P2 Donor care and bedside processing

P 2.1 Risk and prognosis of blood donation related complications – a nationwide prospective Danish study
B Aagaard1, B Samuelsen1, J Jørgensen1, K Tittelstad2 and SP Johnsen3
1Blood Transfusion Centre and 2Department of Clinical Epidemiology, Aarhus University Hospital, Denmark

Aim: Complications among blood donors were examined with the aim to reduce the risk.

Materials: Complications related to blood donation are in Denmark registered in a central database in the period 1997–2002. Data include symptoms, donor characteristics, and economical compensation.

Results: Complications were reported in 433 of 1 833 000 donations, i.e. 24 complications per 100 000 donations. This ratio is in accordance with previous finding in a smaller but representative area. The complications included – 319 needle stick injuries (ratio: 18), 107 vasovagal reactions (ratio: 5), and seven other complications (ratio: 1). Prolonged symptoms (>12 months) occurred in 64 (ratio: 3), and a more than 5% reduction in working capacity was registered in 26 (ratio: 1). Severe symptoms developed especially in high age donors (>50 years), after needle injuries, or after an immediate debut of symptoms. Needle injuries followed by parasthesia was strongly associated with an increased risk of prolonged symptoms.

Conclusion: The absolute risk of donor related complication was low. Nevertheless, for blood donors the risk is still unacceptable high, in particular for prolonged and life lasting symptoms or some degree of disablement. Changes of needle insertion technique and treatment of complications have been introduced to reduce the risk of severe complications and improve the prognosis.

P 2.2 Evaluation of ALYX automated collection of double red blood cell dose
C Areal*, F Hernández, A Castrillo, A Castro, M Abalo, J Flores, M Adelantado, J Cid and A Eiras
Centro de Transfusión de Galicia, Santiago de Compostela, Spain

Purpose: As blood transfusion demand is increasing, and availability of donors is shrinking, double red cells (RBC) collection has become a powerful tool, especially when done in a mobile unit. Evaluation of ALYX Component Collection System® (Baxter) for 2xWBC-depleted RBC (2xLRBC) automated collection is summarized.

Methods: Data on donor comfort and post-donation conditions, operator friendliness and ease of use, collection and filtration times, and product quality parameters (volume, Hct., Hb., haemolysis, residual WBC) were recorded. Statistics was done using MS Excel.

Results: 2xLRBC collections were performed on 21 donors and no statistical difference between the paired units (P > 0.05) for volume (278.5 ± 14.6 ml; mean ± sdev), Hct. (55.2 ± 2.8%), Hb (51.3 ± 4.2 g), haemolysis (0.29 ± 0.38%) and residual WBC (0.15 ± 0.22 x 10⁹) were observed. Collection time was 24 ± 4.1 min, and filtration time 6.23 ± 4.03 min. Donor Hct. was decreased 8.9 ± 1.3% with dilution effect (immediately after donation), and dilution free estimated loss was 6.7 ± 0.5%. Donors had a positive experience, particularly with the short collection time. No adverse reactions were observed during donation. Operators also found it very easy to use.

Conclusion: Highly standardized 2xLRBC units were collected in a very easy process for both donor and operator, facilitating its use in mobile units. As Hb collected per unit was higher than CE guideline (40 g Hb), collected volume could be reduced, shortening the process time even more.

P 2.3 Vitamin supplements to accomplish a preoperative autologous blood collection program
J Cid*, X Ortín, R Milá, V Merino, E Contreras and E Elies
Centre de Transfusion I Banc de Teixits, Tarragona, Spain

Background and aim: It is a common practice to administer vitamin supplements to blood donors who are candidates for a preoperative autologous blood collection program (PABCP). We investigated the role of iron and folic acid as vitamin supplements to accomplish our PABCP.

Material and methods: We prospectively, randomly assigned 147 autologous blood donors who underwent to elective orthopedic surgery to receive no vitamin supplement, iron (105 mg of elemental iron/day), or iron + folic acid (5 mg). All of the donors had a haemoglobin value ≥115 g/l at the start of the study. Supplement vitamins were started 1 month before the donation of the first autologous unit. We used a weekly schedule for blood collection and we drew two (for knee surgery) or three (for hip and scoliosis surgery) autologous units. Blood collection was performed if haemoglobin value was ≥105 g/l.

Results: There were 136 donors who were eligible to study. They were 49 men and 87 women with a median age of 69 years (range: 16–82). The PABCP was accomplished in 37 (86%) of 43 donors who receive no vitamin supplement, in 41 (85%) of 48 donors who received iron supplement and in 38 (84%) of 45 who received iron + folic acid supplement.

Conclusion: The use of vitamin supplements is insufficient to accomplish our PABCP.

P 2.4 Prevalence for 9 serological markers in blood donors tested in CTSP-UNR (2003–2004), Argentina
L Di Tullio Budassi*, MJ Spoletti and O Fay
Centro de Tecnología en Salud Pública – Universidad Nacional de Rosario, Argentina

Objective: The aim of this study was to determine prevalence of obligatory markers for blood transmitted diseases.

Design: Retrospective observational study including the last 12 months.

Material and methods: 2429 serum samples from the University Centenario Hospital were sent to our service for performing pretransfusion serological studies. We have tested nine screening markers suggested by law: HBsAg by EIA, Hbc-antibody by EIA, HIV antigen-antibody by EIA, HIV antibody by EIA, Trypanosoma pallidium-antibodies by TPPA, Brucella abortus- antibodies by agglutination test, Trypanosoma cruzi-antibodies by ELISA test and Trypanosoma cruzi-antibodies by indirect haemaglutination test and HIV 1,2 /Ag-Ab by EIA.

Results:

| Screening test | Positive donors | Prevalence |
|----------------|-----------------|------------|
| HBsAg EIA      | 14              | 0.56%      |
| HBc-Ab EIA     | 87              | 3.60%      |
| HCV-Ab         | 22              | 0.92%      |
| HIV 1,2 /Ag-Ab | 5               | 0.21%      |
| HTLV III-Ab    | 12              | 0.49%      |
| T.cruzi-Ab HAI | 111             | 4.59%      |
| T. cruzi-Ab ELISA | 106         | 4.18%      |
| B. abortus-Ab  | 1               | 0.07%      |
| T. pallidum-Ab TPPA | 56         | 2.3%       |

Conclusions: In our region, the prevalence for blood transmitted disease markers have shown coincidence with other national public services, except for Trypanosoma cruzi antibodies (ELISA and HI tests), which shows a higher prevalence at Rosario city. Although we are not in Chagas disease endemic area, this is probably due to internal migration of donors from endemic areas to Rosario city in the last 15 years.

P 2.5 How do you use Haemocrome analysis for blood units validation?
G Facco*, L Albin, T Zilli, L Valle and A Massaro
Transfusion Medicine Department AO OIRM-Sant’Anna – Turin, Italy

Haemocrome (H) is compulsory, according to Italian law, not only as one of donor’s selection criteria but also for Blood Units (BU) validation. Since 1971 we perform Haemoglobin levels before every standard Blood Donation (BD) and H at the end. Results are used to define donors’ general health conditions, donation eligibility and screen haematological alterations (HA). From 1998 H is also applied for BU validation to improve BU safety and quality. So, after post donation
P 2.6

Donor comfort and safety of the ALYX component collection system

T Hundthauer1, A Doescher1, V Franck1, W Gebauer1, EK Peterhans1, T Mueller1 and F Schunter1
1DKR-Blood Donation Service NSTOR, Germany and 2Baxter Healthcare BV, Belgium

Background and purpose: Blood component collection by apheresis could serve to optimize collection efficiency in times of shrinking donor numbers. The present paper evaluates donor comfort and safety of the new mobile apheresis device ALYX.

Material and methods: Thirty-six experienced male blood donors were enrolled for an ALYX double-dose red cell collection. Observed side effects during apheresis and the following 5 days were assessed by standardized questionnaires. Clinical data including heart rate, blood pressure, hemoglobin concentration (Hb) and several sensitive coagulation parameters were taken pre and post donation.

Results: Thirty-four procedures were completed successfully. Ninety-four percent of donors felt no impairment of physical performance and would be willing to donate with ALYX on a regular basis. Seventy percent were free of ACD-induced side effects. No serious side effects were observed and vital parameters remained basically unchanged. Apart from an elevated thrombin-antithrombin complex (TAT) concentration usually also seen after conventional blood donations, coagulation parameters remained normal. Except in one donor, hemoglobin concentration postdonation did not fall below 11.0 g/dl.

Conclusion: Among experienced blood donors ALYX is well received. In praxi it proves to be safe and feasible. Side effects are similar to those known from other apheresis devices. Hemoglobin concentration seems to be a critical parameter, as some European countries require a Hb of at least 11.0 g/dl after donation.

P 2.7

Cancer screening of blood donors by electron spin resonance spectroscopy (ESR/EPR)

G Matthies1-3, K Schnurr1, U Siwertz1 and G Fischer4
1Institute of Transfusion Medicine, Leipzig, 2MedInnovation GmbH, Wildau and 3Red Cross Institute of Transfusion, Chemnitz, Germany

Background: Current tests for screening of blood do not include tumor marker. There is little evidence for transmission of cancer by transfusion, the scientific question is still unresolved. To contribute to an answer look back samples from donors who suffered of cancer after their last donation) were analyzed by a new ESR/EPR spin probe method to detect early stages of cancer in these donors.

Materials and methods: The ESR/EPR tumor marker test is indicating an active malignant process and offers the possibility to detect in donor serum all cancer types, with an outstanding diagnostic sensitivity and specificity. One hundred and twenty-three look back samples from 48 donors with postdonation report on cancer (cancer types: breast, prostate, kidney, cervix, skin) were analyzed.

Results: In look back samples of these donors cancer 39% did show a positive ESR/EPR test, up to 31 months before cancer diagnosis. There are time periods (3–5 months) of active and silent tumor activity, in prostate, kidney and bladder cancer. In 284 healthy donors could not be obtained any abnormalities.

Conclusions: We assume that in several cases blood components taken from donors with an active tumor have been transfused. To prevent transmission of cancer by blood components, ESR/EPR tumor screening may be added to guidelines to increase transfusion safety.

P 2.8

High eligibility and willingness to donate double red blood cells in two European donor populations

J Muncunill1, W Nussbaumer2, J Zamora1, D Schoenitzer1, N Nussbaumer3, MT Jimenez1, E Gizona1, MA Bernal1, A Forteza1 and F Baesa4
1Banc de Sang i Teixits, P. Mallorca, 2Transfusion Medical Department, Innsbruck, 3Biostatistics Department, Univ. Complutense and 4Baxter Europe, Madrid, Spain

Purpose: Double red blood cell (2xRBC) collection is one way to solve availability shrinkage in blood centres. Donor eligibility frequency under CE and AABB guidelines was tested in two very different European populations.

Methods: Population 1 (n = 385; >1000 m altitude; Tyrol) and population 2 (n = 166; sea level; Mallorca) were tested for location, sex, height, weight, age, donation history, willingness to donate, Hb, Hct. Standards for 2xRBC: CE guideline >70 kg, Hct >42% and Hb >14 g/dl; AABB requires Hct >40% for all, >167 cm and >48 kg for women, and >155 cm and >59 kg for men. Data in access database were analyzed with SPSS.

Results: Twenty-five per cent donors are simultaneously eligible for CE guideline and willing to donate 2xRBCs, and for AABB that frequency ranges 20–40% depending on the volume to donate. Seventy-five per cent donors had only been whole blood donors, >15% new donors and <10% were previous apheresis donors. Lower eligible rate among women than in men for CE guide (4.4% in T and 0% in M vs. 69.6% in T and 37.7% in M), and for AABB (14% vs. ~93%). Observed general lower eligibility rate in M than in T for CE guide (28% vs. 39%).

Conclusion: Blood centres should seriously consider taking advantage of the >20–30% of 2xRBCs eligible + willing donors, which could reach 36% by stimulating donor willingness with education. Donor ineffectiveness on automated collection is not a restraint to 2xRBC donation. These conclusions are quite powerful as they come from two very different populations in Europe.

P 2.9

Prevalence of anemia in a donor population in Chile

MA Rodriguez2, A Vázquez, MS Urquieta, C Valenzuela, M Spencer, C Lyng, Cl. Cerón et al.
Banco de Sangre Hospital Naval Almirante Nef, Centro de Sangre Valparaı́so, Chile

Objectives: To know the prevalence of anemia in a population of blood donors from the region which has reached the higher levels of voluntary blood donation in the country.

Material and methods: We studied blood donors from the Naval Hospital and Valparaiso Blood Centers between December 2001 and May 2003, selecting those which were rejected for low hemoglobin. Hemoglobin was measured using hemoglobinometer or with the cupper sulphate method. We included male donors with hemoglobin below 13 g/dl and females below 12 g/dl. All anemic donors had blood counts using a Coulter Max-M counter, Serum Iron, Total Iron Binding Capacity, Serum ferritin by ELISA and erythrocite protoporphirine. Anemic donor were counselled and given a full report of findings.

Results: There were studied 10 664 blood donors, 4460 from Hospital Naval Blood Bank and 6204 from Valparaiso Blood Center. One hundred (0.93%) blood donors were anemic, six males and 94 females. Hemoglobin levels between 11.6 and 12.9 g/dl in males, and 8.3–11.9 g/dl in females, 33 (33%) had an iron deficiency anemia, 64 had normal iron status and three were diagnosed as having minor beta thalassemia.

Conclusion: It was found a 0.93% blood donor rejection for anemia in a population of donors which reached 40% voluntary blood donation. Most of the anemic donor were females (94%) which may be related with nutrition and childbearing related iron and other deficiencies.

P 2.10

The standardized finger prick: improving the quality of capillary blood sample collection

T Scanes1 and L du Plessis
The South African National Blood Service

Introduction: Significant variabiliy in donor finger-prick haemoglobin determinations (cHb) has been noted using the HemoCue Donor Hb Checker device. An attempt was made to reduce this cHb variation.

