiV in a Japanese individual is described.
Materials and methods: A Japanese propositus exhibiting the DiV phenotype was analysed by standard serological methods and molecular techniques. The RH transcripts of the propositus were sequenced and compared with those of normal donors. Results: The propositus typed D+CE-c+Et+E-c+Go(a+). D epitope mapping revealed the absence of D epitopes 1, 2, 3 and 9 and the presence of the other D epitopes. In sequence of the cDNA, a Japanese Inva were appeared to be encoded by an RH-CE-D(CE-D) hybrid. The complete primary structure of the 417 amino acid polypeptide differ from the normal D protein at position of Asp350His (GAT350CAT). In molecular basis of Inab, two different types, proband Inaba and proband HA, have been identified. The Inab phenotype propositus described here, who has the same mutation as HA type. The antigen recognized by the human monoclonal anti-DAF was identified in SCR1 of DAF.
P 17.24
Analyses of anti-Rhd, -RhC, -RhG, and -Rhe antibodies in primary immunized persons
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Several groups show that the Ig genes of anti-D antibodies (Ab), in hyperimmunized donors, are restricted to the IGHV-3-30 genes. We investigated the IGIV genes in anti-D Ab after primary immunization and in other anti-Rh Ab. Two-plateau display libraries (PDL) were made from two pregnant women who were primary immunized in their first pregnancy. Woman 1 (rr phenotype) had anti-D, -C and -G Ab and woman 2 (RJ2R phenotype) had anti-C and anti-e Ab. Anti-Rh phages from PDL1 were obtained after selection rounds with red cells of either the R1R1, R2R2 or r' phenotype. Anti-Rh phages from PDL 2 were obtained after selection rounds with red cells of the RJ2R or rr phenotype. Red cells with different Rh phenotypes were used to determine the anti-Rh specificity of the phages. IGIV genes of selected phages were sequenced. From PDL1, we selected 29 anti-D-, 11 anti-G- and 2 anti-C phages. After DNA-fingerprinting, sequences of 13 unique anti-D clones were analyzed. Ten clones had the IGIV-33 gene, two had the IGIV-31-30 gene and one had the IGIV-31-11 gene. Six of the 11 anti-G phages were sequenced. Three clones had the IGIV-69 gene and three clones had either the IGIV-33, IGIV-30 or IGIV-20-30 gene. Both anti-C pages had the IGIV-30 gene. From PDL-2, we selected two anti-e phages which had both the IGIV-30 gene. We found a restriction of IGIV genes in anti-D phages obtained from two primary immunized women. Furthermore, we have shown a similar restriction in phages with other anti-Rh-specificities.

P 17.25
A clonal anti-Rhd response in a hyperimmunized donor
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Several groups show that the Ig genes of anti-D antibodies, in hyperimmunized donors, are restricted to the IGIV-3-30 genes. We now describe an anti-D donor that even produced a clonal B-cell response against RhD. We made two different phage display libraries (both IgG1 and IgG3-based) of one hyperimmunized anti-D donor. Anti-Rhd-specific phages were selected from both libraries and IGIV genes were sequenced. Both libraries had heavy chains with the same IGIV-30-30 - IGIVD6-13 - IGIVKb rearrangement. These phages were identified as one group of clones that had different patterns of somatic mutations. Many different Rh-epitopes are recognized by these highly similar clones. All clones that differed in heavy chain also differed in light chain, which clearly excludes contamination of the libraries. There were no PCR artifacts because reactions were performed independently. The frequency of these clonal B-cells was 1:2000 as determined by limiting dilution PCR. We also determined the RhD-peptides to which the alloreactive CD4+ T cells in this donor respond and found only one stimulatory peptide. After 10 years the anti-D titer in this donor could not be raised by boosting anymore. We conclude that during long-term hyperimmunization clonally related B cells started to dominate the humoral response in this donor. Whether the induction of tolerance is due to the exhaustion of the clonally related B cells or to the limitations of the helper-T cells remains to be investigated.

P 17.26
Genomic blood group typing for quality assurance of reagent test RBC – do you know your cells?
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Background: To fulfill quality assurance (QA) standards, we rely on well validated reagents tlo phenotype reagent red blood cells (rRBCs). Prediction of antigen dose is based on the expected allele frequency; however dose of RhD cannot be determined serologically. Furthermore, FY typing can be misinterpreted due to FY'T0 and FY'X, which silence/weakens FY' expression. In some instances, e.g. in the Dombrock system (DO), reagents are rare and of poor quality. As part of QA, we used validated assays to determine RhD, FY and DO genotypes of rRBCs.

Materials and methods: Genomic DNA was isolated from our rRBC panels. Standard PCR techniques were used. RH bear analysis was performed on rRBCs samples (n = 16).

FY analysis was performed on Fya(a+b+) (n = 37) and Fya(a-b+) (n = 25) samples. DO genotyping was performed on 38 samples of unknown Do phenotype.

Results: Two of 36 (6%) Rhd+ samples were unexpectedly heterozygous for the RhD gene, i.e. R'D' and R'D' instead of R'D and R'D '. Six of 62 (10%) samples presumed homozygous for either FyaA or FyaB, were in fact heterozygous, with FY'A (n = 4) or FY'B (n = 2) alleles present. Genomic DO/AB typing gave clear, easily interpretable results.

Conclusion: Serologically determined RH and FY phenotypes were inaccurate in 8% of all typings evaluated. Furthermore, genotyping for DO has improved our knowledge of the rRBC antigen profile. Genomic analysis is a valuable tool to ensure that antibody screening rRBCs comply with international QA requirements.

P 17.27
Determination of the molecular background of the Caribbean antigen occurring with a weak C antigen
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The low incidence Caribbean antigen (RhD3) was first recognized because of reactivity of a D+ sample with a polyclonal anti-D serum. The antigen was found primarily in Blacks, and most were R2R2 (J.J. Moulds, P. Bitni, observations), although exceptions were found. Recently, Crawford was associated with a novel RHCE allele (c(eF)) that encodes W16C, Q233E, and L245V (Schlamer et al. Transfusion Suppl. 2003; 43, 34A). A donor’s RBCs, previously typed as D-negative, reacted with two monoclonal anti-D known to detect Crawford. The cells were (C+), c+, E–, E+ and reactive with anti-V/VVS.

Our aim was to determine the molecular basis for expression of weak C and for the Crawford antigen. DNA was isolated and RH transcripts were synthesized by RT-PCR and cloned. Twenty-four clones were characterized by restriction enzyme digestion, and 12 representative clones were sequenced. Genomic DNA was also tested. Three clones corresponded to the D-C-E-D hybrid found in the (C(eF) V/SV– haplotype in Blacks. This hybrid is associated with weak C expression, and explains the reactivity with C-.

Five clones were identical to the Crawford c(eF) allele, and four were conventional RHCE (c(e)). Genomic analysis indicated heterozygosity for RHD. These results confirm the molecular basis for the Crawford antigen and its association with rh observed in early studies. Importantly, they raise the possibility that the D-C-E-D hybrid may contribute to expression of D epitopes in this Crawford background.

P 19.1
Is there a relationship between anti-HPA-1a and severity of neonatal alloimmune thrombocytopenia? 
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Recent research into the relationship of anti-HPA-1a and severity of neonatal alloimmune thrombocytopenia (NAIT) has yielded conflicting results. In order to investigate this relationship further, we determined the amount of Ab in ab-positive HPA-1a using a quantitative ELISA (employing purified anti-HPA-1a as a standard curve) and, in selected cases, compared it with a commercial assay. Sixty-eight samples collected from the women at various stages of pregnancy were tested. Selected samples were also assayed by GTIpak12 G. A National HPA-1a ab standard (NIBSC 93/710), designated as one arbitrary unit/ml (AU/ml), was used in each ELISA to standardize the purified anti-HPA-1a from units of µg/ml to AU/ml. As such, the purified anti-HPA-1a standard was measured at 20 AU/ml with minimal standard deviation of ±0.7, indicating a high degree of consistency over the eight assays. The ab quantity was significantly correlated with the ab titre in the 68 samples studied (R = 0.57, P < 0.001). Furthermore, there was a significant correlation between GTIpK12 and the quantitative ELISA in a selected number of cases with or without NAIT (r = 0.76, n = 10; 0.001 < P < 0.01). On the other hand, there was no correlation of ab quantity with NAIT incidence (R = −0.046). Our study indicates that there is no relationship between anti-HPA-1a quantity or strength and severity of NAIT where ELISA is used, although the correlation between ELISA and other methods such as MAIPA remains to be determined.
P 19.2 Development and validation of a rapid monoclonal antibody immobilisation of platelet antigen assay
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Platelet immunoassays in England are provided by four NBS laboratories, each using the MAIPA test as its cornerstone technique for the detection of HPA antibodies (abs). However the actual MAIPA techniques vary considerably between laboratories. We therefore decided to develop a common standardised protocol with the aim of reducing assay time, but without losing sensitivity. A modified MAIPA assay taking only 5.5 h was developed. The assay was validated in each laboratory using 61 samples distributed ‘blind’, of which 58 contained antibodies detectable by other MAIPA assays. All the antibodies were detected by the new modified MAIPA, and included the following HPA specificities: HPA-1a (n = 21), HPA-1b (five), HPA-3a (three), HPA-5b (12), and single examples of anti-HPA-3b, –5a, –1a + 5b, –1b + 3b + 5b, –1a + 2b + 3a, –3b + 2b, and 3a + 5a. HLA class-I antibodies were detected in 32 samples, in 28 cases these were in combination with HPA abs. Glycoprotein reactive antibodies without HPA specificity were detected in six samples. Titration of the NIBSC anti-HPA-1a standard (93/710) increased to 1:64 with the new assay compared with 1:16 with the existing assays. We have therefore developed a MAIPA assay that reduces testing time by approximately 50% without decreasing sensitivity, and enables laboratories to meet national requirements for documentation and reagent standardization.