Materials and methods: Thirty finger-prick samples were collected by standard procedure with samples taken at 15-s intervals for a 2-min period. In a further 30 vol-

Results: Regression on standard collection serial cHb results showed a significant downward trend (P b = 0.05) while cuff collection results did not (P b = 0.05). The first cHb of the series was considered to be the cHb on which donors were passed or failed. These results, from the cuff collections, showed significantly less variance (P-test, P < 0.05) than those from the standard collection.
Discussion: To ensure effective donor screening, accurate and reproducible Hb results are essential. Collection of capillary samples at standardized pressures appears to reduce Hb variation.

Conclusion: The application of a blood pressure cuff, inflated to 40 mmHg, during capillary blood collection appears to increase accuracy and precision of Hb. This could improve donor safety by avoiding inappropriate bleeding of ineligible donors.

P 2.11

Donor screening proficiency testing as an educational and motivational tool
T Scanes* and L du Plessis
The South African National Blood Service, South Africa

Background: Phase 1 of replacing the CuSO4 with the HemoCue Donor Hb Checker was instituted and an IQAS started. This presentation reports on progress for the first 24 cycles.

Materials and methods: Monthly red cell samples were sent to operators of each instrument. Hb was measured twice daily for 3 days. From the results, scores reflecting operator and instrument performance were calculated. Performance and corrective action reports were prepared. In these reports operators and instruments were rated as excellent, average, poor or bad.

Results: The number of instruments has grown (Cycle 1 = 115 instruments; Cycle 24 = 136). A steady improvement in result returns (cycle 1 = 90% and cycle 24 = 100%), total result precision (CV%) of 4.8% for 616 results for cycle 1 and 1.8% from 815 in cycle 24 and operator ratings (tabulated below) have been seen over the 24-month period.

| Period  | Excellent (%) | Average (%) | Poor (%) | Bad (%) |
|---------|---------------|-------------|----------|---------|
| Cycle 1 | 78.3          | 10.4        | 5.2      | 6.1     |
| Cycle 24| 98.5          | 1.5         | 0        | 0       |

Discussion: Participation is voluntary and the increase in participant numbers, the improvement in returns and performance parameters indicate the motivational and educational benefits of the scheme.

Conclusion: The institution of a scientifically sound proficiency testing program, in a technical analysis system operated by clinically trained operators, can be motivational and educational if the program design avoids the big stick attitude and the reporting system is kept simple and stimulating.

P 2.12

MacoPharma ABC+: a new concept to improve the quality of whole blood collections
F Schooneman*, D Aguettaz, JJ Huart et al
EFS Nord de France, Lille, France

Every day, blood bag units are discarded because of poor mixing or inappropriate anticoagulation. The MacoPharma ABC同胞 has been designed for the automated collection of a unit of whole blood by gravity with proportional addition of the anticoagulant solution throughout the donation. All types of bags, with and without in-line filters, can be collected. Each individual bag system is fitted with a special line connecting to a small satellite bag containing the CPD anticoagulant solution. During the donation, a small peristaltic pump adds CPD to the blood maintaining a constant ratio of the fluids (1 ml of CPD to 7 ml of blood). By achieving this optimal anticoagulant ratio, the ABC machine is able to avoid potential clots. Preliminary results from the first evaluation have shown a recovery of high-quality red cells, demonstrated by measuring good levels of erythrocyte haemoglobin and haemolysis during storage. When comparing blood product parameters achieved using standard blood techniques, the results support the use of the ABC: no coagulation activation, increased concentration of plasma proteins. The ABC is convenient for both mobile and static donation units, for the collection of both adult and paediatric blood bags. The data management software ensures full traceability of the blood collection and accurate data transmission.

P 2.13

Donor safety and comfort for two red blood cell units donation on the ALEY system
F Schooneman*, D Aguettaz and JJ Huart
Etablissement Français du Sang Nord de France

The first protocol available for the new ALEY component collection system (Baxter Healthcare Inc.) allows automated collection of two Red Blood Cell (RBC) units from one donor. The primary objective of our evaluation was to assess donor safety and comfort. Thirty procedures were performed on eligible donors according to French best donation practices. Eligibility criteria were defined in order to ensure a postdonation hemoglobin concentration of 11 g/dl minimum. Predonation ferritin level was also checked. 360 ml of absolute RBC were collected from each donor. Donors physiological parameters and haematological profile were measured immediately before and after donation. Adverse events and donor reactions were observed during the procedure and followed daily during 5 days after donation. Hemolysis in RBC units was followed until end of shelf life (42 days). All procedures came to end without incident in 24 ± 3 min. Donors physiological and haematological parameters changed in a normal and clinically well tolerated range. No serious adverse events were reported during and after donation. Typical minor hypocalcaemic apheresis reactions were reported during procedure at a normal frequency. No calcium compensation was required. All donors confirmed they would agree to donate 2 RBC units again on this system. RBC products hemolysis at day 42 was below 0.8%. This evaluation indicates that 2RBC donation is feasible on the ALEY system, comfortable and safe for eligible donors.

P 2.14

Evaluation of Hemoglobin Color Scale for hemoglobin measurement in blood donor setting
JS Shukla* and RK Chaudhary
Department of Transfusion Medicine, SGPIMS, Lucknow, India

Background: The hemoglobin (Hb) level is the most-used parameter for screening blood donors for the presence of anemia. Presently, CuSO4 specific gravimetric method is the most commonly used technique in the blood donor setting. Recently, the Hemoglobin Color Scale (HCS) has been developed by the World Health Organization as a simple, reliable and inexpensive method for Hb screening in blood donors.

Method: The present study was to compare three methods of Hb measurement in a blood donor setting.

Aim: The purpose of the present study was to evaluate three methods of Hb measurement in a blood donor setting.

Materials and methods: Serial blood samples from 1400 blood donors were screened for the presence of anemia by CuSO4 specific gravimetric method, the HCS and Cyanmethaemoglobin method.

Results: The HCS was more reliable and user friendly than the CuSO4 specific gravimetric method. The HCS method gave 1.5% total false results compared to 6.1% with CuSO4 method (P < 0.0001).

Conclusion: The HCS can be a better replacement for copper sulphate method for blood donor screening for the presence of anemia.

P 2.15

A comparative study: haemocrits LDP REV C and LDP REV C4 protocols using haemocrits MCS+
B Wright*
The South African National Blood Service

Introduction: Our study aim was to compare the collection efficiency, leukodepletion performance and overall performance of the Haemonetics MCS+ using the LDP revision C protocol vs. the LDP revision C4 protocol.

Materials and methods: Fifty voluntary donors donated on the MCS+ according to the LDP REV C protocol and subsequently with the LDP REV C4 protocol. Procedure targets were the same for both groups. Twenty-three males and 27 females donated. The average age was 41 years ranging from 20 to 76 years. The average total blood volume of the donors were 4,914 ml, ranging from 3930 to 7009 ml.

Results:

|                  | REV C  | REV C4 |
|------------------|--------|--------|
| Duration [min]   | 98     | 87     |
| Platelet yield [x10^11] | 4.72   | 5.38   |
| Platelet yield [x10^11] | 2.62   | 3.33   |
| WBC residuals [x10^11] | 0.356  | 0.0922 |
| Citrate reactions | 6.5% mild | 3.5% mild |

Conclusion: The efficiency of platelet collection is significantly increased using the Rev C4 protocol. WBC contamination is lower with Rev C4 protocol and less citrate reactions were observed. The shortened procedure time is an advantage to donors. Continuous leukocyte filtration takes place during collection and no further manipulation is required.
P4 Viral diagnostics

P 4.1 Evaluation of a triplex NAT assay for simultaneous screening of HIV–1, HCV and HBV in blood donations
A Assal*,1, P Morel2, JY Py1, M Baudoucourn1, N Baudoucourn1, F Auger1 and G Andreu1
1EFS Centre Atlantique, Tours and 2EFS Bourgogne-Franche-Comté; France

Objective: The purpose of this study was to assess the analytical sensitivity and the ability of the Procleix Ultro assay to close the window period (WP) for HBV by testing commercial seroconversions panels. The specificity and the practical feasibility were tested as well.

Materials and methods: Analytical sensitivity was assessed by testing dilution series of the WHO standards for HIV-1 RNA, HCV RNA and HBV DNA. The 95% detection limit was calculated by Probit analysis. The WP period closure for HBV was estimated by comparison of the Ultro Assay with the Prism HBsAg assay (Abbott). Panel members were tested neat and diluted (1:2, 1:16 and 1:24). Specificity was evaluated by testing 5648 donations in 706 pools of eight samples in a French blood center. The practical feasibility of the new test was assessed in routine conditions.

Results: The 95% detection limits are about 13 IU/ml for HIV-1 RNA, 3 IU/ml for HCV RNA and 6 IU/ml for HBV DNA. The Ultro Assay would close the window period by an average of 19 days on neat samples, 5 days on pools of 8, 4 days on pools of 16 and 2 days on pools of 24 samples. Specificity is 100%.

Conclusion: The Ultro Assay demonstrated good analytical sensitivity and specificity on HBV screening without compromising HIV-1 or HCV detection. HBV was detected by Ultro earlier than by the Prism HBsAg assay on all dilutions. The use of Ultro did not impact the current workload using pools of eight samples.

P 4.2 Different susceptibility of B19 virus and Minute Virus of Mice to low pH treatment
N Boschert1*, J Niederhauser and J Blümel et al
ZLB Bioplasma AG; Paul-Ehrlich-Institute

Background: Parvoviridae are highly resistant, small, nonenveloped viruses. As low pH is frequently applied to process intermediates or final products, we assessed the impact of such conditions on the human cytopathic virus B19 (B19V) and the mouse parvovirus (MVM) which is frequently used as a model for B19V.

Methods: Virus inactivation was monitored by decrease of infectivity and loss of capsid integrity, endonucleases were added after virus inactivation was monitored by decrease of infectivity and loss of capsid integrity, endonucleases were added after virus inactivation was monitored by decrease of infectivity and loss of capsid integrity. Viable virus was determined by titration in KB cells and luciferase activity of infected cells was measured. The impact of pH on capsid integrity was analyzed by transmission electron microscopy and the level of capsid integrity was determined by Western blot analysis.

Results: B19V was inactivated >5 log after 2 h at pH 4, whereas MVM was resistant towards wet heat conditions this is the second report showing, that the HCV RNA was detected in all follow up samples; in two of four under observation for >12 months, the HCV RNA clearance was observed. ALT activity during wp was slightly elevated in 23 of 50 donors and highly elevated (114–2043), at least once in the follow up, in 11 of 16 donors. The probable source of infection was found in seven of 50 donors: sexual transmission – 3, parental exposure – 3, infection within family – 1.

Conclusion: In most donors seroconversion occurred within 3 months of HCV detection by NAT, ALT activity was slightly elevated in some wp donors whereas occasionally highly elevated in most of them after seroconversion, (1) HCVAg was detected in 62% of HIV RNA pos/anti-HCV neg donors, (2) spontaneous HCV RNA clearance occurred in two of four donors under observation for >12 months, (3) probable sources of infection were determined for some donors because of additional epidemiological questioning.

P 4.5 Lack of correlation between HBsAg and HBV DNA levels in blood donors positive for HBsAg and anti-HBC
MC Kuhns, SH Kleinman, AL McNamara, B Rawal, S Glynn and MP Busch*
Abbott Laboratories, Abbott Park, IL, Westat, Rockville, MD and Blood Systems Research Institute, San Francisco, CA, USA

Background: Studies showing a significant correlation between HBsAg and HBV DNA levels have focused on the HBV seroconversion window. Methods: We (1) analyzed HBsAg levels relative to HBV DNA results in 200 confirmed HBsAg positive, anti-HBC reactive blood donors using quantitative PCR (detection limit 400 copies/ml), two research PCR assays with increasing sensitivities (65 gen/ml and 1.3 gen/ml, respectively), and a quantitative HBsAg assay; (2) correlated HBsAg and HBV DNA levels with HBV serological profiles; and (3) evaluated the potential for replacing HBsAg screening with NAT.

Results: Serological profiles for over 90% of the samples were consistent with chronic HBV infection. Correlation between HBsAg and HBV DNA levels was weak (correlation coefficient = 0.33). Seventy-two of 200 (36%) of donor samples had DNA levels under 400 copies/ml. Retesting of the 72 samples by more sensitive PCR assays showed that 60 of 200 (30%) were positive by PCR with sensitivity of 65 gen/ml while six of 200 (3%) required PCR sensitivity of 1.3 gen/ml for positivity. Six of 200 (3%) were negative by all three NAT assays.

Conclusions: HBV DNA levels in HBsAg positive blood donations can be extremely low. About 6% of donations would be negative by current minipool HBV NAT methods. About 3% of donations would remain undetected by sensitive single donor NAT. These results have important implications for future HBV screening policy. It is unlikely that HBV NAT can replace HBsAg screening.

P 4.6 Yield of West Nile Virus (WNV) nucleic acid testing (NAT) of US blood donors
S Glynn, S Kleinman, M Busch*, D Todd, S Schreiber, I Katz, I Powell, L Pietrelli, G Nemo and C Bianco
America's Blood Ctrs., USA, Westat, USA, Gen-Probe/Chiron, USA, Roche USA, NHLBI, USA and Blood Systems Res. Inst., USA

Background: WNV NAT was implemented on July 2003. We present WNV yield at blood ctrs collecting ~50% of US blood supply.
Methods: WNV RNA screening was performed at multiple labs using Gen-Probe/Chiron Procleix TMA assay in minipools (MP) of 16 or on indivi donations (ID); or Roche TaqScreen PCR in MPs of 6. Seventy-two US cts reported screening data at 2-week intervals to a coordinating ctri. Reactive donations were confirmed by alternate NAT and WNV IgM Ab assays [data obtained from test manufacturers].

Results: From July 1 to October 31, 2003, 2.5 million donations were screened for WNV RNA; 874 were NAT-reactive (discard rate: 3.5/10,000 units), 472 confirmed (viremia rate: 1.9/10,000). Rates varied significantly by area and time: the epidemic peaked July 16–31 in cts in northern Texas; Aug 1–15 for cts in Colorado, So Dakota, Wyoming; and Aug 16–31 for cts in Nebraska, No Dakota, Montana. Peak lowly confirmatory pos rates at these cts varied from 33.0 to 104.4/10,000 units. Comparison of MP and ID NAT reactivities in selected areas gave positive predictive value of 94.6 and 11.2%, and false positive rates of 0.8 and 28.3 per 10,000 units, respectively.

Conclusions: WNV screening was highly efficacious in interdicting viremic units, likely reducing transfusion-transmitted infections in the US. Our data reporting system shows that surveillance can be conducted at multiple sites for an agent with varying geographical distribution. ID NAT had much higher unit discard rate than MP NAT.

P 4.7
Multiplex real-time quantitative RT-PCR assay for HBV, HCV and HIV-1
D Candotti*,1, J Temple2, S Owusu-Ofori3 and J-P Allain2
1 National Blood Service, UK; 2 Div. Transfusion Medicine, University of Cambridge, UK; and 3 KATH Blood Bank, Dept. Medicine, Kumasi, Ghana

Background: NAT assays were developed to reduce the risk of transfusion-transmitted infection with blood-borne viruses.

Objective: To lower the cost of NAT, a multiplex real-time QRT-PCR assay was developed for simultaneous detection, identification and quantification of HBV, HCV and HIV-1 in plasma or serum.

Methods: Viral DNA and RNA were purified simultaneously from plasma and loaded in a RT-PCR reaction containing three primers/probe combinations located in the S gene of HBV, the 5'-UTR of HCV and the LTR of HIV-1. Amplification and detection were performed using the Mx4000 Multiplex Quantitative PCR System (Stratagene).

Results: The assay provided direct, single step, identification of amplified viral genome by using three spectrally discernable fluorescent oligoprobes. Genomic amplification of one virus was unaffected by the simultaneous amplification of the other two. Competition between HBV and HIV-1 amplification slightly affected the yield of HIV-1 amplification. Quantification was possible when a single virus was present. The 95% detection limits were 30, 167 and 680 IU/ml for HBV DNA, HCV RNA and HIV-1 RNA, respectively. The multiplex assay detected with similar efficiency strains of HBV genotypes A-F, HCV genotypes 1–6, and HIV-1 subtypes A-G. Applied to 267 10-plasma pools from blood donors, the assay was reproducible, sensitive, and specific.

Conclusion: With further improvements, this assay has the potential to be used for large-scale nucleic acid testing of blood donations.

P 4.8
Improved hepatitis B virus (HBV) immunoassay to detect mutated forms of the surface antigen
D Chien*, A Tahrizi-Wright, Y-L. Fong, B Jaitner, G Ching and B Phelps
Blood Testing Division, Chiron Corporation, Emeryville, CA USA

Background: Due to its mode of replication by reverse transcription of its pregenomic RNA, HBV has a high rate of mutation relative to other DNA viruses. Amino acid substitutions have been described in all HBV DNA encoded viral proteins such as polymerase, nucleocapsid protein (HBcAg) and surface antigen (HBsAg). There are increasing concerns about the contribution of variant HBsAg to vaccine escape, immune prophylaxis failure and false negatives in serological HBV diagnosis. The group-specific 'a' determinant region (amino acids 124–147) has attracted most attention, not only because mutations in the 'a' determinant were found in 10–20% vaccine escapes, but also because of the fact that high-affinity antibodies in response to this region have been used in most HBV diagnoses.

Methods: Rabbit and murine monoclonal antibodies were characterized based on the binding patterns as well as their linear vs. conformational recognition specificity.

Results: [1] Common HBsAg mutations can be detected with mixed monoclonal antibodies incorporated in the assay. [2] Rabbit monoclonal antibody has demonstrated a broad immunoreactivity and higher affinity to HBsAg mutant antigens.

Conclusion: By understanding the HBsAg major immunodominant region structure and by using a combination of rabbit and murine monoclonal antibodies with specificity covering all key mutation locations, maximal anti-HBs-based protection and highly sensitive diagnosis to HBV variants can be achieved.
Conclusions: Based on the 95% detection limit, the probability of detecting HBV DNA for the individual donation when present in a pool of 6 or 24 is respectively 30 and 120 IU/ml. The assay proved to be robust and could easily be implemented in the current setting with a modification of the pooling protocol by preparing intermediate small pools.

P 4.12
Evaluation of a multiplex assay for HCV/HBV-1/HBV NAT screening in The Netherlands
M Koppelman, H Reesink, M Sjerris and HT Cuypers*
Sanquin Diagnostic Services – Amsterdam, The Netherlands
Objective: HIV/HCV NAT testing in Sanquin is performed on pools of 48 donations with semi-automated silica extraction and cDNA PCR. Sanquin is examining introduction of HBV DNA testing and performed an evaluation of the Procleix Ulitro assay in small test pools. The objective of the evaluation is to investigate sensitivity and robustness.
Materials: Dilution series of WHO-4S for HBV-DNA, HCV/HIV-RNA; commercially available reference panels for HBVgtA, HCVgt1 and HIV-1gB (PeliCheck, Sanquin-VQC). Twenty-four replicates were tested. Dilutions of HBV genotypes A to G samples were tested. Robustness was evaluated by screening of 800 donations in pools of 8.
Results: Analytical sensitivity: 95% success rate for a positive result (95% CI) by Probit analysis on log converted concentrations. All genotypes were detected at 100 Geq/ml or better. No false positive result was found and 99.9% of the results were valid.

Panel
|                  | HBV-DNA gA | HCV-RNA gt1 | HIV-1 RNA gtB |
|------------------|------------|-------------|---------------|
| WHO-4S (IU/ml)   | 9.0 (6.0–15) | 3.0 (2.0–5.0) | 32 (15–111)   |
| PeliCheck (Geq/ml)| 313 (186–686) | 101 (61–212) | 89 (47–235)   |

Conclusions: Based on the limit of detection, the probabilities of detecting HBV DNA, HCV-RNA and HIV-RNA for the individual donation when present in a pool of 8 are respectively 72, 24 and 256 IU/ml. HBV genotypes (A–G) are detected with comparable sensitivity. The assay proved to be robust. HBV NAT introduction with the Ulitro assay in small test pools of 8 might be done with a limited expansion of workload.

P 4.13
Implementation of anti–Hbc screening of blood donors: first year’s experience at Héma-Québec
G Delage*, M Germain, F Bernier and S Gélinas
Héma-Québec, Montréal, Canada
Background: In April 2003, Héma-Québec introduced screening by the anti–Hbc test of all blood donations as an additional safety measure.
Aim: The purpose of this presentation is to summarize our first year’s experience with this test.
Material and methods: All donations were tested for anti–Hbc using the Hepatitis B Virus Core Antigen (Recombinant) Ortho HBc Elisa Test System. All repeat-positive specimens were tested for anti–Hbs using the AUSAB ELISA (Abbott). Donors were permanently deferred if they tested repeat positive for anti–Hbc on two separate donations.
Results: Of 177 558 donations tested to date, 2097 (1.2%) tested repeat reactive for anti–Hbc. Of these, only 20 were HbsAg positive. The positivity rate peaked at 1.45% in July and then came down to 1% in December. 1617 were donations found anti–HBc. Of these, only 20 were HBsAg positive. The positivity rate peaked at 1.45% in July and then came down to 1% in December. 1617 were donations found anti–HBc. Of these, only 20 were HBsAg positive. The positivity rate peaked at 1.45% in July and then came down to 1% in December.

P 4.14
Sensitivity of the Procleix®Ultro® assay in the Procleix and fully automated TIGRIS® systems
J Dockter*, C Militar, A Umali, S McDonough, M Deras and C Giachetti
Sanofi Diagnostics Pasteur, Courbevoie, France
Aims: The Procleix Ulitro Assay is a Transcription-Mediated Amplification assay for the simultaneous detection of HIV-1, HCV and HBV in blood donations. The HIV-1, HCV and HBV Discriminatory Assays, containing specific probe reagents, are used to discriminate the three viruses in Ultro reactive specimens. Here we present the analytical sensitivity of the four assays in the Procleix System and in the fully automated TIGRIS System.
Methods: We tested serial dilution panels of the HIV-1, HCV and HBV WHO International Standards, as well as generic variants, in the Ultro and discriminatory assays on the Procleix and TIGRIS systems.
Results: Probit analyses of the analytical sensitivity results indicate that the predicted 95% detection levels for HIV-1, HCV and HBV were approximately 19, 3 and 7 IU/mL, respectively, on both systems. In the Ultro assay, 40 unique specimens infected with HCV generic variants (types 1–6) were detected at 30 copies/ml in both systems. Forty-one unique H BV-1 specimens (groups M (A–G), N and Q) and 55 unique HBV specimens (types A–G) were detected at 300 copies/ml in each system. Similar results were observed in the discriminatory assays for each system.
Conclusion: These results indicate that the Ultro and HIV-1, HCV and HBV Discriminatory Assays are very sensitive for the detection of prototype and subtype viral isolates. Assay performance was similar between the Procleix System and the fully automated TIGRIS System.

P 4.15
Acute HCV seroconversion in a Scottish blood donor
B Down, M Munro, J Buchanan, K Ferguson, F Davidson, C Lecy, L Jarvis, T Jordan and S Lumley
Scottish National Blood Transfusion Service Microbiology Reference Unit, Glasgow; SNBTS Edinburgh and Inverness
Objective: To describe the investigation of a Scottish blood donor who was seroconverted for HCV.
Methods: The donor was identified from a pool of 48 donations tested for HCV-RNA and HCV-IgM. All donors were screened for anti-HCV using a number of screening methods available in Scotland. Details of the investigation were recorded for up to 6 months.
Results: HCV-RNA was detected in a pool of 48 donations tested for HCV-RNA and HCV-IgM. All donors were screened for anti-HCV using a number of screening methods available in Scotland. Details of the investigation were recorded for up to 6 months. This donor was counselled and all further samples were negative.

P 4.16
A fully automated platform for the COBAS Ampliscreen™ HBV, HCV, and HIV-1 tests
Y Yang, Y Wang, D Ohde, M Patel, E Kyger, A Maxwell and J Gallarda*
Roche Molecular Systems, Pleasanton, CA, USA
Objective: The COBAS AmpliPrep™ (CAP) instrument is an automated sample preparation system designed to generically extract viral DNA and RNA from plasma samples. The CAP system lyses viruses and nucleic acid is released and captured by magnetic glass microparticles. This report presents the feasibility of using the CAP system to automatically extract HBV DNA and HCV/HIV-1 RNA simultaneously, and to detect viral nucleic acid using the existing COBAS AmpliScreen™ (CAS) HBV, HCV and HIV-1 Tests.

Materials and methods: A sensitivity study was performed using dilutions of a triplex viral stock prepared from the three viruses. Each diluted sample was extracted with the CAP system, as well as with the manual Multiprep method, and tested by the CAS HBV, HCV and HIV-1 Tests. The specificity of the CAP system was also evaluated by testing HBV, HIV-1 and HCV seronegative plasma. In both studies, one extraction (~200 µl) was split among the three CAS Tests.
Results: The LOD results indicate that the analytical sensitivity of the CAS Tests with CAP extraction was approximately 5 IU/ml for HBV, 25 IU/ml for HCV and 70 IU/ml for HIV-1, which is comparable with the results of the manual extraction method. The observed specificity was 100%.
Conclusions: The CAP system provides an automated sample preparation method for CAS HBV, HCV and HIV-1 Tests. Use of separate PCR assays allows the user flexibility to select any or all of the three assays with this system.
P 4.17
HBV DNA nucleic acid test (NAT) for blood screening: individual vs. minipool testing
Y Yang, Y Wang, D Oude, M Patel and J Gallarda*
Roche Molecular Systems, Pleasanton, CA, USA

Background: Screening of donors for HBV DNA with a sensitive NAT assay can further increase blood safety. The COBAS AmpliScreen™ (CAS) HBV Test, a PCR-based assay designed for blood/plasma screening, was evaluated for both nonclinical and clinical performance.

Materials and methods: Five hundred and fourteen seropositive HBV samples, including 49 acute patient samples, were tested by the CAS HBV Test undiluted and at 1:24 dilution to simulate ID NAT and MP NAT (pools of 24), respectively. To determine if the CAS Test would reduce the window period, 40 serocconversion panels were similarly tested with and without dilution. The CAS Test was also evaluated in a clinical study at five sites in the US.

Results: Of the 514 HBV-positive samples, 514 (100%) and 503 (97.9%), were ID NAT- and MP NAT-positive, respectively. By testing the 40 HBV serocconversion panels, ID NAT detected HBV DNA a mean of 22 days and 19 days prior to Ortho HBsAg ELISA Test System 1 and the Abbott PRISM HBsAg Test, respectively. With MP NAT, the Roche PCR assay detected HBV infection a mean of 17 and 14 days prior to the Ortho and Abbott HBsAg assays, respectively. Four HBV window cases were identified from about 400 000 donations during the US clinical trials using MP NAT.

Conclusions: Both ID NAT and MP NAT detected the presence of HBV prior to licensed HBsAg tests. The CAS HBV Test successfully detected HBV DNA in the intended use setting and may be used for blood screening with MP NAT and/or ID NAT.