P 19.3 Improved detection of HPA-15 antibodies by selection of panel donors and capture monoclonal antibody
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The biallelic HPA-15 system (Gov) is carried by the protein, CD109. The incidence of HPA-15 alloantibodies is high, and only exceeded by HPA-1 alloantibodies. Detection of HPA-15 antibodies has therefore become important for improving patient care. HPA-15 antibody detection is problematic as CD109 is not present on recovered platelets and HPA-15 phenotyping by MAIPA suggests variability in platelet CD109 expression. Our aim was to improve detection of HPA-15 antibodies by optimizing both donor selection and choice of capture monoclonal antibody (MoAbs) used in the MAIPA test. The reactivity of MoAbs D2 with platelets of 100 random donors was determined using flow cytometry. Reactivity was weaker than seen with most other platelet proteins expressing HPA-1, but showed a near normal distribution, covering approximately a 3x increase in fluorescence. This is a wider distribution than found with other platelet antigens. In addition, increased CD109 expression was observed in ~2% of donors, and was associated with a 10x increase in CD109 mRNA levels. We tested 10 CD109 MoAbs (8A3, 8A1, TEA2/16, BE-47, 7C5, 7D1, D2, E063, C210, IS288) on 78 random donors and 61 samples, in 28 cases these were in combination with HPA abs. Glycoprotein reactive antibodies without HPA specificity were detected in six samples. Titration of the NIBSC anti-HPA-1a standard (93/710) increased to 1:64 with the new assay compared with 1:16 with the existing assays. We have therefore developed a MAIPA assay that reduces testing time by approximately 50% without decreasing sensitivity, and enables laboratories to meet national requirements for documentation and reagent standardization.

P 19.4 Early placental expression of platelet glycoproteins GPIIb and GPIIIa during pregnancy
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Background: Fetal and neonatal alloimmune thrombocytopenia (NAIT) due to anti-HPA-1a alloantibodies usually occurs in a first pregnancy. HPA-1a/1b is on GPIIIa (CD62, the β3 integrin subunit). β3 associates with αIIb (GPIIb, the α3 integrin subunit). αIIb/β3 associates with αV (CD51)/β3 (CD63). GPIIb/IIIa is important in platelet aggregation and adhesion to the subendothelium.

Aim: To determine if these molecules are expressed on placenta, in particular on the villous trophoblasts (VT) which are epithelial cells of fetal origin and are in direct contact with maternal blood.

Materials and methods: Cryostat sections of first trimester (10-11 weeks gestation, n = 3) and term (36-41 weeks gestation, n = 3) placenta were stained with monoclonal antibodies (mAbs) by immunocytochemistry. Binding of FITC-conjugated human anti-HPA-1a (2E10) was detected with a murine anti-FITC mAb.

Results: The strongest staining of VT by two anti-GPIIIa mAbs was on first trimester cytotrophoblast and the weakest on distal syncytiotrophoblast (differentiated VT) of term placentas. Mabs to αV or αV/β3 integrins stained VT in a similar manner to anti-β3 mAbs. In contrast, GPIIb was absent from placental tissues. Purified staining of maternal fibrinoid material was observed with anti-GPIIb and anti-GPIIIa but not anti-β3 or anti-αV/β3 mAbs. Anti-HPA-1a reacted with isolated villosus stromal cells of HPA-1a-positive term placentas.

Conclusion: The presence of GPIIIa on placental villous trophoblasts by 10 weeks of pregnancy may account for NAIT in first-born infants.

P 19.5 Thrombocytopenia induced by sodium valproate
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Background: Thrombocytopenia induced by specific drugs is not uncommon. Sodium valproate has been implicated in some cases of thrombocytopenia, usually related to antiplatelet autoantibodies. A case of thrombocytopenia in a 39-year-old man with cerebral palsy and epilepsy treated with sodium valproate and topiramate is reported.

Conclusions: The ELISA assay was positive in the presence of sodium valproate, but not in the presence of topiramate, with specificity for glycoprotein IIb/IIIa. After discontinuation of sodium valproate treatment, the number of platelets returned to normal values (190 000/μl).

Conclusion: It is possible to detect antiplatelet autoantibodies related to specific drugs by using an ELISA test.

P 19.6 Fetomaternal alloimmune thrombocytopenia in south-east England: the Cambridge experience
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All suspected cases of fetomaternal alloimmune thrombocytopenia (FMAIT) occurring in south-east England are investigated at NBS Cambridge. The aim of the study was to collect clinical data and determine the relationship between HPA antibody and this disease. Maternal samples were tested for HPA antibodies by PIFT and MAIPA. HPA genotypes were determined on maternal, paternal and neonatal samples. Clinical details were obtained by telephone and questionnaires. Over a period of 77 months, HPA alloimmunization was confirmed in 170 of the 1144 referrals. A single antibody was responsible in 165 pregnancies: anti-HPA-1a in 132 (78%); ~5b in 25 (15%); ~15b in five (3%); ~2a in two and ~1b in one. Combined antibodies were found in five: anti-HPA-1a + 5b in three; ~5b + 15a in one and ~5b + 15b in one. There was no previous history of alloimmunization in 107 (33 primi and 74 multiparae). Five intrauterine deaths occurred in this group. Of the 104 live births, 76 neonates required treatment. 15 neonates suffered intracranial haemorrhage (ICH) of whom one died on day 1. Sixty-three pregnancies were known to be previously alloimmunized. Forty of these women required antenatal treatment including intratetralone transfusions (ITTs) in 34. 6 of the fetuses that received ITTs died in utero and 59 were born alive. Two of these neonates developed ICH. Twelve babies who suffered ICH were followed up and in nine, neurological problems still persist. This study has provided useful data on the clinical spectrum of FMAIT and the different HPA antibodies.

P 19.7 Alloimmunization against HPA-1a in an HPA-1a positive mother
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Introduction: Alloantibodies against human platelet antigens (HPA) are involved in neonatal alloimmune thrombocytopenia (NAIT), post-transfusion purpura and refractoriness to platelet transfusion. In NAIT, maternal immunization occurs against paternally inherited antigens with alloimmunization against HPA-1a causing
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provision of HLA-matched platelets

gene frequencies and variants in a Chinese population from the

FCGR3B

History: due to immunization against HPA-1a in a HPA-1a positive mother.

thrombocytopenia in one in 1200 neonates. Here we describe an unusual case of NAIT

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thrombasthenia carriers.

The mother phenotyped as HPA-1a negative using directly

labelled monoclonal anti-HPA-1a. The maternal serum was positive with paternal

platelets and HPA-1a positive platelets in immunofluorescence and MAIPA. SSP-PCR

genotyping indicated that the mother was HPA-1a/b heterozygous. Sequencing of the

GPIIIa gene confirmed the HPA-1a/b heterozygous status of the mother and also

identified a heterozygous single nucleotide insertion in exon 10 of GPIIIa. This results

in a frameshift and premature termination of GPIIIa at amino acid 471.

Conclusions: Exposure to the HPA-1a antigen during pregnancy can result in

immunization against HPA-1a and NAIT in HPA-1 heterozygous Glanzmann’s

thrombasthenia carriers.

P20 Granulocyte immunology

P 20.1

FCGR3B gene frequencies and variants in a Chinese population from the
Province of Zhejiang

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Background: The human neutrophil antigens (HNA)
carriers.

c

alleles were screened by allele-specific PCR

sequences of the FCGR3B-gene and the FCGR3A-gene, which codes for the FcγRIIIa isoform. These DNA-fragments were
duplicated and sequenced.

Results: The gene frequencies were 0.565 for FCGR3B1*, 0.430 for FCGR3B2*0 and 0.0
for FCGR3B3*. Two donors (0.48 %) were completely negative for the FCGR3B-gene. In
seven out of 19 individuals genetic variants, differing in one or more of the poly-
morphic nucleotide positions 141, 147, 227, 266 and 377 were found.

Conclusion: In contrast to the German or an African population, the FCGR3B2*-gene
is more frequent than the FCGR3B1*-form in the Chinese cohort, while the FCGR3B3*-
gene seems to be absent. FCGR3B-deficiency and individuals with genetic variants
were found in each population.

P21 HLA

P 21.2

HLA epitope mismatches for the selection of matched platelet donors

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Patients immunologically refractory to random platelet transfusions may be

successfully supported using platelets HLA matched at the specificity level according
to defined match grades ‘A’, ‘B’ or ‘C’. In this study, we have retrospectively assessed the
impact of molecularly defined epitope mismatching in the outcome of platelet

transfusions in a group of 259 immunologically refractory patients including 35 highly

sensitized patients (HSps) with panel reactive antibodies (PRA) >80%. The degree of

epitope mismatching was defined, using the computer algorithm ‘HLAMatchmaker’ and the T-cell epitope prediction databases ‘SYFPEITHI’ and ‘ProPred’. Mismatched epitopes were categorized by the HLAMatchmaker as zero, immunogenic or nonim-
munogenic. Platelet increments in patients receiving zero or nonimmunogenic epitope

mismatched platelets were significantly higher (P < 0.000001), than those in patients

who received immunogenic epitope mismatched platelets. Furthermore, platelet

increments in 13/35 patients transfused with zero or nonimmunogenic epitope

mismatched platelets, were also significantly higher than those obtained in patients

receiving platelets containing immunogenic epitope mismatches (P < 0.0004). The use

of platelets with nonimmunogenic HLA-epitope mismatches results in an improved

treatment of transfusions in immunologically refractory patients and it may also

increase the probability of identifying matched products in a restricted pool of platelet

donors.

P 21.3

Changes in soluble HLA variants after bone marrow transplantations

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The significance of soluble HLA class-I molecules (sHLA-I) was observing in prediction and differential diagnostics among posttransplantation complications (GVHD, graft rejection and infections). Even more sensitive indicator of immunologic changes could be the evaluation of sHLA-I variants. There are at least three variants of sHLA-I in serum with diverse molecular weights: 41, 39 and 35 kDa. They are produced by different mechanisms and apparently have diverse immunomodulating effects. We have introduced Western blot method for detection of sHLA-I variants in the blood serum and examined the dynamics of sHLA variants in nearly 30 hematologic patients [about 300 blood samples] after bone marrow transplantation (BMT). Our results indicated that the dynamics of sHLA variant’s levels were very specific for each patient and depended in addition to seriousness of post-transplant complications also on other factors, especially on used pharmacological and immuno-

therapy. The concentrations of respective sHLA variants responded to post-BMT complications in different manner. The 43 kDa variants raised mainly in GVHD, the 35 and 39 kDa variants increased rather in infections. sHLA-I levels mostly decreased in relapses, particularly the 35 and 39 kDa variants. The correlations between levels of sHLA-I variants and the course of diseases are demonstrated as individual

casuistics.