P 4.18
Automated and enhanced semi-automated TMA-NAT plasma screening methods for parvovirus B19 and HAV
D Getman*, M Deras, S Vijaysri, M Blankenbiller and L Stringfellow
Gen-Probe Incorporated, San Diego, CA, USA

Aims: To evaluate sample pooling schemes for detection of parvovirus b19 (b19) DNA and hepatitis A virus (HAV) RNA in human plasma using Transcription-Mediated Amplification (TMA) assays with the Procleix® enhanced Semi-Automated System (eSAS) and the automated Tigris® System.

Materials and methods: The b19 and HAV assays use Gen-Probe's magnetic target capture, TMA, and Hybridization Protection Assay technologies. The assays were developed for the eSAS and the Tigris System. Both assays contain an internal control that is captured, amplified, and detected along with the target viral nucleic acids. Dilutions of plasma specimens containing b19 and HAV were tested in 8- to 96-member pools to assess assay sensitivity using different sample screening algorithms.

Results: The HAV TMA assay showed equivalent sensitivity on the eSAS and Tigris platforms, with 95% detection below 100 copies/ml for HAV RNA. A modified, low-sensitivity version of the b19 TMA assay was tested with high titer b19 panels either neat or in multiple pool sizes. This modified b19 assay was sensitive to 10 000 IU/ml, which allowed detection of high titer, but not low titer, b19-positive samples in plasma pools. Conclusion: These results show that the Procleix-format HAV assay showed equivalent sensitivity on the eSAS and on the Tigris System. A reduced sensitivity b19 assay facilitated screening of pooled plasma samples for detection of clinically relevant high titer, but not low titer, b19-positive units.

P 4.19
HCV/HIV1 AB pos. not viremic donors correlation between ratio Ch.Lia RIBA pattern – score and viremia
P Giacca*, M Chiara, G Demarini, G Demarchi, G Cornaglia, M Ricciti, L Albin, P Catapano and AL Massaro et al
Transfusion Medicine Service AO O.R.M., Sant’Anna Tarus, Italy

Background: Serology Lab implemented Ab screening with ABBOTT PRISM for HBsAg, HCV1/2, and HIV since 11 August 1998. Molecular biology Lab implemented NAT Test for HIV1 and HCV on all blood donations from 4 November 2001 with Chiron Procleix HVI1 HCV Assay. Till 31 December 2003 190 000 donations were tested from 50 000 donors/year (7000 new donors/year). Aim of the study is evaluate: not viremic HCV-HIV1 Ab confirmed positive donors, significance of confirmatory assay pattern and band score related to pathologic ALT correlation between Ratio (R) result for serology and viremia.

Method: We compared R results of HCV/HIV1 Ab positive donors by PRISM and ORTHO RIBA bands and score with Procleix TMA HVI1-1 HCV Assay results.

Results: Percentage of HCV Ab confirmed positive not viremic is 30% according to international results on selected blood donors population (normal population 20%). HCV confirmed-positive specimens express all bands with a prevalence = C33 92.8%, C22 90.4%, C100 59.5% and N55 50%. Among this group, donors with pathologic level of ALT-RNA positive show the highest score 4+ for all bands. In HCV indeterminate specimens only c22 (50%) and c33 (50%) are expressed. NAT test shows a good separation between neg. and pos. (all pos. have R > 9). Ch.Lia test’s discriminatory power is not so high despite a strong correlation (100%) between a 6–9 R Prism and RIBA pos. Correlation goes down to 50% with R Prism lower.

Conclusion: Why perform RIBA when Ch.Lia and TMA Ratio are 9?

P 4.20
Three-year review of HIV antenatal screening
E Jackson* and M Reed
NBS, Oxford, UK

Background: HIV testing was first made available to antenatal patients as an opt in system (with midwife counseling) in April 2000. The government introduced this in order to help reduce the numbers of children acquiring HIV from their mothers. Studies based in the western world show that 14.4% of babies born to HIV-infected mothers will themselves become infected with HIV. This can be reduced to 5% if the HIV status is known and appropriate treatment is taken. Targets suggested for all health authorities included an increase in uptake of antenatal HIV testing to 90% by 31 December 2002. This would result in 80% of HIV infected pregnant women being identified during antenatal care.

Objectives: To access the percentage of pregnant women being tested and to determine if the government targets have been met.

Results: Each year the National Blood Service, Oxford, test approx. 14 000 antenatal booking samples. The percentage uptake for HIV testing is shown in Table 1.

Table 1. Antenatal HIV testing April 2000–October 2003

| Month       | April 2000 | April 2001 | April 2002 | April 2003 |
|-------------|------------|------------|------------|------------|
| % HIV tested| 27         | 53         | 68         | 71         |
| % Initial positives | 0.09   | 0.26 | 0.46 | 0.4 |
| Number of HIV positives | 3   | 8 | 12 | 7 |

Conclusion: The government target of 90% uptake for the HIV test has not been met. Therefore new measures need to be developed in order to increase awareness of the high importance of HIV testing for antenatal women.

P 4.21
External quality assessment scheme (EQAS) for donor screening by NAT in blood laboratories
D Jardine*, SJ Best and EM Dax
National Serology Reference Laboratory, Australia

Objectives: Recent developments in technology have introduced Nucleic Acid Testing detect early infection with HIV or HCV in blood donors. To help monitor the quality of NAT the National Serology Reference Laboratory, Australia (NRL) implemented an EQAS. Design, materials and methods: EQAS panels were designed to examine issues including assay sensitivity, and cross-contamination. Results were analysed in the NRL and reported compared laboratories performances. Results: Ten laboratories from Australasia and Singapore and use either Chiron TMA HIV-1/HCV multiplex or Roche AmpliScreen assays. EQAS results identified problems in testing within laboratories, such as results for the NAT EQAS in 2003 showed. All negative samples in the panel were nonreactive in the TMA assay demonstrating no evidence of cross-contamination. Two negative samples in one panel were reactive in the Roche Ampliscreen assay, suggesting sample contamination had occurred, in one testing laboratory. Three of nine 500 Gen/ml HCV RNA positive samples were not detected by the Roche HCV AmpliScreen assay. Two of 32 results for one sample containing low HIV-1 viral load were nonreactive in the TMA assay. The Roche HIV-1 Ampliscreen assay (version 1.5) did not of detect Group O isolates of HIV-1.

Conclusions: By participating in EQAS laboratories are alerted to potential deficiencies in their testing processes and can initiate necessary improvements.

P 4.22
Performance features of the new Bio-Rad HCV antigen and antibody assay: monolisa HCV AG–AB ultra
N Lambert*, L Vermet, M Border, A Clement, M Costaille, V Priegent, O Flecheux, A Sanjuan et al
Bio-Rad, Marne-la-Coquette, France

Objective: Evaluation of the new Bio-Rad MONOLISA HCV Ag-Ab ULTRA test, for simultaneous detection of HCV antigen (Ag) and antibodies (Ab), designed to shorten the prescreening window period over conventional HCV Ab screening assays.
**Methods:** This two-step ELISA assay is based on the combination of an indirect test for the detection of Ab (core, NS3, NS4) and a sandwich test for core Ag detection. No specimen pretreatment is required. The sensitivity was evaluated on HCV seroconversion and versions and results were compared with viral RNA detection and conventional HCV Ab screening assays. Specificity was evaluated using random blood donor samples.

**Results:** Due hundred and twenty-seven of 138 (92%) HBV RNA-positive and Ab-negative specimens from 32 seroconversions were detected, thereby reducing the window period by 22.4 days compared with Monolisa HCV Plus V2 test. The mean delay in detecting HCV infection between HCV RNA and HCV Ag/Ab assays was 0.8 days. With 10,067 anti-HCV-negative sera and plasma tested, the repeat reactive rate was 0.18%.

**Conclusion:** MONOLISA HCV Ag/Ab ULTRA test allows simultaneous detection of HCV core antigen and anti-HCV (core, NS3, NS4) antibodies, thus significantly reducing the time gap between the initial detection of HCV RNA and the first appearance of detectable anti-HCV antibodies. By improving the safety of blood supply, this test could constitute an alternative to NAT for the diagnosis of HCV infection when the cost-effectiveness has to be taken into consideration.

**P 4.23 Nucleic acid testing (NAT) performance in HBsAg positive blood donations**

F Bouchardea, A Girault, A Razer, A Servant-Delmas and L Lapereche*

**Virology Unit, Institut National de la Transfusion Sanguine, Paris, France**

**Objective:** To evaluate the detection of Hepatitis B virus (HBV) DNA by Procleix Ulitro Assay in HBsAg positive blood donations (BD).

**Materials and methods:** Two hundred and fifty-three HBsAg and anti-HBc positive BD samples were simultaneously investigated neat and diluted 1 in 8 to mimic individual donor testing (IDT) and mini-pool (MP) respectively.

**Results:** All HBsAg positive samples were positive in MP and IDT. Of the 203 anti-HBc positive BD, 153 (75.4%) were MP and IDT positive, 35 (17.2%) MP negative and IDT positive, one was MP positive but IDT negative, five (2.5%) were negative with MP and IDT and nine gave invalid results with one of the two procedures (four in MP and five in IDT). After control, 19 of 35 MP negative/IDT positive remained MP negative (six had HBV DNA < 200 IU/ml); three of five MP and IDT negative were still negative with both procedures and two became positive only with IDT (HBV DNA < 200 and 400 IU/ml). The sample MP positive and IDT negative and all invalid results were subsequently found positive.

**Conclusion:** HBV NAT performed in MP and IDT was able to detect 100% of HBsAg/ HBcAg BD. Of the 203 HBsAg/anti-HBc positive samples 19.7% could not be detected in pool of 8, however only 2.9% were missed by IDT. Knowing that in France about 12% of HBsAg positive donations are HBcAg positive, MP-NAT could fail to detect in pool of 8, however only 2.9% were missed by IDT. Knowing that in France the time gap between the initial detection of HCV RNA and the first appearance of detectable anti-HCV antibodies, by improving the safety of blood supply, this test could constitute an alternative to NAT for the diagnosis of HCV infection when the cost-effectiveness has to be taken into consideration.

**P 4.24 Reduction of the HIV diagnostic window with genescreen ultra HIV Ag/Ab**

D Leloucy, J Sice, C Le Sager, S Denis, E Billy, JM Jego and S Gadelle

**Bio-Rad, France**

**Background:** In order to reduce the window phase between time of human immuno deficiency virus infection and laboratory detection, a new generation screening assay, Genscreen Ultra HIV Ag/Ab has been developed.

**Methods:** Genscreen Ultra HIV Ag/Ab is a combined antibody-antigen, sandwich enzyme immunoassay, using specific anti-p24 monoclonal/polyclonal antibodies for Ag capture, HIV1 and HIV2 antibodies for HIV 1/2 Ab capture. To validate the performance of this combined assay, 86 seroconversion panels and a large number of HIV1 and HIV2 Ab subtype samples from different geographical locations were used for evaluating the assay specificity and a population of unselected blood donors were used for evaluating the assay sensitivity. The results were compared to Ab screening assay Genscreen HIV 1/2 version 2 and to several combined antibody-antigen assays: Vironostika HIV Uni-Form II Ag/Ab and Enzygnost HIV Integral, Assym HIV Ag/Ab Combo, and Murex HIV Ag/Ab Combo.

**Results:** Based on compiled data on 85 seroconversion panels, mean detection is earlier with Genscreen Ultra HIV Ag/Ab than the other assays. The Ag sensitivity has been evaluated at 14 pg/ml. The window period is decreased from 13 to 8 days, compared with Genscreen Plus.

**Conclusion:** Genscreen Ultra HIV Ag/Ab allows earlier diagnosis of HIV infection, enabling significant reduction of the window phase.

**P 4.25 Validation of the Procleix Ultrio Assay in Poland**

E Brojer, M Ledzowska*, J Medynska, D Kubicka-Rusel, A Gronowska and G Liszewski

**Institute of Haematology and Blood Transfusion, Warsaw, Poland**

**Objective:** To evaluate the detection of Hepatitis B virus (HBV) DNA by Procleix Ultrio Assay in HBsAg positive blood donations (BD).

**Materials and methods:** Two hundred and fifty-three HBsAg and anti-HBc positive BD samples were simultaneously investigated neat and diluted 1 in 8 to mimic individual donor testing (IDT) and mini-pool (MP) respectively.

**Results:** All HBsAg positive samples were positive in MP and IDT. Of the 203 anti-HBc positive BD, 153 (75.4%) were MP and IDT positive, 35 (17.2%) MP negative and IDT positive, one was MP positive but IDT negative, five (2.5%) were negative with MP and IDT and nine gave invalid results with one of the two procedures (four in MP and five in IDT). After control, 19 of 35 MP negative/IDT positive remained MP negative (six had HBV DNA < 200 IU/ml); three of five MP and IDT negative were still negative with both procedures and two became positive only with IDT (HBV DNA < 200 and 400 IU/ml). The sample MP positive and IDT negative and all invalid results were subsequently found positive.

**Conclusion:** HBV NAT performed in MP and IDT was able to detect 100% of HBsAg/ HBcAg BD. Of the 203 HBsAg/anti-HBc positive samples 19.7% could not be detected in pool of 8, however only 2.9% were missed by IDT. Knowing that in France about 12% of HBsAg positive donations are HBcAg positive, MP-NAT could fail to detect 17% of HBsAg donations and IDT only 2.5%.

**P 4.26 Hepatitis B surface Antigen escape mutant in a first time blood donor**

S Levicnik-Steinzarin*, U Rahne-Potokar and P Nograsek

**Blood Transfusion Centre, Slovenia**

**Background:** In many countries, detection of HBsAg is the only marker to identify HBV infected donors. Mutant forms of HBsAg thus represent a risk as they may remain undetected by certain commercial immunoassays. HBsAg escape mutants arise in individuals under immune pressure and certain mutations are known to be stable and horizontally transmittable. We present a case of a blood donor with a novel mutation that alters the common ‘a’ determinant of the HBsAg in a way that several immunoassays have missed to detect this HBV infected donor.