Acknowledgement: This work was supported by VZ UKRT 237160001.
P 21.4
NK cell immunoglobulin-like and cytotoxicity receptor repertoire in patients with PSA
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Background: Natural killer (NK) cell-mediated cytolsy is stimulated and downregulated through the interaction of distinct human leukocyte antigen (HLA) class-I molecules on target cell with specific killer cell immunoglobulin-like receptors (KIRs). It is currently discussed, that NK cells may contribute to autoimmunity.

Materials and methods: We have therefore studied the genetic distribution of inhibitory (IL) and stimulatory (DS) KIRs among 19 patients with psoriatic arthritis and 143 healthy controls. Analysis of KIRs was performed as described previously (S. Becker et al., Human Immunol 64, 2003) using PCR amplification with sequence-specific primers for KIR gene segments. In addition the cell surface expression of activating receptor NKP30, NKP44 and NKP46 war analysed by flow-cytometry.

Results and conclusions: Our results indicate that psoriatic arthritis patients are characterized by an altered KIR gene repertoire. The inhibitory receptor KIR2DL1 and KIR3DL2 were decreased among psoriatic arthritis patients in comparison with controls ($P < 0.01$) and a shift in KIR haplotype distribution. Interestingly, individual psoriatic arthritis patients showed the presence of activated-NK cells as indicated by the expression of NKP44. Variation in KIRs could considerable alter the NK and/or T-cell mediated immune response and may contribute to the disease pathogenesis.

P 23.1
Using audit, education and communication to change clinical practice
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The Government Health Service Circular 2002/009 included a plan of action for the NHS to avoid the unnecessary use of blood components in clinical practice. As the results of previous audits in this hospital showed that about 30% of fresh frozen plasma (FFP) was being used inappropriately, it was felt that this area should be addressed as a priority. An attempt to change practice was made using an education and communication program. The results of the previous audits were presented to staff in high use areas and a culture of communication between clinicians and laboratory staff was encouraged. Subsequently requests noncompliant with the guidelines were referred to a haematologist. A reaudit was undertaken in 2003, to determine whether there had been a change in practice, and to complete the audit cycle. The reaudit was undertaken over an 8-month period in 2003, during which 163 requests were received and 460 U of FFP were transfused. The percentage of FFP used inappropriately had fallen to 9%. Our results have demonstrated that, although audit alone cannot change clinical practice, combining audit with education and communication can have dramatic effects. There has been considerable reduction in the inappropriate use of FFP within this hospital, there is also now a more open culture of communication and discussion between clinicians and laboratory staff and a better awareness of the guidelines for blood components.

P 23.2
Cefotetan and haemolytic anaemia
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The study of drugs has provided insight into the mechanisms of haemolytic anaemias. Mechanisms such as 'penicillin type', 'cephalosporin type' and 'alpha-methyl dopa type' have been described. Cefotetan was implicated in 1992 as a cause of fulminant haemolysis and a review in 2002 included 85 cases, 15 fatal. This drug continues to cause severe haemolytic anaemia. Cefotetan is used as a prophylactic antibiotic at the time of surgery. The purpose of this communication is to record one centre’s recent experience. The estimate of the population is 600,000 over a 3-year period. During this time seven cases of Cefotetan-induced haemolysis were suspected and confirmed – a positive direct antiglobulin test in the presence of the drug with appropriate controls. The haemoglobin values ranged to 47 g/l and all patients required transfusion. The blood film showed the expected change with spherocytes and reticulocytosis. There was biochemical evidence of haemolysis in all cases. The diagnosis was confirmed with a positive direct antiglobulin test in the presence of the drug, with appropriate controls. All patients were treated with steroids until the diagnosis was confirmed; the steroid was then ceased. In addition to the issue raised in relation to the continuing use of the drug, other points worthy of consideration are the difficulty in finding the drug prescription in the patients’ records (drug, anaesthetic, premedication sheets) and possible overlap with other drugs of this class.

P 23.3
Changing clinical practice

P 23.4
Blood product utilization practices in a university hospital: an interventional study
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Background: Transfusion may be life-saving, however the possibility of an adverse outcome because of transfusion exists. It is essential that the utilization of blood products is appropriate and accurate.

Aims: To assess the appropriateness and accuracy of blood product utilization in a general hospital and determine the effect of a clinical transfusion form on the transfusion process.

Methods: The study was conducted in three phases. I: transfusions were assessed for appropriateness, completeness of the blood order form and documentation of response to transfusion; II: reassessment after the introduction of a form requiring the ordering physician to confirm that the transfusion was indicated; III: reassessment after removal of the transfusion form.

Results: Transfusions (5.8%) were inappropriate in phase I, 2.4% in phase II and 6.2% in phase III ($P < 0.05$). Clinical aspects of the blood order forms were complete in 34% of orders in phase I, 64% in phase II and 74% of orders in phase III. Post-transfusion notation was absent in 11.2% of transfusions in phase I, 5.6% in phase II and 8% in phase III. Post-transfusion notation in the patients’ charts was complete in 50% of transfusions in phase I, 66% in phase II and 77% in phase III.

Conclusion: A clinically oriented form improved the appropriateness of blood utilization, completeness of the blood order form and post-transfusion documentation. Routine use of such a form may improve the transfusion process.
P 23.5
Quality of life measurement in patients with post-partum anaemia in blood transfusion practice
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Introduction: Anaemia is a common and important complication after delivery. Fatigue, assumed to be anaemia-related, is one of the most important symptoms. Patients are treated with blood transfusions to improve health-related quality of life (HRQoL). Although improving HRQoL is the major goal of blood transfusion, the HRQoL has not been investigated empirically. In this study three internationally established HRQoL measures were evaluated after delivery and the association between the severity of the anaemia and the HRQoL was investigated.

Material and methods: A total of 120 randomly chosen patients (60 vaginal deliveries and 60 cesarean sections) completed the multi-dimensional fatigue inventory, the EuroQol VAS and the SF-36 directly, 1, 3 and 6 weeks after delivery. Hb level was measured on t = 0. Psychometric analysis focussed on feasibility and reliability.

Results: The questionnaires showed a high feasibility and reliability. A significant improvement of the HRQoL was found for both patient groups during the first 6 weeks after delivery. A correlation between Hb level and HRQoL was found.

Conclusion: This study provides detailed insights in the suitability of established HRQoL measures after delivery and the relation with blood loss. The HRQoL measures are complementary and seem to be useful tools in future clinical trials on the assessment of the effect of red cell transfusion and to confirm the role of HRQoL in the decision whether red cell transfusion is necessary.

P 23.6
Interactive e-learning package for safe transfusion practice
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The e-learning interactive animated software package, safe transfusion practice, has been custom designed and produced by Bourne Training. SUHT is one of the biggest and busiest teaching hospitals in the UK. A central laboratory provides blood products for transfusion at numerous locations. A main priority of the trust is to facilitate education and training. A programme for transfusion awareness training is being led by the specialist practitioner of transfusion, supported by the Hospital Transfusion Committee and the Hospital Transfusion Team. Training a multi-professional team with varying shift patterns and on several sites is an overwhelming task. The challenge is to reach all relevant staff from consultant to porter. The HIT provided the clinical and scientific data drawn from better blood transfusion 2, the SUHT Blood Transfusion Policy and SHOT, enabling Bourne Training to create the software. The package can be accessed at any networked terminal within the Trust. It includes an introduction, six teaching modules and an assessment. The modules cover the entire transfusion process, each having informative, interactive, animated and self-test screens. The user can select modules in any order, but cannot skip screens within a module. If a 100% score is achieved in the assessment, a certificate is printed. A trust database collects the identity of those accessing the programme and the number of certificates produced. This aids audit and monitoring of training.

P 23.7
Increasing transfusion safety by control of and training for correct blood sampling
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An important area of risk in transfusion safety are errors occurring in pretransfusion specimen collection and labeling. Effect of control of nonconformities and training for correct blood sampling was evaluated.

Material and methods: Three error categories were defined: (i) least severe – incomplete patients’ or phlebotomist’s identification data. Analysis of sample rejection due to mislabeling was carried out on 48 330 samples during 2003; (ii) moderately severe – discrepancy between identifying information on sample and request form was assessed during 1995–2002 (10 000 samples/year); (iii) most severe – discrepancy between current blood group and historical or second sample (misdraw), data before training (1991–1994) and after training (1995–2002) was compared.

Results: (i) Frequency of sample rejection due to mislabeling was 2.09%. The most frequent error was incomplete information of phlebotomist (91%); (ii) frequency of sample rejection due to discrepancy between sample and request form information was 0.025%; (iii) frequency of misdraw before training was 0.067%/year. A decrease in misdraw rates to 0.028%/year (improvement of 46%) was observed following the training.

Conclusion: Nonconformities in pretransfusion blood sampling can be classified as near-miss events, that can be used to identify root causes for failures in the process of blood transfusion. Control that includes feedback to the phlebotomist and training of personnel reduces frequency of errors in blood.

P 23.8
HaemoNet TC001: the database in the apheresis unit
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We are evaluating the utility for our apheresis unit of the data-base HaemoNet TC001 as a mean to better control the apheresis processes. Five MCS* cell separators are connected to a central monitoring system. All procedures data are automatically stored. The system utilize a barcode reader to identify each step: kit, solutions, technical/ medical personnel, donor and unit. Procedures data and donor characteristics are also automatically collected. Medical staff could follow the cell separators working simultaneously from the central monitor even if it is in a different room. Alarms appear on the PC giving the same information as the cell separator does. Besides, the HaemoNet system allows the statistical analysis of the procedure’s data: processed volume, ACD used, ACD to donor and into blood components, solutions and additives used and many others. Moreover, data of quality controls are allowed by additional entry. In 2 months we have collected data from 301 apheresis without trouble and waste of time. The staff is no more obliged to cumbersome data collection and registration, the information is no more difficult to find out, the errors are fully analyzed in real time and not now and then on a subjective basis. Our conclusion is that the routine use of a central fully automated data-base could be very helpful for an apheresis unit and simplify the staff work, guarantee a full procedure traceability and give an objective mean to overcome problems.

P 23.9
Erythrocytapheresis (EA) and erythropoietin (HfHuEPO) in hereditary hemochromatosis (HH) therapy
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Introduction: Phlebotomies and chelating agents allow the iron found in the blood to be removed. In the past, HfHuEPO has been used to help lower ferritin levels. We present our experience with the use of EA in five patients with HH.

Aims: To evaluate the efficacy and the safety of EA in association with HfHuEPO in reducing ferritin levels.

Methods: Six pts (five males, one female) were enrolled. Five of them had HH, one secondary hemochromatosis. Mean age was 43 ± 7 years. Data at admittance: mean haemoglobin (Hb) = 14.9 ± 1 g/dl, mean ferritin = 813 ± 321 nmol/L. Treatment protocol: HfHuEPO 40 000 IU subcutaneously/week + folic acid 10 mg/day per os + EA when the hematocrit ≥38%. The RBC, amount collected for EA was within 20% of the circulating RBC, mass. Haemonetics MCS 3p separator was used to perform the procedures. The end point was ferritin lower than 50 ng/ml in pts with HH, 100 in pt with secondary hemochromatosis.

Results: In all the pts target ferritin value has been reached in a median period of 11 weeks (range 8–19). No adverse events or anaeamisation were observed (Hb always ≥11 g/dl). Mean interval between EA was 14 ± 9 days. Mean total RBC volume removed for pt was 2860 ± 1620 ml. Mean Hb at the end of the treatment was 11.7 ± 0.8 g/dl.

Conclusions: In HH the association EA + HfHuEPO is safe and induces a quicker reduction of ferritin than conventional treatment.

P 23.10
Transfusion education and requesting blood – an audit
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This prospective audit recorded the decision making process involved in blood transfusion requests at two hospitals over 28 days. A sample of cross-match requests stratified by specialty was taken using a questionnaire administered by specially employed nurses. A total of 244 cross-matches were audited (64% were requested by beneficiaries).
house officers and senior house officers (junior doctors) whilst 30% were requested by higher grade doctors). Nurses and midwives accounted for 6% of requests. The decision to transfuse was made by higher grade doctors in 70% of requests whereas junior doctors made the decision in 29%. Nurses were responsible for 1%, 34% of cross-matches were requested by staff who said that they had received no training in blood transfusions in ‘electronic education’, prepared 10 modules on blood procurement and processing (1), clinical use of red cells, platelets, plasma and cryoprecipitate (2), transfusion reactions (3), blood conservation and alternatives (2), fractionated products (1) and transfusion in sickle cell disease (1). These underwent expert review by the panels prior to acceptance as the final version.

Conclusion: A comprehensive electronic learning program in blood transfusion has been made available on ‘the web’. At time of submission the program is being evaluated for credits in continuing education for physicians, nurses and technologists.

P 23.12 Preparing for practice – transfusion practitioner education programme
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Introduction: As part of a Government sponsored program to improve transfusion practice and enhance blood conservation, an electronic learning program has been compiled, supported by a concise handbook summarizing the content and containing references.

Methods: Four expert panels reviewed literature on use of blood products, transfusion reactions, blood conservation and alternatives, and fractionated products, and provided advice on content. Based on the recommended content, the authors, working with specialists in ‘electronic education’, prepared 10 modules on blood transfusion. Of these, 65% of transfusion practitioners were given the new module for education and practice were included in the report.

Results: In October, 2003 the 10 modules comprising 306 screens using audio, text, charts, illustrations and animated illustrations were posted at www.sunnybrook-andwomen.ca (Research and Education). The poster will illustrate a sample screen from each module and the presenter will have a CD version available for demonstration by arrangement.

Conclusion: A comprehensive electronic learning program in blood transfusion has been made available on ‘the web’. At time of submission the program is being evaluated for credits in continuing education for physicians, nurses and technologists.

P 23.13 Blood transfusion practices in a developing country (4 years on)
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Introduction: A study comparing blood transfusion practices between a developing country and a developed country had been carried out in 1999; Sudan and the UK were chosen as examples. 4 years on, a follow-up study funded by the BBTS has been conducted.

Aim: To observe the changes that occurred during this time, apply proposed solutions and identify areas of weakness, and to propose further solutions to improve the transfusion practices in Sudan, with minimal fiscal implications, using the UK practice.

Study design: Fieldwork, observation and interviews were conducted to glean data.

Results: Minimal changes occurred during the 4 years. HBV infectivity rates remained high (approximately 6%), and HIV infectivity rates increased (by approximately 0.9%). In contrast, large changes were implemented in the UK over this period.

Discussion: Changes have been introduced to improve the transfusion practice in the Sudan. Ideas for further improvement have been proposed, and their feasibility considered. The system needs a complete overhaul, with changes from donation system, donor care, and donation testing, to the use of blood.

Conclusion: Immediate help is needed to improve the transfusion service in Sudan, especially to halt the rate of infection by HBV and HIV. Follow-up plans: a transfusion practice course, sponsored by the NBS, will be held in Khartoum in February 2004. It aims to improve the knowledge of the blood bank staff, nurses and doctors.
P 23.16  
Assessment of bar code tracking of the ‘end-to-end’ hospital transfusion process  
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Baseline audits revealed 14% hospital patients without wristbands, and procedural errors in blood collection from fridge and blood administration of 20 and 40%, respectively. We have therefore evaluated bar code control of the complete transfusion process, consisting of:  
1 Computer printed wrist band identification (SATI), carrying linear and 2-dimensional (2D) bar codes;  
2 Demand printing of a bar coded label for the blood grouping sample tube obtained by scanning the 2D wrist band bar code;  
3 Positive sample identification in the transfusion laboratory  
4 A computer (BARKS, DiaMed), on the blood refrigerator linked to an electromagnetic lock, providing ID-controlled staff access, data transfer from the laboratory computer of blood loaded, and controlled blood;  
5 Issue with bar code matching of blood and label from case notes;  
6 Data transfer from BARKS to a bedside scanner (PBARKS), allowing verification of ‘correct blood’ via patient wrist band when administered.  
Full audit trail of staff activity, confirmation of blood administration and cumulative ‘out-of-fridge’ time. During a 12-month pilot, there was no down-time due to the system itself, but we saw repeated lost of function due to hospital power failures or computer network problems. A programme of deliberate challenges to the system is ongoing, and reaudit of the transfusion process will be reported. Success of computerized identification depends on resilience and support of hospital network systems.

P 23.17  
The WHO basic information sheet (BIS) as an audit tool for blood transfusion  
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Objective/design: The transfusion basic information sheet (BIS) was created by the Birmingham WHO Regional Office to serve as a simple bedside clinical performance management tool. We evaluated the BIS for collecting transfusion date in different clinical settings in a prospective study in Croatia.

Methods: Transfusion episodes (TE) were monitored for 2 months in 18 centres, using the BIS. Clinical areas covered were gastroenterology, hematology, pediatrics, gynecology and intensive care. Data required included: patient identification, diagnosis, date/time of transfusion, baseline clinical and laboratory data, transfusion targets, therapy and transfusion outcomes.

Results: A total of 1,203 TEs were reviewed. Patient identification was adequately documented in 76% of total TE. The time/date of transfusion was recorded in 86% and transfusion targets in 50%. The pretransfusion IB and platelet count were documented before PRBC and platelet transfusions in 60%. The PT/(APTT) were recorded before the use of FFP in 40%. Post-transfusion data was >80%. PRBCs were administered to before PRBC and platelet transfusions in 60%. The PT/APTT were recorded before the therapy and transfusion outcomes.

Conclusion: We suggest that the BIS can be incorporated into routine practice. BIS has served as a useful audit tool by identifying areas for clinical practice development.

P 23.18  
Development of an electronic system for the remote issue of blood in hospitals  
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The electronic issue (EI) of blood is now accepted transfusion practice providing certain criteria are met. These include: the ABO and RhD groups are tested twice, no current or historical red cell antibodies, the blood bank computer will prevent the issue of ABO-incompatible blood. Our laboratory has used EI for 2 years with no reported delayed haemolytic transfusion reactions, with approximately 60 000 red cell units transfused. An enhancement of EI would be the issue of blood at a fridge distant from the laboratory – ‘remote issue’ (RI). The project objective was to provide a means for assessing the eligibility of patients for EI using computers at remote blood fridges linked to the blood bank computer (Sifith) via BloodTrack (Olympus). If the patient is eligible, compatibility labels are printed and attached to the units of blood at the fridge. If the patient is ineligible the release of blood must be prevented. A total of 400 random patients were tested using the new RI software to determine their eligibility for EI. Their eligibility for EI was checked against the standard EI protocol and found to be correct in all cases. This was repeated with 150 patients with known antibody specificities, these results were also correct. Other problematic situations were also tested, for example, patient on an exclusion list (recent transplant), all these results were correct. In conclusion, the software appears suitable for use for RI. The next stage is to pilot the system in a clinical setting.

P 23.19  
An audit of patients wearing correct and complete ID wristbands in Leeds Teaching Hospitals Trust  
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Patient misidentification is known to be a major cause of incorrect blood components being transfused with 64% of reports sent to SHOT in 2001–2002 attributed to this. An audit of patient identification wristbands within LTH NHS Trust was performed to assess compliance with current identification policies. This would also provide baseline data prior to the introduction of a new Patient Identification Policy (PIP) within the Trust. The audit comprised four of six sites, adult inpatient wards only [PI] and five random patients per ward, totalling 429 patients, which gave a 95% confidence interval. Data was collected over 21/2 days, and we recorded if a wristband was worn and the information present was correct and complete. The audit form was compiled and analysed using form recognition software. Results showed that 122 (28.44%) of the patients surveyed were not wearing a wristband, and 115 (37.56%) of those who were had incorrect or missing data. Of great concern 10 (12.2%) of those not wearing a wristband were unable to identify themselves to us. It was observed that compliance with current identification policies was very poor which creates a potential risk for patients receiving a transfusion. It is intended that the new PIP be introduced with a high profile throughout the Trust, with transfusion training sessions highlighting the need for thoroughness in positive patient identification and reaudit planned for later this year.