**Case report:** A first time blood donor was found highly reactive by PRISM HBsAg (Abbott). Subsequent testing with an alternative HBsAg assay (Biohit) gave a negative result. Additional testing with different HBsAg assays yielded discrepant results. The sample was characterized to be reactive for anti-HBc and anti-HBx as well as low titre HBV DNA. Sequencing of the sample resulted in a mutation in a critical region of the ‘a’ determinant which led to the nondetection in some assays.

**Conclusion:** This case stresses the importance to reliably detect mutant forms of HBsAg. As several commercial assays for HBsAg used in screening are not able to cope with mutants, the risk of undetected chronic HBV infection in the normal blood supply is increasing. Adaptation of HBsAg assays is indicated to overcome these deficiencies or alternative methods need to be considered like anti-HBc screening or the use of HBV DNA pool testing.

**P 4.27 Comparison of Procleix®WNV assay on the semi-automated system vs the automated TIGRIS® system**

JM Linnen*, M Deras, S Burgess, W Wu, A Broulik, M Cass, S Miller, M Lewis, J Cline, M Alden, M Shih, G Dennis, T Horn, C Giachetti and S McDonough

**Gen-Probe Incorporated, San Diego, CA 92121, USA**

The Procleix WNV Assay is an investigational nucleic acid test (NAT) for West Nile Virus (WNV). In 2003, US blood centers using this test on a semi-automated platform intercepted over 800 WNV-infected donations. This study compares performance between the semi-automated platform and the completely automated TIGRIS system. To compare analytical sensitivity, WNV was tested at 20 copies/ml. Over 990 replicates of this low titer virus sample were tested on the TIGRIS system; 720 replicates were tested on the semi-automated system. Confidence intervals for sensitivity were based on the exact binomial distribution. No false positive results were observed. This case stresses the importance to reliably detect mutant forms of HBsAg. As several commercial assays for HBsAg used in screening are not able to cope with mutants, the risk of undetected chronic HBV infection in the normal blood supply is increasing. Adaptation of HBsAg assays is indicated to overcome these deficiencies or alternative methods need to be considered like anti-HBc screening or the use of HBV DNA pool testing.

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Analysis of data from four TIGRIS instruments revealed no significant differences between instruments. Analytical sensitivity results indicated that the TIGRIS results were not statistically different than those observed on the semi-automated system. Specificity was very similar for both platforms. These results indicate excellent comparability between platforms.

P 4.28
Mimicry between HCV protein NS5A and human proto-oncogene LCK: consequences for serological assays
T Veljkovic* and N Vasiljevic
Institute for Nuclear Sciences ‘Vinca’, SCG; Serbian Blood Transfusion Institute, SCG

Background: It has been suggested that molecular mimicry between HCV proteins and some human proteins, representing targets of autoantibodies, could be a source of false positive results obtained by serological assays. We performed informational analysis of all human proteins in order to identify possible antigen which could mimic the non-structural protein NS5A of HCV.

Materials and methods: Informational analysis of NS5A and human proteins from SWISSPROT database was performed by the informational spectrum method. Eighty HCV-negative sera collected from healthy blood donors were tested for reactivity with lck-derived peptide by an ELISA assay.

Results: Informational analysis revealed the proto-oncogene lck as human protein which could be immunologically and functionally crossreactive with NS5A protein. Further analysis revealed high homology between peptide SDFADLIEAL (P-NS5A) derived from NS5A and peptide SDFADLIEAL (P-lck) derived from the lck protein. Six of 80 HCV-negative sera showed strong reactivity with peptide P-NS5A in an ELISA assay.

Conclusions: Presented theoretical and experimental results point out that the molecular mimicry between NS5A and human lck protein could cause false positive results in screening of human sera with diagnostic assays encompassing this HCV antigen.

P 4.29
Detection of proviral DNA HTLV I and II in Portuguese anti-HTLV I/II positive
I Neves*, J Pereira, M Diniz, F Resende, A Ribeiro and G Sousa
Centro Regional de Sangue de Lisboa/Instituto Portugués do Sangue

Introduction: The Human T cell Lymphotropic virus type I (HTLV-I), was identified in 1978 and the Human T cell Lymphotropic virus type II (HTLV-II) in 1982. These virus can be transmitted by sexual contact, sharing needles, from mother to child and by cellular blood products transfusion, from infected blood donors. In the Centro Regional de Sangue de Lisboa we started the anti-HTLV screening in all blood donors in 1992. This screening became mandatory in Portugal since 1995.

Material and methods: The screening has been performed with EIA methods from Ortho Diagnostics and Abbott. The immunoblotting confirmatory assays used were Western blot from Genelabs Diagnostics and InnoLia HTLVII from Innogenetics. The PCR was introduced in 1996 [Amplico HTLV-II-Roch]; in 2002 it was changed: the DNA was extracted with QIAamp DNA blood mini Kit (Qiagen) and the amplification and detection with HTLV DNA Presence from Symbiosis.

Results: Between 1992 and 2003 were collected 387,119 blood units. One hundred and sixty-seven EIA positive samples with negative results by immunoblotting, were also negative by PCR. Twenty-seven EIA positive samples with indeterminate results by immunoblotting were negative by PCR. All EIA positive samples confirmed HTLV I positive by immunoblotting (19 of 387,119) were PCR HTLV I positive. Four samples (1992–1995) were studied retrospectively.

Conclusion: We did not find any HTLV-I positive sample by Immunoblotting and PCR. We found 19 HTLV I positive blood donors, corresponding to 0.005%.

P 4.30
Improving blood safety through double screening: 1-year study at general hospital, Lagos Nigeria
R Olatunji* and A Akamnu
Gen. Hos. Lagos, Nigeria; Univ. of Lagos Teaching Hos, Lagos, Nigeria

Objective: An observation of a steady increase in the prevalence of Transfusion Transmitted Infections (TTIs) brought the issue of double screening of blood into play at the Lagos State Blood Transfusion Service (LSBTS). This was aimed at ensuring the safety of blood to be used for transfusion in the state.

Method: All donors were screened for HIV 1 & 2 and HBsAg using rapid screening reagents before donation. Only those who were negative were bled into bags and all collected pints were rescreened using the conventional ELISA technique for HIV 1 & 2 and HBsAg. All positive samples from the second screening were discarded. Some but not all of these were subjected to confirmatory testing using immunocomplement kit.

Results: Of 3888 donors seen at the donor clinic, 43% (11.13%) were not allowed to donate as they were positive for either HIV or HBsAg; of the 3450 who were bled, 268 pints (8.4%) were discarded because of positivity for either HIV or Hepatitis during the second ELISA screening. Of the 268 units positive on second screening, 104 were due to positivity for HIV. Forty-seven of these 104 were subjected to confirmatory testing. Twenty-three, 18 and six were negative, indeterminate and confirmed positive respectively.

Conclusion: We conclude that rapid screening for HIV alone in donor blood is not safe. Conventional ELISA screening is necessary as a check for units that previously tested negative.

P 4.41
Efficacy of blood donor predonation screening for viral markers with rapid tests
S Owusu-Ofori*, F Sarkodie1, J Temple2, D Candotti1 and J-P Allain2
1 Department of Medicine, KATH, Kumasi, Ghana, 2 Department of Haematology, University of Cambridge, UK and National Blood Service, UK

Background: In high endemic areas for HBV, HIV & HTLV, predonation viral screening of blood donors saves blood bags, allows to inform deferred donors and to store only safe blood.

Objectives: To determine the efficacy of rapid test viral screening by testing negative units for HIV, HCV & HTLV.

Methods: Pools of 10 individual plasmas were tested with a triplex NAT directly identifying HIV, HCV and HBV genomes. Individual samples from positive pools were resolved with single virus NAT and positive donations were re-tested with corresponding rapid test and EIAs.

Results: From 517 pools, 1 HIV, 11 HCV and 8 HBV genome-containing samples were identified as testing errors related to breaches of SOPs. No HIV or HCV RNA-containing donations were rapid test failure. In a subset of 72 pools of donations negative with 5 ng/ml sensitive dipstick, 8, 17 and 29 individual samples (16 from negative pools) were detected with a 1 ng/ml HBsAg sensitive dipstick, 0.2 ng/ml sensitive EIA and HBV NAT (20 IU/ml), respectively. HBV NAT in pools of 10 samples does not detect 50% of HBV DNA-containing donations.

Conclusions: Predonation screening of blood donors is effective provided the testing procedures are adequately followed. Both EIA and rapid tests leave low viral load HBV-containing samples undetected. The implementation of quality assurance schemes appears critical to ensure safe blood in resource-poor settings. NAT in pools of 10 plasmas is insufficiently sensitive for HBV DNA screening.

P 4.42
Comparison of three serologic assays for the detection of HHV-8 in blood donors
E Pena*
Department of Clinical Sciences, Department of Pathology and Laboratory Medicine, University of Kentucky and Department of Laboratory Medicine and Pathology, The Mayo Clinic, USA

Background: The seroprevalence of HHV-8 in blood donors ranges from 0 to 23%. In this study, 685 healthy blood donors were examined to detect serologic evidence of HHV-8 infection.

Methods: All specimens were tested using the HHV-8 latent antibody ELISA designed to detect IgG antibody to latent protein ORF-73 (ORF). All ORF-positives and equivocals and 12.5% of the negative specimens were tested using the whole virus lysate-based HHV-8 IgG Antibody ELISA (WL) and the HHV-8 IgG IFA (IFA) [all from Advanced Biotechnologies Inc, Columbia, MD, USA]. The ELISA results were compared to IFA (the gold standard) and the sensitivity and specificity calculated for each assay. Results: Of the 685 serum samples tested by the ORF, 40 were positive, 641 were negative and four equivocal. A subset of 116 donors (44 ORF-positives or equivocals and 72 randomly selected negatives) resulted in 15 positives, four equivocals and 97 negatives by the WL and two positives and 114 negatives by IFA. The two IFA positives also were positive by both ELISAs. Comparison of the two ELISA assays demonstrated concordant results for only 60.3% of specimens tested. Although the sensitivity of both ELISAs was 100% compared with IFA, the ORF had a specificity of only 65.5%, and the WL a specificity of 88.2%.

Conclusions: The two ELISAs used did not correlate well in predicting IFA positive donors. Both assays gave false positive results compared with IFA.
Prospective study to evaluate screening of plasma pools from blood donors for West Nile Virus RNA
L Pietrelli1, J Gallarda, L Lee-Haynes and the WNV Study Group
Roche Molecular Systems, Inc., CA, USA

Background: The clinical study objective is to confirm the effective use of a West Nile Virus nucleic acid test (NAT) for the detection of West Nile Virus (WNV) RNA in pools of size 6 (and in individual donor specimens at multiple facilities).

Materials and methods: The clinical study was conducted at 11 blood centers in the US. Additional testing was conducted for specimens that were individually WNV-positive. The additional WNV-specific testing included IgM, IgG, alternate NAT, WNV NAT quantitation, and sequencing. In addition, donors from which the WNV-positive samples were obtained were eligible for enrollment in a follow-up study.

Results: The first blood center began testing June 17, 2003. Through November, 2003 over 234,000 pools of six have been tested representing over 1.4 million specimens. Eighty-nine individual specimens have been identified as WNV RNA-positive. Seventy-four of the associated donors (83%) have been enrolled into the follow-up study. Based on follow-up testing, 71 have been confirmed as WNV-positive, one has been confirmed negative, and two are pending test results. Fifteen donors were not enrolled; 14 were presumed to be WNV-positive based on additional testing of the index donation and one was WNV-negative. Seventy-two samples have been quantitated and the median viral titer is 19,500 cfu/ml and a mean of 51,348 cfu/ml (Range: 100-760,000).

Conclusions: The COBAS TaqScreen™ WNV Test has successfully identified WNV RNA-positive specimens.

Analysis of anti-HBC data from a prospective study to evaluate volunteer blood donations for HBV DNA
L Pietrelli*
Roche Molecular Systems Inc, CA, USA

Background: The objectives were to determine the effectiveness of the COBAS AmpliScreena® HBVTest in detecting HBVDNA in pools of 24 and in single unit plasma specimens obtained from volunteer blood donors, in detecting HBVDNA in individual donor units seronegative by licensed HBV tests, and to determine HBV DNA positivity in anti-HBc reactive donors negative for HBsAg.

Materials and methods: Specimens that are anti-HBc+/HBsAg-/HBV DNA Pools negative had Individual Donor Nucleic Acid testing (IDNAT);50 specimens from each five donors from which the WNV-positive specimens were obtained were eligible for enrollment in a follow-up study. All samples negative by IDNAT were negative by HBsAg testing and the other three sites used Abbott. Four of 14 IDNAT positive donors were enrolled in the follow-up study. All samples negative by IDNAT were-negative by alternate NAT, 150 specimens were tested by anti-HBs, and all IDNAT-positive specimens were tested by alternate NAT, quantitated, and donors requested to enroll in a follow-up study.

Results: About 72,000 samples were tested. Two sites used Ortho for anti-HBc and HBsAg testing and the other three sites used Abbott. Four of 14 IDNAT positive donors enrolled in the follow-up study. All samples negative by IDNAT were negative by alternate NAT. Overall, 56% of 750 anti-HBc+ units had anti-HBsAg; 66% Ortho, 49% Abbott.

Conclusions: IDNAT detects HBV DNA in some anti-HBc positive, HBsAg negative donors using currently licensed assays; screening for anti-HBc is likely to prevent the transmission of HBV. IDNAT may be useful in determining re-entry of anti-HBc positive donors.