P 23.20  
Transfusion nurse specialists: a 5-year experience  
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Background and aims: We report 5 years experience of hospital-based transfusion nurse specialists, and the effectiveness of these posts in improving the quality of the transfusion process.

Results:  
1 New protocols and continuous audit of orthopaedic and cardiac surgery blood use has documented reduction of red cell and product use without under-transfusion. Total hospital blood use has fallen by 15.3% despite increasing clinical activity.  
2 We have carried out comprehensive education and training of all staff involved in the transfusion process from porters to consultants.  
3 All transfusion incidents, complaints and adverse clinical reactions are rapidly investigated. Improved communications and a clear contact for clinical staff have improved relations between blood bank and clinical areas and led to increased reporting to SHOT as a result of better recognition and investigation of adverse reactions.

4 Several major new developments in transfusion practice have been enabled by the presence of transfusion nurses. These include blood collection procedures, transport procedures, new administration procedures, platelet ordering procedures, and introduction of near patient coagulation testing and cell salvage in several clinical areas.

Conclusion: Transfusion specialist nurses have enabled significant advances in transfusion quality within our hospital, with a reduction in overall transfusion budget.

P 23.21  
Pilot study of the use of RFID in transfusion-medicine  
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Traceability, safety and full documentation of all parameters is the aim of the procedure of a blood bank, started from collection till to application in the hospital. The use of radio frequency identification (RFID) can be a more efficient alternative to the actual barcode system. In a first trial, three series of RFID with a Chip I-Code 1 were tested in plasma preparation. The chip was integrated in a special bag including methylene blue pill used for pathogens inactivation of plasma. The process of
inactivation includes an illumination step achieved with a dedicated equipment in which RFID readers were integrated. Those readers allow automatic detection of the bags in the machine therefore a better safety process. Before and after the inactivation step, data were automatically transferred to each label by the integrated antennas. In a preliminary test all collection-data were transferred by an operator to the RFID chip by means of the illumination equipment barcode reader. In the second part, all identification data were preregistered by an independent device (SLG) so no barcode scan were necessary. After illumination, the virus inactivated plasma was stored at −30°/−40 °C in bags with RFID. Including all data of the illumination, no loss of data was measured after freezing and defrosting.

P27 Transfusion and alternatives in surgical patients

P 27.1

Is swab washing detrimental to the quality of salvaged blood in aortic surgery?

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Washing swabs can increase the efficiency of intraoperative cell salvage (ICS). The ‘salvaged cell syndrome’ theory suggests however that processing dilute salvaged blood results in leucocyte activation and release of cytokines which, on reinfusion, can cause disseminated intravascular inflammation. Blood losses from the surgical field and from swabs washed in 1000 ml isotonic saline containing 10 000 IU of heparin were harvested using a Haemonetics Cell Saver 5 (CSS) in 10 patients undergoing elective aortic aneurysm repair. All volumes were recorded and samples taken before and after processing each subsequent unit were assessed for cellular and cytokine content: leucocyte Bi, (LTB4), tumour necrosis factor-α (TNF-α) and granulocyte-colony stimulating factor (G-CSF). The median [IQR] haematocrit (Hct) of blood from the surgical field was 0.2 (0.14–0.23) compared with 0.06 (0.05–0.08) from the swab wash (P < 0.001). Although cytokines were present, they were eliminated effectively by the CSS with median [IQR] percentage clearances of 95.90% [93.53–98.09]% for LTB4; 76.03% [65.37–87.50]% for TNF-α; and 86.57% [76.64–92.61]% for G-CSF. Cytokine levels declined as Hct declined and residual concentrations in packed red cells derived from the swabs were not significantly higher than in the units derived from blood from the surgical field. ICS does not lead to patients being exposed to clinically significant levels of LTB4, TNF or G-CSF and swab washing does not effect this.

P 27.2

Intravenous infusion of iron in patients suffering from anemia

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Objective: Preliminary examination of patients planned for cardiac surgery procedures, showed the presence of anemia in relatively large number of patients which more often receive allogeneic blood transfusions.

Aim: To show importance of iron intravenous administration in order to achieve fast anemia correction and to reduce the number of allogeneic blood transfusions in patients planned for surgery.

Patients and methods: Our investigation included 390 pts (257 male and 133 female), that were planned for cardiac surgery procedure. In anemic patients iron (Ferrilecit) were infused. Infusions (1–3 ampoules, dissolved in 200–300 ml of 0.9% NaCl solution) were repeated on the third day.

Results: In 151 patients (39%) Hgb values were >130 g/l (120 g/l for female). In 92 pts (24%) concentration of iron in serum was <11 μmol/l, and ferritin level was <15 μg/l. After applied intravenous iron (12–18 ampoules), concentration of Hb increased by 21–32 g/l. Transfusion of allogene red blood cells (RBC) was reduced in postoperative period. Regarding historical period, use of allogene RBC was reduced by 55%.

Conclusion: Need for transfusion of allogene RBC and blood can be significantly reduced by application of iron through intravenous infusions. In our country there is extremely high rate of anemic persons (>30%), and our medical staff and other relevant social structures should take this problem into serious consideration.

P 27.3

Changes in transfusion requirements for liver transplantation over 15 years at a single institution

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Background: The pattern of transfusion therapy in hospitalized patients continues to change and is influenced by a variety of factors including, but not limited to, patients’ diagnoses and ages, surgical procedures and techniques, and physician ordering practices. A liver transplant program, initiated at our hospital in 1988 was associated with high blood product use and, therefore, greatly impacted the blood bank. A survey of intraoperative blood product use for patients undergoing orthotopic liver transplantation in 1991 revealed greater use at our institution than the average use of other institutions surveyed. This review demonstrates a remarkable decrease in use of blood products for our liver transplant patients through 2003.

Methods: The transfusion records of patients receiving liver transplant in 1988, 1991, 1999, 2000 and 2003 were reviewed.

Results: As shown below, the mean transfusion requirements of patients undergoing liver transplantation has markedly decreased.

Table 1. Mean blood product usage during liver transplantation

| Year | RBC | PLT conc | FFP | CRYO |
|------|------|----------|-----|------|
| 1988 | 32.1 | 37.1     | 17.5| 9.6  |
| 1991 | 37.1 | 17.5     | 9.6 |      |
| 1999 | 17.5 | 9.6      |     |      |
| 2000 | 9.6  |         |     |      |
| 2003 | 5.6  |         |     |      |

Conclusion: The significant reduction in blood usage reported here (>50%) was primarily the result of changes in surgical techniques implemented in mid-2000 in association with the arrival of a new surgeon.

P 27.4

Prevalence of anaemia and red cell transfusion in patients undergoing elective orthopaedic surgery

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Introduction: Few large studies have examined the relationship between preoperative Hb and the prevalence of blood transfusion for elective orthopaedic surgery (EOS).

Objective: To determine the prevalence of preoperative anaemia in patients undergoing EOS and the associated peri-operative red cell transfusion rate.

Material and methods: Retrospective cohort study based on computerized data registers. All patients admitted to the main teaching orthopaedic hospital, Edinburgh over a 12-month period were studied. The patient database was merged with the haematology and transfusion databases.

Results: There were 3417 admissions; full blood count data were available for 1749 (51.2%). Of these, 299 (17%) were anaemic (Hb males <130, females <115 g/l). For the anaemic admissions: 64% had normocytic normochromic anaemia, 26% demonstrated hypochromasia. A total of 305 admissions received red cells. The table shows the relationship between preoperative Hb and prevalence of transfusion. A total of 838 red cell units were used. 83% of these units were transfused to admissions undergoing major joint surgery.

Table 2. Preoperative Hb and proportion transfused

| Hb range | # | % |
|----------|---|---|
| ≤124     | 54|
| 125–134  | 27|
| 135–144  | 13|
| ≥145     | 2 |

Conclusions: Admissions (17%) with available Hb data were anaemic. The majority had normocytic normochromic indices. Prevalence of red cell transfusion is associated with preadmission Hb. P27.5
**P 27.5**

**Oral tranexamic acid in total knee replacement: results of a randomized study**

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**Background:** Total knee replacement causes minimal intraoperative but extensive postoperative blood loss. Intravenous tranexamic acid can significantly reduce postoperative blood transfusion. A comparison between oral and intravenous TA has not been performed.

**Aim:** To compare postoperative blood loss and transfusion requirements in TKR patients treated with three tranexamic acid regimens compared with untreated controls.

**Patients and methods:** A total of 80 patients were randomized in this controlled, trial to one of four treatment groups: TA-Long, [i.v. bolus of TA, 15 mg/kg i.v. infusion of 10 mg/kg/h for 12 h]; TA-Short, [i.v. bolus of TA, 15 mg/kg i.v. infusion of 10 mg/kg/h for 12 h, oral TA, 1 gr, after 6 and 12 h]; TA-Oral, [TA 1 gr p.o. 60 min prior to surgery, 1 gr postop q6 h for the next 18 h]; control, [no TA].

**Results:** All three TA groups had decreased postoperative blood loss and reduced blood transfusion requirements compared with the control group (P < 0.05) as shown in the table below.

|                | Control  | TA-Long | TA-Short | TA-Oral |
|----------------|----------|---------|----------|---------|
| **Blood loss (ml) 24 h** | 444 ± 138 | 222 ± 65 | 205 ± 44 | 338 ± 70 |
| **Postoperative transfusion** | 12 | 18 | 10 | 14 |

No adverse effects including venous thromboembolism were associated with TA use.

**Conclusions:** TA significantly reduces postoperative blood loss and transfusion requirements in TKR. The oral regimen in this study was as effective as the iv protocols and may be more convenient to administer.

**P 27.6**

**Effectiveness of blood conservation in CABG surgery**

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**Purpose:** Cardiac surgery is associated with a high rate of allogeneic transfusion. With increasing awareness of the adverse effects of transfusion, a transfusion nurse was appointed to assist in implementation of blood conservation measures to reduce the likelihood of allogeneic transfusion.

**Methods:** Patients undergoing elective primary coronary artery bypass graft (CABG) surgery were evaluated for blood conservation options by the transfusion nurse 21–42 days prior to surgery. Where suitable, preoperative autologous donation (PAD), erythropoietin and/or CryosealTM fibrin sealant options were offered.

**Results:** At baseline, prior to implementation of blood conservation measures, 61% of patients received allogeneic transfusion. Twelve months later, 33% of patients overall received allogeneic transfusion. Similarly, overall, the mean number of allogeneic units administered per transfused patient decreased (3.8 vs. 2.5). The allogeneic transfusion rate for patients treated with erythropoietin was 27%, for those managed by PAD 25%, and for those who received both erythropoietin and PAD 13%. Combined use of PAD and CryosealTM further decreased the proportion of patients receiving allogeneic transfusion.

**Conclusions:** Blood conservation measures can be successfully applied in elective primary CABG patients, reducing need for scarce blood resources, as well as increasing patient safety and satisfaction.

**P 27.7**

**Effectiveness of a network of Ontario transfusion nurse coordinators:** ONTraC

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**Purpose:** To enhance transfusion practice outside the blood transfusion laboratory and promote blood conservation in surgery patients.

**Methods:** A total of 23 hospitals were funded for a transfusion nurse salary. At specific time periods detailed anonymized information was collected in a defined number of all consecutive patients admitted for the three designated surgical procedures (knee arthroplasty, abdominal aortic aneurysm (AAA) surgery and primary coronary artery bypass graft (CABG) surgery).

**Results:** Data collected at baseline and at 12 months showed considerable variation between institutions in proportion of patients transfused with allogeneic red cells and in the amount administered per transfused patient. At the 12 month time point, most, but not all, hospitals had decreased use of allogeneic blood and there was an overall 24% reduction in patients undergoing knee surgery, 14% in AAA and 23% in CABG. While the percent of patients donating autologous blood increased over this period, the percent of autologous units transfused decreased. Over this initial time relatively little increase in use of other modalities occurred. Postoperative infection rates and length of stay were significantly higher in recipients of allogeneic blood.

**Conclusions:** The ONTraC pro–gram represents important savings in costs associated with blood components, hospital stay and work in transfusion laboratories, as well as enhancing patient satisfaction and safety.

**P 27.8**

**Blood salvage with blood irradiation in cancer surgery**

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The contraindication of intraoperative blood salvage (IBS) in cancer surgery can be overcome by blood irradiation, since effective elimination of tumor cells by irradiation with 50 Gy and high quality of the irradiated RBC have been demonstrated. We report on 8 years of experience with this method in clinical practice.

**Method:** Intraoperative blood salvage was performed (CS5, Haemonetics) during surgery of primary tumors or metastasis. Washed RBC were transferred (1–3 U) to an irradiation bag (BIT500, Dideco) labeled in great detail for identification, and irradiated with 50 Gy (IBL417, CShi) prior to retransfusion.

**Results:** IBS with blood irradiation was performed in 847 cancer patients including surgery of abdominal and bone tumors, spinal and hip metastasis, liver resection and transplantation. Efficient reduction in allogeneic blood transfusions and saving of blood resources was observed. Blood irradiation was made available for Jehovah’s Witness patients. The extension of the indication to cancer surgery has doubled the use of blood salvage in general surgery at our hospital. In more than 90% of analyzed cases tumor cells were identified in the wound blood.

**Conclusions:** Blood irradiation is necessary prior to retransfusion to eliminate proliferating tumor cells contaminating salvaged blood. The combination of blood salvage and blood irradiation is feasible, supports the blood supply in major cancer surgery, reduces transfusion risks, and saves blood resources.

**P 27.9**

**Does swab washing improve red cell recovery in aortic surgery?**

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Intraoperative cell salvage (ICS) reduces homologous transfusion requirements in aortic surgery by recovering red blood cells (RBC) from the surgical field. Blood lost to swabs however is not normally recycled. We investigated the contribution of swab washing to RBC recovery during ICS. Ten patients undergoing aortic aneurysm repair were studied. Volumes and haemoglobin concentration (Hb) of the blood suctioned into a Haemonetics Cell Saver 5 reservoir directly from the surgical field were measured both prior to and after processing. Swabs were washed in 1000 ml isotonic saline containing 10 000 IU of heparin. At the end of the operation, this solution was suctioned into the cell saver reservoir, and volume and Hb measurements repeated. Mean (SD) estimated blood loss was 991 ml (403), resulting in a mean (SD) salvaged RBC volume of 380 ml (124). The mean (SD) haematocrit of salvaged RBCs was 64 (8.40). The median (IQR) Hb collected from direct suction was 84.9 g (61.8–131.4), of which 50.1 (45–71.5) were returned to the patient after processing, a median yield of 68 (49–77%). Swab wash produced a median (IQR) 39.4 g (28.4–64.9) of Hb, of which 26.2 g (16.8–31) were rein infused, a 67% (33–98) yield. Swab wash thus contributed with a median (IQR) of 31% (24–39) of the total RBC recovery. Swab washing produced a third of salvaged RBC volumes. The resulting improved efficacy of ICS may translate into further reductions in homologous transfusion when large blood losses occur.
P 27.10
Intraoperative cell salvage as an alternative for blood transfusion in neurosurgery
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Objective: The purpose of this study was to evaluate the benefits of intraoperative autotransfusion.

Methods: Automated reinfusion of packed red blood cells prepared from blood lost during removal of tumors was the main component of transfusion therapy in 192 patients (197 operations) with brain and spinal tumors. All patients developed massive blood loss of 0.5–5 TCB during the intervention, reinfusion device cell saver C.A.T.S 2–02 (Fresenius, Germany) was used. Special attention is paid to parameters of hemostasis during automated autotransfusion and the problem of tumor contamination of reinfused suspension. This latter problem was solved by using the leukodepletion filter RC–400 Klev (Pall, Germany).

Results: The average intraoperative blood loss was 4.5 l [1.5–20]. The amount of autologous blood transfused was 600 ± 590 ml (230–3000). Automated reinfusion of packed red blood cells effectively compensated for massive intraoperative blood loss, on condition of correction of hemostasis disorders by fresh frozen plasma and purificaction of reinfused suspension from tumor cells by filtering through leukodepletion filters.

Conclusion: Autologous blood transfusions were demonstrated to be safe in patients undergoing neurosurgery and obligatory in case of supermassive hemorrhage. Intraoperative autologous blood transfusions may be used alone in more than half of the patients requiring transfusions during neurosurgery and decrease the amount of allogenic blood used.

P 27.11
Age and transfusion therapy: autologous blood predonation in primary total hip arthroplasty (pTHA)
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Objective: To compare the use of autologous and homologous blood in patients undergoing pTHA, ages 65 years.

Materials and methods: We retrospectively analysed transfusion therapy of 560 pts underwent pTHA over last 3 years, divided in two age groups. Group 1: 362 pts <65 years, mean age 52.3 (20–62). Group 2: 198 pts >65 years mean age 70.9 (66–83). Patients from both groups predonated in average 1.6 U (1–2) of autologous blood.

Results: Perioperatively received blood transfusion therapy was: (i) autologous blood – 236 patients, 1.55 U/pat (group 1), 135 patients, 1.60 U/pat (group 2); (ii) autologous + homologous blood 111 patients, 3.53 U/pat (group 1), 61 patients, 3.98 U/pat (group 2); (iii) total 347 patients, 2.18 U/pat (group 1), 194 patients, 2.38 U/pat (group 2), mean preoperative and postoperative hematocrit (Ht) values (%) were for the group 1, respectively, 35.3 and 30.1%, and in group 2, 34.8 and 29.6%. Preoperative and postoperative hemoglobin (Hb) values (g/l) were for the group 1, respectively, 117.9 and 100.2 and in group 2 116.1 and 99.2. No significant differences between these two groups were noted.

Conclusion: This study did not reveal any significant difference in transfusion therapy, as well as in preoperative or postoperative mean Ht and mean Hb between participating patients younger and older than 65 years. This finding suggests that older patients can successfully participate in preoperative autologous blood donation.

P 27.12
Blood transfusion: patient knowledge and opinions
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Aim: To determine perceived levels of knowledge of blood transfusion, acceptability of blood transfusion and information provided to patients regarding transfusion.

Methodology: Self completed questionnaire given to consecutive patients at orthopaedic outpatient clinic. Questions related to (i) knowledge about blood donation, availability and testing of blood; (ii) Safety of transfusion and explanation given by Health Care Professionals and (iii) knowledge of, and acceptability of, alternatives to homologous blood.

Results: A total of 1023 forms were distributed, four were illegible therefore 99.6% were analysed. 42% (448) had some knowledge of transfusion, 38% (387) had previously donated. 59% (601) were aware of difficulties in supplying blood, 84% (856) knew donated blood is tested before transfusion. The majority would want blood from a UK donor. 80% (815) thought blood transfusion was safe and 47% (497) would be happy to accept a transfusion. 90% (917) felt an explanation would be required pre-transfusion. 21% (214) had had a previous transfusion, 41% (88) of these did not get any explanation of this. 55% (560) would wish to predonate blood if possible.

Conclusion: Transfusion is perceived as safe. Adequate information is not being provided to blood transfusion recipients. Means of improving this should be addressed. Autologous predonation is perceived as an acceptable alternative to homologous blood.