Prospective study to evaluate volunteer blood donations (VBD) for presence of HBV DNA
L Pietrelli1,2, J Gorlin2, PV Holland, M Strong, C Cousins and G Tegtmeier
1RMS, Pleasanton, CA, 2Memorial, Minneapolis, MN, 3BloodSource, Sacramento, CA, 4Puget Sound, Seattle, WA, 5Gulf Coast, Houston, TX and 6Community, Kansas, MO, USA

In this multicenter clinical trial, the COBAS AmpliScreena® HBVTest was evaluated for effectiveness in detecting HBV DNA in plasma pools of up to 24 Volunteer Blood Donations (VBD), in undiluted plasma specimens from VBD, and in detecting HBV window period cases. Development of serological viral markers in HBV/AIDS negative, PCR positive donors was evaluated and clinical sensitivity and specificity of the assay were assessed. Further testing was required for HBVDNA negative and anti-HBc and/or HBsAg positive or HBV DNA positive and anti-HBc and/or HBsAg negative specimens. Testing may have included Individual Donor NAT (IDNAT), alternate NAT and anti-HBc. All IDNAT positive specimens were tested by alternate NAT and quantitated.

Results: 24,000 pools of 24 VBD were tested; 581,790 donations. Of the 2,988 specimens that were anti-HBc+/HBsAg-/HBV DNA, -11 were IDNAT positive, 2,888 were negative; 89 were not done. Of the 16 specimens that were anti-HBc+/HBsAg+/HBV DNA, -10 were IDNAT positive, five were negative; one was not done. Two confirmed window period cases were identified in pools of 24. One donor became HBsAg+ 17 days postdonation (PID) and anti-HBc+ 60 days PID. The other donor was anti-HBc+ day 22 PID and was not HBsAg+ through 78 days of follow-up.

Conclusions: The COBAS AmpliScreena® HBVTest has identified preseroconversion period window cases.

First report of a HBV genotype G infection by blood transfusion in Germany – case report
M Schmidt1, M Chudy2, M Nuhl3, E Grunet1, E Seifried1 and WK Roth1
1German Red Cross Institute Frankfurt, Germany, 2Paul Ehrlich Institut, Germany and 3German Red Cross, Institute Cottbus, Germany

Background: An acute hepatitis B infection was diagnosed by pool PCR and HBsAg in a 60-year-old blood donor in May 2003. The virus load was 1642 IU/ml and fell to zero 1 month later. The donor presented without clinical symptoms and normal ALT. Anti-HBc (IgM) and anti-HBs were detectable 14 days after diagnosis. He donated alternatingly apheresis plasma and platelets every week.

Material/methods: Single sample HBV PCR was performed on archived samples and recipients samples. Amplified HBV DNA was sequenced and subjected to phylogenetic analysis.

Results: HBV sequences could be amplified from archived samples of two previous thrombophlebitis donors and the respective recipients. Sequences were identified as HBV genotype G and phylogenetic examinations demonstrated close relationship between all HBV isolates. This is the first report describing HBV genotype G infections by blood transfusion in Germany. The delayed diagnosis of the HBV infection of the platelet donor may be explained with the HBV primer design of the screening PCR which was directed at the HBV core region. This genomic region is less conserved between different genotypes than the surface gene region.

Conclusion: For an early diagnosis of HBV infection a PCR with primers targeted to the highly conserved region of the HBV S gene is recommended. Frequent apheresis and double concentrate donations may increase transfusion risks if donors donate blood during the infectious preseroconversion phase.

WNV testing in the US: summary of results for 2002 preclinical and 2003 clinical studies
SL Stramer1, AG Wagner, JE Paolillo, GA Foster, ML Beyers, JT Davis, JP Brodsky and RY Dodd
Scientific Support Office, American Red Cross, USA

Introduction: Investigational NAT for WNV was qualified using donations collected in 2002; screening was introduced in June 2003. Donations from US areas of greatest WNV risk were screened individually (IDT) rather than in minipools (MP).

Methods: WNV NAT was performed in MP5 of 16 or by IDT (Gen-Probe), and confirmed by PCR (National Genetics Institute) and IgM (Abbott); IgG testing (Abbott) was performed on selected samples. 48,620 frozen samples donated in September 2002 from six high-risk US regions were subjected to IDT WNV NAT. Over 2.9 million donations collected in 2003 were screened by MP NAT; IDT was used in two epidemiologic areas (Nebraska and Kansas).

Results: 2002 prevalence of WNVRNA, confirmed-positive donations was 0.95/1000 (95% CI = 0.69–1.26) vs. CDC estimates of 0.24–1.23/1000. Of the 46 confirmed positives, 26 (56%) were detected in MP of 2–16; all MP-reactive/IDT-reactive donations were IgM positive. IgM reactivity was retained for 398 days or longer in 73% of confirmed-positive donors. Prevalence in 2003 was 1:5700 (414 confirmed positives) with the highest prevalence in Nebraska (1:175) and Kansas (1:243). MP NAT in these two regions failed to detect 97 of 179 (53%) confirmed positives; all but six of 97 were IgM positive.
Conclusions: WNv transfusion-transmission was not documented in this system following the MP and IDT WNv NAT protocols for 2003; the strategy of WNv NAT MP ‘surveillance’ followed by IDT for epidemic regions was effective.

P 4.38
The ability of Procleix Ultrio assay to detect HBV DNA in HBsAg positive blood donors
A Taggart1,2, ML Rihel3, E Menatt1, A Zanetti4 and F Azzaro5
1Institute of Virology, University of Milano and 2Immunoematol. Med. Trasf. Niguarda Ca’ Grande, Milano, Italy
Aims: To evaluate the ability of Procleix Ultrio Assay (co-developed by Gen-Probe Inc. and Chiron Blood Testing) to detect HBV DNA in a group of Italian HBsAg positive blood donors.

Materials and methods: In a blood center of Milano 30 000 first-time donors were screened for HBsAg between 1992 and 2003 and 115 (0.45%) tested HBsAg positive. All but five had normal ALT values, were HBsAg negative and anti-HBe+. These five donors had an acute HBV infection and were followed up to a sustained HBsAg clearance. HBV DNA was detected in serum by an in house PCR with nested primers (nPCR) from 5 gene region encompassing a ‘determinant.’ All nPCR products were directly sequenced.

Results: Of 135 HBsAg positive donors, 115 (85%) tested HBV DNA positive by nPCR. About 85% of 5 gene sequences were classified as type D, the remaining as type A. Of the HBsAg positive donors 116 (86%) were reactive by Ultrio. There were seven Ultraio positive, nPCR negative donors and six nPCR positive, Ultraio negative donors. In the five donors with acute HBV infection both Ultraio and nPCR detected HBV DNA from the first to the last HBsAg positive bleed samples and in one donor both Ultraio and nPCR detected an additional HBV positive sample after HBsAg was negative.

Conclusions: Testing by Ultraio adds an additional value for HBV detection and these data support the utility of the test for blood screening. HBV DNA positive donors should be evaluated for the presence or development of chronic liver disease.

P 4.39
West Nile Virus detection by nucleic acid testing of blood donors in the midwestern United States
GE Tegtmeier*, SE Henderson, RL Edwards and JE Menitove
Community Blood Center of Greater Kansas City
Kansas City, MO, USA
Background: In 2003, it was recognized that blood transfusions could transmit WNV to patients, sparking the development and implementation of NAT screening for WNV RNA by July 2003. We report data from WNV NAT of blood donations from the Midwestern US.

Materials and methods: Donor plasma samples were pooled robotically, six per pool, extracted for total nucleic acids and assayed for WNV RNA using the Roche TaqScreen100 WNV Test. Positive pools were deconstructed by individual testing.

Results: The data represent testing from July 1 to October 31, 2003. The first positive sample was detected on August 18 and the last on September 12, 2003. The detection rate during that period was 1:2500. Nine of 10 donors enrolled in a follow-up study had no detectable WNV antibodies in their NAT-positive donation samples. All were NAT-negative 2 weeks thereafter but positive for WNV IgM, IgG and neutralizing antibodies.

Two of the nine donors experienced symptoms compatible with WNV illness.

Donations tested 95
Pools tested 884
Pools positive 16
Pools resolved 314
Detection rate 1:2500

Conclusions: Testing of pooled donor samples with a WNV NAT interfected donations from infected donors thus preventing WNV infection. Detection of WNV was restricted to a 4-week period in late summer.

P 4.40
Relative performance of three West Nile Virus antibody assays in US viremic blood donor specimens
LH Tobler*, H Prince, G Hafner, WW Andrews, C Harrington, J McCausley, W Winkelman, S Caglioni and MP Busch
Blood Systems Laboratory and Research Institute, USA, Focus Technologies, USA, Panbio Ltd, AU, Chiron Corporation, USA
Background: West Nile Virus (WNV) is a Flavivirus transmitted to humans from birds by a mosquito vector. Documented cases of transmission-transmitted WNV occurred during the 2002 epidemic. At the time, prevalence of viremia and serology markers among blood donors was unclear. During the 2003 epidemic, assays were in place to evaluate these markers in blood donors. We assessed all available WNv antibody assays by analyzing viremic donations detected during nucleic acid screening (NAT).

Methods: Ninety-nine confirmed viremic units identified by NAT were evaluated under code for anti-WNV antibodies. Two manufacturers developed both anti-WNV IgM and IgG EIA (Focus and PanBio); while the third manufacturer developed an EIA that captures all anti-WNV antibodies as well as an immunoblot assay that captures anti-WNV IgA, IgM, IgG, and antibodies to WNV envelope antigen(s) (Chiron).

Results: Based on detection of IgM, 86, 85, and 76% of the 99 specimens were drawn prior to seroconversion; while 4, 5, and 3% were drawn during the IgM only phase. Conversely, 10, 9, and 7% were drawn after full seroconversion.

Conclusions: The need to protect transfusion recipients from infection, with recurring WNv epidemics, presents an opportunity to critically evaluate the natural history of infection among healthy individuals. This can only be achieved if the performance of WNv NAT and serology assays is critically examined. Evaluation of follow-up specimens from these donors is in progress.

P 4.41
Screening of parvovirus B19 –DNA using the Magna Pure nucleic acid isolation method
K Tsuomi*, S Westberg, P Palomaki and H Laitinen
Department of Virus Diagnostics, Finnish Red Cross Blood Service, Helsinki, Finland
Blood donor parvovirus screening was started in Finland in January 2002 as an in-process control to limit the viral load of fractionation plasma pools. Nucleic acid isolation with NucliSens technology was combined with LightCycler real-time PCR (Roche). In May 2003 the nucleic acid isolation method was changed to MagnaPure based on magnetic-bead technology. Donor samples are pooled in maxipools (96) and further combined in maxipools representing 480 donor samples. Nucleic acids are isolated from 1 ml sample. 215 000 donations have been screened since May 2003 using the Magna Pure method. Six of 452 maxipools contained B19-DNA above the 104 IU/ml cut-off level. Six individual positive donations were traced, two of these with a B19-DNA load >106 IU/ml. One of these high positive samples caused cross-contamination in our simulation study with B19-DNA positive (105–107 IU/ml) and negative plasma samples and B19-DNA of 12% (11%) negative samples showed amplification and two of these (1.8%) were above the cut-off level. The automated Magna Pure nucleic acid isolation method is labour saving and increases capacity and throughput of the screening process. However, contamination that is observed with samples containing high B19-DNA load might cause logistic cross-pooling problems during epidemic seasons.

P 4.42
Acute hepatitis C in a repeat donor by sexual transmission
H Vrielink*, PJM van den Burg, M Koot, MGM Koppelman and HW Reesink
Sanquin Blood Supply Foundation, Amsterdam, The Netherlands
A female unrenumerated repeat blood donor tested +ve in HCV-NAT. HCV antibody assays (ELISA and RIBA) were –ve. Five days after the donation, the donor was retested (anti-HCV ELISA, RIBA, cDNA-PCR) and a standardized interview was performed. Again, HCV antibody tests were –ve and the donor tested +ve in the cDNA-PCR (bDNA: 23 × 106 copies/ml). Counselling revealed no risk factors for HCV infection, except for a new heterinosexual relationship 6-7 weeks prior to the donation. There were no indications for co-existing venereal diseases. Eight days after the donation, the donor seroconverted (ELISA pos, RIBA ind). That day a therapy with Peg-Interferon and Ribavirin was started for 24 weeks. We did a weekly follow-up for HCV antibody and cDNA-PCR testing. After 5 weeks of anti-viral therapy, the donor became –ve in consecutive cDNA-PCR testing. Two weeks later the RIBA changed from +ve to ind. One year after the donation, the donor was discharged for follow-up from her HCV infection. The male sexual partner was traced for testing and counselling. The interview revealed risk factors for HCV infection (IVDU and tattoo 1977). Tests showed a HCV infection (ELISA, RIBA, cDNA-PCR +ve, bDNA: 15 × 106 copies/mL, anti-HIV –ve). Genotyping showed a HCV type 3a in both donor and her sexual partner. Sequencing of the NS5b part of the HCV genome demonstrated identical viruses in donor and partner. We report a case of acute hepatitis C in a repeat donor caused by sexual transmission.

P6 Malaria

P 6.1
Prevalence of malarial parasite in blood donors in North India
RN Makroo*, V Raina, Rosamma and B Rita
Department of Transfusion Medicine, Apollo Hospital, Sarita Vihar, Mathrua Road, New Delhi, India
Objective: This study was carried out to find the incidence of malarial parasite positive donors in our blood transfusion centre using a rapid malaria detection kit, based on the antigen capture technique using the pLDH enzyme (OpiniMAL).
Design and methods: This study was undertaken from October 2003 to December 2004. A detailed donor interview was carried out for all blood donors prior to blood donation. Donors with history of malaria in past 6 months were excluded from the study. A total of 20 949 cases were screened for malarial antigen using a rapid malarial antigen detection kit based on the detection of Plasmodium Lactate Dehydrogenase enzyme (OptiMAL). The positive cases were then confirmed by microscopic examination of the Giemsa-stained smears.

Results: Out of a total of 20 989 blood donors screened for malaria, six (0.03%) were found to be positive for malarial parasite. Four of these positive donors were *P. falciparum* and two were *P. vivax*. Microscopic examination of the smears revealed the low parasite counts in all positive donors.

Conclusion: In a malaria endemic country like India, it is essential to have a practicable and sensitive method for malarial parasite screening in blood donors than the conventional peripheral smear examination to prevent transfusion-associated malaria.

P 6.2 Direct antiglobulin tests do not correlate with malaria infection

J Moulds, D Ataliio, O Dumbo, C Fowere and A Tounkara

Drexel University College of Medicine, USA, University of Bamako-MRTC, Mali, University of Maryland-CVD, USA, and Centre National de Transfusion Sanguine, Mali

Background: A positive direct antiglobulin test (DAT) has been reported in severe malaria. However, many early studies were performed using reagents that contained high levels of anti-C4 or anti-transferrin and few studies had any follow-up. Thus, we reinvestigated the incidence of positive DATs in malaria patients using monoclonal reagents.

Methods: A total of 1371 samples from Mali were tested using rabbit polyclonal anti-AHG reagents and similar anti-scrub typhus reagents. Samples were tested with monoclonal anti-C3b/d and anti-IgG-positives were subclassed using a microtitre plate method. A 4-year sampling in Bandiagara included children with mild or severe malaria and case matched controls (*n* = 81) drawn both at enrollment and 6 months later. In addition, 200 healthy children from Donemequeoungou and 340 healthy adults from Bamako were tested.

Results: The incidence of DAT+ ranged as follows: healthy children, 1.8–7.4%; mild malaria, 1.6–9.4%; severe malaria, 3.6–11.8%; and healthy adults, 2%. The incidence of positives increased beginning April 2000 (low transmission) and peaked in September 2001 (high transmission). The most common subclasses were IgG1 > IgG3 > IgG2. The severe malaria cases tended to have more C3 on their RBCs, which correlated, better with anaemia than a positive DAT obtained with polyspecific AHG.

Conclusion: There was a high incidence of DAT+ among both healthy children and adults who did not have malaria suggesting that there are other causes for these previous observations.

P 7.2 Detection of PrPSc with reagents designed and screened to bind PrPSc

M Michelitch, C Gao, X Wang, M Connolly, R Zuckermann, J Hall, T Horn, A Gyenes, B Shimizu, L Pack, B Phelps, D Chien and C Hu

Blood Testing Division, Chiron Corporation, Emeryville, CA USA

Background: vCJD and BSE have caused great public health concern and impacted blood supplies worldwide. Chiron has initiated a project to develop sensitive tests for PrPSc. Most tests presently available employ antibodies that bind to both the normal and diseased forms of prions, PrP27-30 and PrPSc, respectively. Scientists in the prion field have been searching for antibodies specific to PrPSc without binding to PrP27-30. However, this has proven to be difficult. Without PrPSc specific antibodies, most assays rely on the protease resistant nature of PrPSc to differentiate it from PrP27-30. Samples are first treated with protease to clear PrP27-30. But protease treatment is not entirely specific. This fundamentally limits the sensitivity and specificity of these assays.

Methods: In our attempt to design sensitive assays for PrPSc, we have taken the effort to develop specific reagents by utilizing the known chemical and structural characteristics of PrPSc coupled with screening methods.

Results: We have identified a few reagents that bind PrPSc with high affinity but not to PrP27-30 and have been working on these reagents to further improve their affinity and specificity. We have used these reagents to develop assays. The sensitivity of the assays and the approaches to enhance the assay performances will be presented.

Conclusions: The availability of these improved reagents will facilitate the development of sensitive assays for PrPSc.

P 7.3 Removal of dendritic cells from blood by leucodepletion filters assessed by real-time PCR

A Morrison and I MacGregor

SNBTS, PHC RHID Group, 21 Ellen's Glen Road, Edinburgh EH17 7QT, UK

Information is lacking regarding removal of circulating dendritic cells (DC), in particular CD83 expressing cells by leucodepletion (LD) processes. This is important since recent evidence suggests that they may play a role in transportation of abnormal protein PrPSc to lymphoid tissues. RT-PCR can be used to detect low cell numbers with both markers. Results were comparable with flow cytometry data. Using RT-PCR we could detect down to 33 cells/ml of whole blood. All assays were linear over a 5 log_{10} concentration range, allowing pre- and post-LD comparisons. In conclusion we successfully developed an assay to quantify suitably low DC cell numbers. If DCs were found to be involved in transportation of PrPSc then this assay would be valuable for confirming removal of DCs by LD processes.

P 8 Transmissible spongiform encephalopathies

P 8.1 Removal of PrPSc from blood products using various methods

M Addas-Carvalho* and S Correia

Blood Testing Division, Chiron Corporation, Emeryville, CA USA

Recently, it has been shown that a polymorphism at codon 129 in the PrP gene (PRFP) confers genetic susceptibility to vCJD, and PrP129Met and PrP129Val are more prevalent in patients with vCJD than in controls. Epidemiological studies showed that this polymorphism is more frequent in Irish and West European or Asiatic populations. Considering the Met/Met genotype group as a higher risk for vCJD, SB population presents an intermediate risk. However, vCJD has never been reported in Brazil, prophylaxis strategies in our country should consider the PRFP polymorphisms.

P 8.2 In vitro assays for the detection of PrPSc in blood products

G Siffert, S Woodward, J Livnat, K Brown, B Shimizu, M Michelitch, C Gao, C Hsu, X Wang, M Connolly, J Stiles, T Horn, A Gyenes, B Shimizu, L Pack, B Phelps, M Li, T Hane and D Chien

Blood Testing Division, Chiron Corporation, Emeryville, CA USA

Recently, it has been shown that a polymorphism at codon 129 in the PrP gene (PRFP) confers genetic susceptibility to vCJD, and PrP129Val is more prevalent in patients with vCJD than in controls. Epidemiological studies showed that this polymorphism is more frequent in Irish and West European or Asiatic populations. Considering the Met/Met genotype group as a higher risk for vCJD, SB population presents an intermediate risk. However, vCJD has never been reported in Brazil, prophylaxis strategies in our country should consider the PRFP polymorphisms.

P 9 Platelet processing and pathogen reduction

P 9.1 Routine Intercept platelets stop bleeding, show no reactions and similar efficiency than standard

C Areal,1 S Enriquez,2 F Peña,1 A Castrillo,1 F Hernandez,1 M Lopez,1 M Rodriguez2 and J Zamora2

1Centro de Transfusión de Galicia, Santiago de Compostela, 2Hosp. Mexiconeto, Vigo and 1Univ. Complutense, Madrid, Spain

Objective: As INTERCEPT pathogen inactivation method for platelets (Baxter-Cerus) was certified for use in Europe in 2002, a haemonovigilance follow up of routine treated platelets transfusion was performed.
Material and methods: INTERCEPT treated apheresis (Amicus Crescento, Baxter) platelet concentrates (PC) and standard ones (SPC), were transfused to hospital patients under routine conditions. Bleeding stop, transfusion reactions and platelet increment (CI) were recorded. Parametric t-test and multivariate regression analysis were used.

Results: Sixty-five SPC and 84 IPC (treatment platelet loss 6% ± 9%) were transfused to 21 and 18 patients, respectively. No observed acute reactions because of transfusion, and bleeding was stopped. CI was measured at 1–6 h after 57 SPC (20 pts; mean = 12.03) and 60 IPC (19 pts; mean = 8.53). Transfusions. Sample time and CI were similar between both groups (t-test; P = 0.420 and P = 0.381, respectively). After multivariate regression analysis, platelet treatment did not show a significant effect on platelet CI (P > 0.5), while sample time did affect efficiency (P < 0.05).

Conclusion: Platelet inactivation process showed <10% platelet loss and no effect on in vitro efficiency. Sample time however had an important effect. A lower platelet yield could possibly account for the slight CI reduction observed in transfused IPC, but this could not be confirmed as SPC pretransfusion yield was not recorded. Additional follow up of routine IPC transfusion is guaranteed.

P 9.2 Postmarket validation of the quality of stored platelet products collected with SDP software Rev. E
A Blair*, C Martin, M Popovsky, T Chevres and J Sweeney
Haemonetics Corporation, The Miriam Hospital, Providence, RI

Background: Extending platelet storage time is desirable. Haemonetics has developed a platelet protocol (SDP-E) that has been previously shown to reduce donation time without affecting donor safety or platelet quality. We evaluated SDP-E for 7-day storage quality.

Materials and methods: A two-site postmarket study was performed and 56 matched single platelet apheresis procedures were performed on 28 donors randomized to start with either the ‘test’ SDP-E or ‘control’ SDP-D protocol. Platelets were stored in CPP bags for 7 days at 22 ± 2 °C under constant agitation. The platelet products were tested on Days 1, 5 and 7 for cellular composition and measures of in vitro quality.

Results: Day 1 Day 5 Day 7

| Component | Day 1 | Day 5 | Day 7 |
|-----------|-------|-------|-------|
| pH(37 °C) | 7.2±0.1 | 7.2±0.1 | 7.2±0.1 |
| MORPH | NA NA | 19±5 | 19±5 |
| ESC(%) | NA NA | 5±20 | 5±20 |
| Glucose (mg/dl) | 345±14 | 355±27 | 281±137 |

Conclusions: t-Test analyses indicated no significant difference, between the Test and Control products at 5 days. While there are modes changes between day 5 and day 7, the results are compatible with current standards for transfusion. These data suggest that SDP-E protocol will support 7-day storage of platelets collected on the MCs+.

P 9.3 Improving the stability of PLT samples for external quality assessment (EQA) of platelet counting
MJ Beard, V Hancock, J Sutherland and R Cardigan*
Components Development, National Blood Service, Brentwood, UK

NBS EQA schemes evaluate platelet (PLT) counting by sending common platelet samples to all sites. However, results may be affected by PC shelf-life were fixed as follows: No fixative (NF); 0.5 or 1.0% final concentration of paraformaldehyde (PFA); 2.0% f.c formaldehyde in Alsever’s solution (C176) was used to stabilize PLT counts. PLT samples prepared on day 5 or 6 of storage quality.

P 9.4 Quality assessment of plasma and additive solution apheresis platelets
A Castillo*, F Hernández, J Cid, A Castro, M Adelantado, M Abalo, A Eiras, J Flores and C Areal
Centro de Transfusión de Galicia, Santiago de Compostela, Spain

The Guideline of European Council requires pH levels >6.8 at the end of platelets shelf life in apheresis platelet concentrates (PC). PCs from two separators were collected and we evaluated some characteristics on days 5 and 7 of storage.

Material and methods: Each cell separator has different principle but the final product is suspended either in plasma (Trima Accel) or in additive solution, T-sol (Amicus Crescento). The evaluation was done on 50 proces using Trima separator and on 40 proces using Amicus separator. pH was determined with the pHmeter Crison at 22 °C and we assessed the swirling phenomenon: good 3, intermediate 2, bad 1, no swirling 0.

Results:

| Component | Plasma 100% | Plasma/T-sol 35/65 |
|-----------|-------------|-------------------|
| pH        | 7.0±0.1     | 7.0±0.1            |
| Glucose   | 3.2±0.2     | 3.2±0.2            |
| Lactate   | 7.2±0.3     | 7.2±0.3            |
| CI        | 12.0±4.5    | 12.0±4.5           |

Intercept treated apheresis (Amicus Crescento, Baxter) platelets pools from BC showed appropriate metabolic and biochemical activities until day 7 of storage; therefore its application for clinical use is possible, in order to increase safety of routine platelet transfusion.

P 9.5 Intercept® treatment of pooled platelets: in vitro evaluation
A Castro*, A Castro, M Abalo, M Adelantado, J Cid, A Eiras, J Flores, F Hernández and C Areal
Centro de Transfusión de Galicia, Santiago de Compostela, Spain

This study assessed the application of a photochemical treatment to platelets from buffy coats (BC) and their functional characteristics in vitro.

Material and methods: Leucocyte-depleted platelet pools (n = 21) from 5 BC were produced using Optipure® PLT (Baxter) with Sepacell PX-5 filter, and suspended in Intercel as an additive solution. The ratio of plasma to Intersol was 35:65. The pools were treated using the Intercept Blood System, amotosalen-HCl (5-59) and UVA light. We measured platelet count, pH, glucose, lactate, aggregation index of platelet by thrombin 0.25 IU and swirling (good: 3, intermediate: 2, and no swirling: 0). Samples were taken post-treatment-day 1- and on days 5 and 7 of storage.

Results: Average loss after treatment was 9.2% (17.5% max, 5.6% min) of which 1.3% was due to sampling losses. All products of pooled platelets have <1 x 10^6 leucocytes/unit by flow cytometry.

Conclusion: The in vitro parameters indicate that the Intercept Blood System for platelets pools from BC showed appropriate metabolic and biochemical activities until day 7 of storage; therefore its application for clinical use is possible, in order to increase safety of routine platelet transfusion.

P 9.6 Similar in vitro function and bacterial inactivation, leukoreduced (LR) vs. non-LR Intercept platelets
B Donnelly*, L Sawyer and L Lin
Cerus Corporation, Concord, CA, USA

Aims: INTERCEPT Blood System for platelets inactivates pathogens and leukocytes (WBC) in platelet concentrates (PC). We evaluated the effect of WBC in PC on pathogen
inactivation (PI) and on in vitro platelet function following photochemical treatment (PCT).

**Methods:** Whole blood PCs were pooled in 35% plasma + 65% InterSol™. From the pool, ~275 ml was leukoreduced (LR) by filtration; a similar volume was not LR. These portions were used to evaluate platelet function. Three non-LR 50-ml portions of each pool were spiked with ~10^7 CFU/ml of K pneumoniae, Y enterocolitica, or S epidermidis to evaluate PI. All portions received 150 µM S-59 and 3 Joules/cm² UVA treatment. Function assays on day 5 included pH, morphology, extent of shape change, hypotonic shock response, pselectin expression and total ATP.