P 27.13
Growth factors in platelet rich plasma and correlation with age, sex and platelet count of donors
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PRP contains platelet growth factors (GF) capable to accelerate the tissue regeneration and used for nonconventional treatment of ulcers and surgical wounds. Our aim was to evaluate the existence of a correlation between age, sex and platelet count (PLT) of donors with the detectable amount GF in PRP. PRP was prepared from 225 healthy donors (162 men and 63 women), aged 19–59 years. GF concentrations and PLT were determined in whole blood and in PRP. The mean PLT in PRP was five times higher than in whole blood (1,545 ± 312 vs. 280 ± 55 × 10⁹/ml). Some GF showed high concentrations respect basal values: platelet derived growth factor AB (PDGF-AB) = 134 ± 56 ng/ml, transforming growth factor beta-1 (TGF-b1) = 172 ± 69 ng/ml and insulin-like growth factor I (IGF-I) = 96 ± 29 ng/ml; while other GF were only found in little amount: PDGF-BB = 15 ± 8 ng/ml and TGF-b2 = 0.9 ± 0.5 ng/ml. No influence by donor’s sex or age on GF was discovered (except for IGF-I). GF in PRP showed a slight correlation with PLT in PRP (P < 0.05), but not with PLT in whole blood (P > 0.35). GF in PRP showed substantial variations among studied subjects, but the factors influencing their concentrations aren’t still fully known. PLT in PRP showed an initial liner positive relation with GF until to go to a plateau. Nevertheless the standardization of procedures for PRP preparation allows us to resolve this problem, in fact laboratory can prepare PRP with the desired concentration of platelets.

P 27.14
A year long study investigating cell salvage for cardiac surgery – does it reduce donor blood usage?
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The National Blood Transfusion Service has warned of not only a reduction in the amount of donor blood available, but also the predicted sharp increase in its cost. This combined with the well known and documented risks of donor blood means alternatives to transfusion must be explored before there is no choice. The study involved 296 patients and was carried out over a 12-month period. The patients were divided into two groups, a noncell salvage group and a cell salvage group. The aims of the study were as follows:

1 Establish the collection pattern of cell-salvaged blood (CSB);
2 Establish what effect CSB has on the patients circulating Hb;
3 Establish whether cell salvage reduces donor blood usage;
4 Investigate the cost-effectiveness of cell salvage;
5 Identify target patients that benefit/do not benefit from cell salvage.

The results showed that on average patients in the cell salvage group received 371 ml of autologous blood with an Hb of 19.8 g/dl. The patients who received NO donor blood increased by 114% with the use of perioperative cell salvage. The overall reduction in donor blood usage in this group was by 1.35 U per patient.

P 27.15
Optimal use of blood components: a benchmarking study in Finland
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Aim: To create a permanent hospital database-grounded program for continuing comparison of blood component use.

Materials and methods: Eight Finnish hospital districts, coded patient-data from pre-existing electronic medical registers designed for administrative, laboratory, operating
room, and blood center service providing information on diagnoses, operations, laboratory values, blood components, and transfusions.

Results: Data set included 53% of blood components delivered in 2002 by Finland’s only blood supplier, the Finnish Red Cross Blood Service: 126 275 red blood cell (RBC), 87 046 platelet (PLT), and 27 328 fresh frozen plasma (FFP) units. Data comprised 27 662 transfused patients (12 266 men and 15 396 women) and included 1794 patients under 17 years of age. Men were transfused more than women per patient (64 023/62 252 RBC, 51 440/35 606 PLT and 16 593/10 631 FFP units). Patients with malignant haematological diagnoses (4.8% of transfused) received 9.9% RBC, 52.9% PLT and 3.6% FFP units. In surgical operations transfusion practices differed between hospitals, for example fourfold for primary total hip and threefold for knee replacement.

Discussion: (i) Data collected from different electronic databases can serve on transfusion benchmarking. (ii) Significant variation exists in transfusion practices for surgery. (iii) National benchmarking aims to include major transfusion practising hospitals to this programme and to improve and standardize clinical transfusion practices.

P 27.16
Algorithm for platelet transfusion in patients exposed to clopidogrel before open heart surgery
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Increased use of platelets in patients (pts) treated with potent antiplatelet drugs before coronary artery bypass graft (CABG) surgery prompted us to use transfusion algorithm. We evaluated effect of an algorithm based on clinical criteria and measuring of coagulation and platelet function. Forty-five pts undergoing CABG who received clopidogrel within 6 days, and 45 controls without clopidogrel exposure were prospectively enrolled. They were transfused based on algorithm. Comparison was made with historical pts undergoing CABG before algorithm implementation: 53 clopidogrel pts and 428 controls not treated with clopidogrel. Clopidogrel pts in comparison with controls enrolled in the same period received more blood components. Prospective group (treated vs. controls): PLT units 9.0 ± 1.7 vs. 1.2 ± 0.5 (P < 0.001); RBC 4.3 ± 0.6 vs. 2.3 ± 0.5 (P = 0.014), and FFP 1.0 ± 0.6 vs. 0.5 ± 0.3 (P = NS). Historical group: PLT 12.5 ± 2.1 vs. 2.3 ± 0.3 (P < 0.001); RBC 6.4 ± 0.8 vs. 2.3 ± 0.1 (P < 0.0001), and FFP 2.9 ± 1.0 vs. 1.0 ± 0.1 (P = 0.046). Algorithm decreased use of PLTs in prospective controls vs. historical controls. P = 0.049. While RBC transfusions were significantly reduced (P = 0.037) between clopidogrel pts use of PLTs was not. Use of algorithm reduced PLT transfusions in pts undergoing CABG. Although PLT transfusions were not significantly reduced comparing clopidogrel pts, decreased RBC requirements might represent effect of appropriate PLT transfusions in these pts with impaired platelet function.

P 27.17
Reinfusion of own blood after hysterectomy
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A total of 130 patients after abdominal hysterectomy (AH) due to 14–24 weeks uterine myoma were evaluated. First group included 30 patients, who were reinfused by their own blood in early postoperative period; control group included 100 patients who received donor’s blood components only. The initial levels of hemoglobin (HB), red blood cells (RBC), hematocrit (HT) and common serum protein (CP) were normal in the both groups. In the first group the taking of 200–250 ml of blood for further reinfusion was performed 4–5 days prior to AH. It was made after appropriate 2 weeks medication by erythropoietin (40–50 IU/kg twice a week), iron, vitamin B12, and folic acid. There were no any complications of the procedure. It was noted that the values of HB, RBC, HT and CP have decreased after AH in the both groups. However, these parameters were significantly higher in the first group than in the control: HB 124.8 ± 4.5 vs. 102.6 ± 2.5 g/l (P < 0.001); RBC 3.84 ± 0.07 vs. 3.6 ± 0.07 * 10¹²/l (P = 0.001); HT 33.7 ± 1.0 vs. 27.4 ± 1.5% (P < 0.001) and CP 66.5 ± 0.1 vs. 52.5 ± 0.1 g/l (P < 0.001). Besides, prolonged fever, purulent complications and suturet incompleteness were observed in 15% of cases and perimetritis in 4% of cases in control group. Thus, the results indicate that reinfusion of own blood is effective and safety method of physiological correction of blood losses after abdominal hysterectomy.

P 27.18
An audit of the effect of cell salvage guidelines on transfusion rates in total knee replacement
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Audit aims: Audit aims was to identify risk factors for transfusion in patients undergoing total knee replacement (TKR) and to produce guidelines for the use of cell salvage. To reaudit after introduction of the guidelines to ascertain if they were effective in reducing transfusion.

Method: In the first part of the audit, data was collected on 179 patients who underwent TKR between February and July 2002. They were analysed to produce the following guidelines for which patients were at higher risk of transfusion and which patients would therefore benefit from cell salvage. TKR performed without tourniquet bilateral TKR women with a preop haemoglobin under 13 g/dl. Men with a preop haemoglobin under 12.5 g/dl. Pre-op anticogulation. After their introduction, we reaudited to establish whether the guidelines were being followed and had resulted in a reduction in transfusion.

Results: In the initial audit there were 137 unilateral and two bilateral TKR’s. A total of 47 U of blood were transfused. In the follow-up audit there were 127 unilateral and six bilateral TKR’s. Of the unilateral TKR’s 98 data sets were compared. Guidelines were followed in 52. Of these one was transfused (2%). The guidelines were not followed in 19 ‘at risk’ patients. Of these four were transfused (21%), three had low preop haemoglobin and one haemophilic. There were 27 patients who were cell salvaged though at low risk of transfusion, none were transfused. Overall 24 U were transfused.

Conclusions: Cell salvage guidelines reduce transfusion rates in TKR.

P 27.19
A restrictive transfusion trigger is a method for blood saving in elective orthopaedic surgery
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Background: A restrictive transfusion trigger (RTT) is said to save up to 80% of allogenic blood transfusions. We conducted a randomised clinical trial in three hospitals to compare a standard transfusion policy (STP) to a RTT in patients undergoing elective orthopaedic hip/knee replacement surgery. The actual STP varied among hospitals, the RTT was fixed.

Methods: Patients were randomized before surgery and stratified for hospital, type of surgery (hip/knee) and risk level (age-dependent).

Results: A total of 57 consecutive patients were enrolled – 563 were available for the intention-to-treat (ITT) analysis. 69.8% patients were not transfused at all. Overall bloodsaving in hospital no. 1 was 0.435 U/patient [95% CI (1.0 – 1.0 + 0.1)]. In hospital no. 2 the overall reduction was 0.3 U/patient [95% CI (0.5, 0.05)]. In hospital no. 3 there was an increase in blood use of 0.26 U/patient [95% CI (0.003, 0.5)]. However, there was a significant interaction with risk and age. The average bloodsavings in hospital no. 1 was highest in low-risk patients (4.5 U, 95% CI (−6.6, −2.4)). In hospital no. 3 the increase was caused by the older patients, who were randomized for a less restricted policy in the RTT of the hospital. There was no difference in hospital stay or mortality.

Conclusions: In all hospitals the most restrictive trigger led to a reduction of blood. The success of implementation of a RTT depends on the STP itself and the risk/age distribution.

P 27.20
Evidence-based protocol for cross-matching in spinal surgery
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Purpose: To optimize transfusion requests in lumbar spinal surgery by creating evidence-based guidelines and prospective audit.

Methods: The data on elective operations performed on the lumbar spine from June 2002 to June 2003 was collected from the spinal unit database and cross-referenced
with the records of the blood transfusion laboratory. From this, the cross-match: Transfusion ratios (C:T Ratio) and the transfusion index (TI) for common procedures were calculated. Based on these results, a maximum surgical blood ordering schedule (MSBOS) was created. The MSBOS was then prospectively audited for 3 months.

Results:

Table 3. Prospective audit

| Operation          | C:T ratio | TI |
|--------------------|-----------|----|
| Discectomy         | 28.67     | 0.034|
| Posterior decompression | 32.67    | 0.051|
| Posterior fusion   | 10.48     | 0.391|
| Anterior/A-P fusion| 28.67     | 0.176|
| PLIF               | 17        | 0.25 |

Conclusion: The implementation of the MSBOS has led to savings in workload for the transfusion department and financial for the trust. Over 1 year it could be expected that this MSBOS would reduce the cross match requests by about 600 U. With the supply of blood under threat in the future, the departments that have analysed and rationalized their requests will be treated preferentially.

P 27.21

Bellovac ABT – audit of effectiveness postintroduction

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The Astratech Bellovac autologous transfusion system was introduced at the Yorkshire Clinic in September 2002. It is used in all total hip and knee replacement operations with an aim of reducing patient exposure to allogeneic blood and its possible risks of infection or transfusion reactions. The system allows blood that would normally be drained and disposed of, to be filtered and reinfused to the patient. An audit was carried out over a 3-month period and data was collected from patient notes and laboratory records. Crossmatching, transfusion rates and pre and postoperative Hb levels were compared, prior to and after the introduction of Bellovac ABT. The audit demonstrates an increase in postoperative Hb levels and a decrease in the number of inappropriate allogeneic transfusions given in theatre. The percentage of patients transfused donor blood for a single hip replacement has fallen from 40 to 11.8% and for a single knee replacement from 17.6% to 5.9%. Over the 12-month period since its introduction, the number of donor units crossmatched has fallen by 42%. The maximum surgical blood order schedule has been reduced from 4 to 2 U for single joint replacements and from 6 to 4 U for bilateral procedures. As a backup system, Bellovac ABT appears to be an effective method of reducing allogeneic blood usage in total knee and hip replacements.

P 28.1

Use and clinical effectiveness of HPA-1a/5b-negative platelet transfusions in NAIT

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Aims and method: The optimal treatment of neonatal alloimmune thrombocytopenia (NAIT) is the transfusion of compatible donor platelets. The National Blood Service in England has established panels of ‘accredited’ donors negative for HPA-1a and HPA-5b, the most commonly implicated alloantigens. To determine the frequency of use and clinical effectiveness of donations from these donors we have retrospectively tracked all units collected over a 13-month period from the Oxford accredited panel.

Results: Hyperconcentrated platelets (HPCs; 95%) collected were issued for intrauterine transfusion to fetuses at risk of NAIT due to presence of maternal platelet alloantibodies and previously affected siblings; 31% of pediatric platelet concentrates (PCCs) collected were issued of which 57% were used for cases of suspected NAIT; 54% of adult therapeutic doses collected were issued; 5% of these were used in cases of suspected NAIT or proven post-transfusion purpura (PTP). Good increments were seen in most NAIT cases transfused with HPCs or PPCs, and a moderate increment in one case of PTP.

Conclusions: We conclude that establishment of accredited panels is justified and enables delivery of a clinically effective treatment for NAIT. Increased use and cost-effectiveness could be achieved by delivery of an educational programme to neonatal unit clinical staff to increase awareness and appropriate treatment of NAIT.

P 28.2

Octaplas and uniplas efficacy and safety in critically ill neonates

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The efficacy and tolerance of solvent-detergent plasma (SDP) was assessed in all transfused neonates in two large maternity hospitals from April 2002 through October 2003. Two patients’ records were not traced and were excluded.

Results: A total of 41 neonates received 67 transfusions of SDP. Octaplas – 36, Uniplas – 5, mean volume 34.3 ± 24.2 ml (18.4 ml/kg). A total of 31 (76.6%) had coagulopathy without haemorrhage; eight (19.5%) had haemorrhage [excluding intraventricular haemorrhage (IVH)], with coagulopathy. Underlying conditions comprised neonatal sepsis, respiratory distress syndrome, necrotizing enterocolitis, IVH, coagulation factor deficiency. There were nine fatalities (22%): two from massive pulmonary haemorrhage in preterm infants, three from extreme prematurity and sepsis, two from perinatal asphyxia, one each from meningitis and congenital echovirus infection. No adverse reactions were observed for SDP infusion. Twenty of 41 and five of 41 patients received platelets and cryoprecipitate respectively. Pre- and post-transfusion APTT was measured in 40 transfusion episodes, PT in 39, fibrinogen in 39. Mean APTT improved from 68.9 ± 37.4 to 44.0 ± 15.6 (t = 4.79; P < 0.001); PT from 28.7 ± 20.3 to 20.7 ± 4.2 (t = 2.64; P = 0.02); fibrinogen from 1.94 ± 1.1 to 2.51 ± 1.44 (t = 3.41; P = 0.002).

Conclusion: Octaplas and Uniplas in therapeutic doses have very good overall clinical tolerance in neonates and are associated with correction of coagulopathy in these patients.

P 28.3

Cord blood transfusion in cold hemagglutinin disease associated with Mycoplasma pneumoniae infection

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We reported a case of cold hemagglutinin disease associated with Mycoplasma pneumoniae infection transfused with cord blood transfusion for correction of anemia. Cold hemagglutinin disease is a hemolytic anemia most commonly associated with cold reactive autoantibody with anti-I specificity. A 6-year-old male patient was transferred to the emergency department from the community hospital with severe hemolytic anemia. The patient showed the confused mental state. The hemoglobin level was 3.8 gm/dl and dark colored hemoglobinuria was noticed. Many irregular RBCs aggregates were observed on peripheral blood smear, DAT was strong positive, cold antibody titer was 1:1024, Mycoplasma antibody titer was 1:1280 positive. We could
not find the compatible blood due to severe cod autoagglutinin. At least incompatible leukoreduced RBCs was issued, but hemoglobin level did not corrected after transfusion. On the basis of the fact that the level of I antigen on cord red blood cells is extremely low, he was transfused with 60 ml of ABO blood type-matched cord blood. No complication from the transfusion was observed. Due to the deficiency in cord blood supply, leukoreduced irradiated RBC 100 ml was transfused three times thereafter. The hemoglobin level began to increase from the fifth hospital day. The patients was discharged without additional transfusion on the 11th hospital day with complete remission.

P 28.4
Supernatants from stored units of cord blood suppress LPS-stimulated TNF-α release in cord blood
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Background: In an ongoing study we examine the quality of stored RBC units of umbilical cord blood (CB) for autologous transfusion to newborns. Premature infants have an increased risk for infections, we therefore wanted to examine if nonleukoreduced CB units contain immunosuppressive substances.

Material and method: Supernatants (sup.) from CB red cells stored in either SAG-M (n = 11) or PAGGS-M (n = 11) were collected 0, 7, 14, 21, 28, 35 days after harvesting the CB and frozen at -20°C. Freshly heparinized CB was incubated with 10% sup. from CB and 50 ng/ml LPS. After 6 h at 37°C, these samples were centrifuged and the sup. frozen at -80°C. After thawing, the amount of TNF-α was subsequently assessed by ELISA.

Results: The LPS-induced TNF-α production decreased with increasing age of the CB units from which the sup. were harvested. The LPS-induced TNF-α production after addition of sup. from day 0 to day 35 varied from 5834 ± 2515 pg/ml to 3087 ± 1733 pg/ml (P < 0.001, repeated measures ANOVA). The LPS-induced TNF-α production did not differ significantly between units stored in SAG-M and PAGGS-M. By including the change in WBC number and hemolysis as covariates in the ANOVA model, the change in TNF-α production over time turned out as not significant.

Conclusion: The observation that LPS-induced TNF-α production decreased when CB was incubated with sup. from CB units of increasing age was probably explained by increased lysis of WBC and/or RBC in the CB units during storage.

P 28.5
ABO haemolytic transfusion reaction (HTR) in a neonate
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Background: Maternal anti-A/anti-B into consideration when selecting blood for neonatal top up transfusions, whereas the neonatal transfusion guidelines do. We report a severe ABO HTR in a neonate who received a top up transfusion.

Case history: A premature infant referral grouped as A Rh D-positive with a negative antibody screen. DAT was negative. No maternal sample was available. A group A Rh D-negative paedipack was requested for transfusion due to mild anaemia (129 g/l), and pulmonary hypertension. Mild hyperbilirubinaemia (166 μmol/l) was also present.

Results: Anemia, severe hyperbilirubinaemia and renal failure occurred after transfusion of the first paedipack unit. An HTR was suspected but not reported. DAT was positive (2+) but not investigated. A second unit was transfused and the anemia and hyperbilirubinaemia increased in severity (Hb 100 g/l; bilirubin 442 μmol/l). DAT was again positive (2+) and anti-A eluted. Maternal anti-A was detected in the infant’s plasma. The infant recovered after exchange transfusion with 0 Rh D-negative blood.

Discussion: Maternal anti-A present in the infant’s plasma was responsible for the HTR, and was aggravated by mild ABO HDN. Not reporting the first HTR or investigating a positive DAT exacerbated the situation. All neonatal samples requiring top up transfusions are now tested for IgG anti-A and anti-B when a maternal sample is unavailable.

P 28.6
Assessment of Safety for the INTERCEPT® blood system for platelets in neonatal preclinical model
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Background: The INTERCEPT Blood System for platelets uses 150 m amotosalen (S-59) and 3 J/cm² UVA light to inactivate pathogens and leukocytes in platelet concentrates with retention of therapeutic function.

Methods: A preclinical neonatal study was conducted to assess the safety of INTERCEPT platelets for use in pediatric patients. Neonatal rats (age 4–28 days), received daily IV doses (10 ml/kg; twice the human dose) of 35% plasma/65% InterSol(Suppl. 3), S17–S92

Results: There were no test article related mortalities or changes in clinical signs, body weight, urinalysis, hematologic, clinical chemistry, gross pathology, or histopathology. The dose of S-59 ranged approximately from 1.4 to 460 mg/kg/day in the test groups. At up to approximately 460-fold the human clinical exposure (1 mg/kg), there was no S-59 accumulation.

Conclusion: There were no toxicologically relevant effects of the INTERCEPT Blood System for platelets in neonatal rats exposed to daily doses of S-59 at least 460 times expected neonatal clinical exposures, suggesting INTERCEPT Platelets are safe for blood transfusion in human neonates.