**Results:** LR decreased WBC dose from ~2 × 10^9 to <0.3 × 10^9 with ~11% fewer platelets in LR portions. Platelet function was comparable with or without LR. Morphology score showed a small but statistically significant difference (2.89 ± 17 non-LR vs. 2.8 ± 15 LR). PI of K pneumoniae, Y enterocolitica, and S epidermidis in non-LR PC was 26.9 ± 0.1, 5.2 ± 0.6, and >6.5 ± 0.1 logs, respectively. Only Y. enterocolitica showed a decrease in level of PI when compared with studies using LR PC (>6.5 ± 0.1 log).

**Conclusions:** PCT effectively inactivated three representative bacteria in non-LR PC. PC had acceptable pH and in vitro function was not statistically different from LR INTERCEPT Platelets.

**P 9.7**

**Helinx® technology inactivates high titers of SARS-CoV, WNV and vaccinia in Intercept platelets**

K Daugas*, A Sampsom-Johannes, K Bernard and S Jones

Cerus Corporation, Concord, CA. USA and New York State Health Department, Slingerlands, NY, USA

**Background:** Helinx® technology, used in the INTERCEPT Blood System for Platelets, utilizes amotosalen HCl (S-59) and UVA light to inactivate a wide variety of blood-borne pathogens in platelet concentrates (PC). Previous studies have shown inactivation of enveloped and nonenveloped viruses, gram negative and positive bacteria, and parasites. To address the threat of emerging pathogens the following studies were performed: SARS-CoV, West Nile virus (WNV) and vaccinia virus (VV).

**Methods:** For SARS-CoV and VV studies 30 ml platelet aliquots, containing ~2.5 to 6 × 10^10 platelets, were prepared from single donor apheresis PC. The WNV study utilized apheresis (~300 ml) PC units. PC collected in 37% plasma/63% platelet additive solution (InterSol®) were incubated with ~10^6 CFU/ml of virus, then treated with 150 µM S-59 and UVA. SARS-CoV and WNV were treated with a single 3.0 J/cm² UVA treatment. VV was treated with a single 1.0 J/cm² and cumulative 2.0 J/cm² and 3.0 J/cm² UVA treatments. Pre and postillumination samples were taken to determine viral titers by plaque assay on Vero (WNV, VV) or Vero E6 cells (SARS-CoV).

**Results:** See table.

| Virus | SARS-CoV n = 2 | WNV n = 4 | Vaccinia n = 4 |
|-------|----------------|-----------|---------------|
| Genome/size | ssRNA/~30 kb | ssRNA/~10 kb | ssDNA/~200 kb |
| Log reduction: (mean ± SD) | >6.2 | >6.0 ± 0.4 | >5.2 ± 0.2 |

**Conclusions:** SARS-CoV, WNV, and VV in platelets were inactivated to below the limit of detection by treatment with 150 µM amotosalen HCl and 3.0 J/cm² UVA.

**P 9.8**

**The effect of transportation on platelet quality during storage**

J Eronen*, K Javela and P Bruce

Finnish Red Cross Blood Service, Helsinki, Finland

**Objective:** To evaluate the effect of over-night transport on platelet quality during storage.

**Methods:** In all 12 eight-unit LD-PC was divided into two using 1000 ml JMS storage containers. The first half was stored for 6 days in a controlled agitation at ±2 °C. The second half was shipped 611 km by car to a blood bank, stored as appropriate and shipped back. The transport times varied from 16 to 20 h. Insulated EPP boxes with temp-stabilizing plates and data loggers were used. Group A was shipped on the first day and sent back on the fifth day. Group B was shipped on the third and the fourth day. Sampling took place at the time of pooling and on the sixth day. The viability of platelets was evaluated by haematological tests, blood gas analysis, metabolic tests (Lact, Gluc), functional tests (ESC, HSR) and tests for platelet activation (CD62P, CD63, sGPV).

**Results:** No statistically significant differences were observed between the shipped and nonshipped plts in group B. In group A, however, the shipped plts had a lower pH at the end of the self-life (6.92 vs. 7.16), a higher pCO₂ (2.7 vs. 1.6 kPa), a lower ESC (8.8 vs. 10.8%) and a higher CD62P (40.3 vs. 35.6%) compared with the nonshipped plts. The differences were statistically significant.

**Conclusion:** The platelets were found to endure two well-controlled over-night transports. Plts retained their best quality when shipped in the middle of their self-life. The viability of plts was adversely effected transporting PCs at the end of the self-life.

**P 9.9**

**Effects of a new pathogen-reduction technology (MIRASOL PRT) on platelet function**

G Escobar*, M Lozano, M Diaz-Ricart, J Li, R Goodrich and AM Galan

Servicio de Hemoterapia y Hemostasia, CDB, Hospital Clinic, 08024 Barcelona, Spain and Navigant Biotechnologies, Inc., Lakewood, CO 80215, USA

Several strategies for pathogen reduction are being developed to reduce risks associated with platelet transfusion. We evaluated the impact of a new technology for pathogen reduction based on riboflavin + 6.2 J/ml UV light (Mirasol PRT) on functional and biochemical characteristics of platelets. Platelet concentrates (PCs) obtained by apheresis (n = 8) were treated with Mirasol PRT and stored at 22 °C. Modifications in major platelet GPs, activation dependent antigens, and apoptotic markers were analyzed by flow cytometry. Adhesive and cohesive functions of platelets in PCs were evaluated using well-established models with circulating human blood. Studies were performed during the preparation (day 0) and during storage (days 3 and 5) of PCs. Mirasol PRT did not cause modifications in platelet counts (1.206 ± 32.5 × 10^10 plts/µl). Levels of GPIIb and GPIIb-IIIa remained stable during storage in PCs treated with PRT and in the same range as those observed in non treated PCs. A progressive increase in P-selectin expression and in Annexin V binding was observed during storage of treated PCs. A similar pattern was also observed in control PCs. Functional studies in perfusion models indicated that Mirasol PRT treated platelets preserved adhesive and cohesive function to levels compatible with those observed in the respective control PCs. In conclusion, platelet function was well preserved in PCs treated with Mirasol PRT and stored for 5 days.

**P 9.10**

**Evaluation of the Intercept system for pathogen inactivation of platelets**

A Espinosa* and E Berg

Department of Immunology and Transfusion Medicine, St Olavs Hospital, Trondheim, Norway

**Background:** At our Transfusion Service in Trondheim we produce approx. 1800 platelet concentrates from buffy coats (BC) and approx. 500 from apheresis.

**Aim:** The aim of this study was to implement Intercept system for pathogen inactivation of platelets at our transfusion service. Special focus was made on internal logistics and blood store flexibility requirements.

**Materials and methods:** A total 214 Intercept treated platelet concentrates from BC were produced in a period of three months (01.10.03–01.01.04). The results were compared with those from untreated BC and apheresis platelets during one month (September 2003). Results: No transfusion reaction was registered neither in the Intercept nor the untreated group. Some of the results are presented as follows:

**Table 1. Inactivation of viruses of emerging concern in PC**

| Virus | SARS-CoV (n = 2) | WNV (n = 4) | Vaccinia (n = 4) |
|-------|-----------------|-------------|-----------------|
| Genome/size | ssRNA/~30 kb | ssRNA/~10 kb | ssDNA/~200 kb |
| Log reduction: (mean ± SD) | >6.2 | >6.0 ± 0.4 | >5.2 ± 0.2 |

**Conclusions:** SARS-CoV, WNV, and VV in platelets were inactivated to below the limit of detection by treatment with 150 µM amotosalen HCl and 3.0 J/cm² UVA.
P 9.11

*In vitro* performance of Mirasol™ PRT platelet system over a wide range of treatment conditions

D Gamp, R Edrich, N Hovenga, J Estrada, J Anderson, S Cartwright, T Rouse, S Keil, E Hansen, RP Goodrich* and A Segers
Navigant Biotechnologies, Inc., Lakewood, CO, USA

**Background:** Mirasol PRT is a photo-activated treatment of platelet products with riboflavin (RB) solution and 6.2 ± 0.3 ml of light to reduce the pathogen load occurring in normal donor blood products. Apheresis platelet concentrates (PCs) of a wide range of volume and concentration were tested after treatment to assess cell quality (CQ) over 5 days of storage as well as pathogen reduction.

**Methods:** PCs were collected on a Trima® Automated Blood Collecting System. Products were prepared with RB and platelet yields ranging from 170–370 ml and 1000–2000 x 10^9/ml in a 11 ELP™ bag with an ISBT donor label attached. PCs were evaluated for CQ over storage were kept in a Helmer shaker for 5 days at 22 ± 2°C and 72 ± 5 cpm. Parameters included lactate and glucose concentrations, pH, P-selectin activation, and platelet swirl. PCs spiked with *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* or porcine parvovirus (PPV) were sampled to measure titer prior to and following treatment.

**Results:** Complete reduction of PPV and >4.0 logs reduction of each bacterial strain tested was achieved for all test conditions. *In vitro* CQ parameters after 5 days of storage were comparable to results from PCs yielding acceptable levels of *in vitro* CQ and in vivo recovery.

**Conclusion:** The Mirasol PRT System for Platelets utilizing RB and 6.2 ± 0.3 ml of light shows robust performance against PCs for acceptable CQ over a wide range of volumes and concentrations.

P 9.12

Photochemical reduction of pathogen load in platelet concentrates using Mirasol PRT

PH Roane*, A Segers, R Edrich, D Gamp, SD Keil, RL Leonard and RP Goodrich
Navigant Biotechnologies, 1215 Quail Street, Lakewood, CO 80215, USA

**Background:** Mirasol PRT is medical device being developed to reduce the pathogen load in platelet concentrates (PCs). The process involves light and riboflavin (vitamin B2) to break down the pathogen’s nucleic acids.

**Material and methods:** Pathogens were added to apheresis platelets. After treatment, the infectivity of each pathogen was measured using established biologic assays. *In vitro* platelet performance was evaluated after treatment and after 5 days of storage using a panel of 10 *in vitro* cell quality assays.

**Results:** In studies with viral pathogens, the system provided average log reduction of 4.46 ± 0.39 for intracellular HIV, 5.93 ± 0.20 for cell associated HIV and 5.19 ± 0.50 for *S. epidermidis* and *E. coli* bacteria. The assay was also tested with observed reduction factors at the limits of detection of 2.0 logs. Platelet cell quality was adequately maintained after treatment and during storage. *P*-selectin expression, glucose consumption and lactate production increased relative to controls, but not beyond accepted levels.

**Conclusion:** The Mirasol PRT device successfully reduced the number of selected pathogens in PCs. Despite significant differences between *in vitro* parameters for treated and control, it is predicted that the clinical effectiveness of treated products will be preserved, based on comparison with historical data on relation between *in vivo* and *in vitro* cell quality parameters.

P 9.13

How many platelet assays do we need?

VS Hormy*, S MacDonald, O Drummond, HA Leaver, IR MacGregor and CV Prowse
Scottish National Blood Transfusion Service, National Science Laboratory, Ellen’s Glen Road, Edinburgh EH17 7QT, UK

The aim of this study was to compare various *in vitro* platelet assays and determine whether or not they assess the same or related changes in stored platelets. Platelet concentrates in 30% plasma 70% PAS-III M were sampled at days 1, 5 and 7. Samples (n = 54 to 72) were assayed for platelet activation (P-selectin and CD63 expression), *in vitro* function (soluble P-selectin and annexin V binding to phospholipid), morphology (extent of shape change), lysis (soluble annexin V) and viability (mitochondrial membrane potential expressed as % platelets with polarized mitochondria). Correlation coefficients between assays were calculated. Significant correlation (P < 0.001) was only found between the following: extent of shape change v annexin V binding (r = -0.433), mitochondrial membrane potential v annexin V binding (r = -0.525) and soluble P-selectin v soluble annexin V (r = 0.757). Storage in PAS-III M had caused various degrees of morphological change, activation, lysis and apoptosis in the platelets. There was significant correlation between some assays measuring these different platelet properties. However, the conclusion is that using a range of assays in the same study will provide valuable comparative information in determining changes relevant to storage and use. The study will be repeated using Composol as the platelet additive storage medium.

P 9.14

ASA ingestion by blood donors – the effect on *in vitro* properties of stored platelet concentrates

T Zeiler, D Gritzka, R Karger and V Kretschmer*
Institute for Transfusion Medicine and Haemostaseology, University Clinics Marburg, Germany

**Background:** Acetylsalicylic acid (ASA) is one of the most widely used over-the-counter medications and up to 9% of platelethephoresis donors may have taken ASA. However the effect of ASA on platelet activation during apheresis and the influence of ASA on *in vitro* platelet function during storage of single donor apheresis platelet concentrates (APC) has not yet been studied.

**Study design and methods:** In a randomized crossover study, 10 healthy donors donated 2 APCs each, taking 500 mg ASA 12 h prior to one of the aphereses (group A), and taking no medication prior to the other donation (group B). In vitro tests of platelet function and morphology were performed in donors before and after apheresis and in APCs during storage (days 1, 3 and 5).

**Results:** ASA ingestion resulted in a significant decrease of induced platelet aggregation in APCs only on day 1 (P < 0.01). TRAP-6 induced expression of P-selectin (CD62p) was significantly reduced in APCs of group A only on day 1 (P = 0.02). There were no significant differences of morphology and *in vitro* function (LDH, lactate, pH, morphology score, CD62p expression, fibrinogen binding) between APCs of group A and B after 53 days of storage.

**Conclusions:** Alterations of *in vitro* quality of platelets during storage of APCs levels the difference of ASA- and non-ASA-products suggesting that ASA ingestion may be without importance for the quality of stored (≥23 days) APCs.

P 9.15

Automated vs. manual preparation of leukoreduced platelet concentrate (PC) from pooled buffy-coats (BC)

L Larrea*, MA Soler, R Roig, MJ Ortiz, T Pamplona, P Solves and V Mirabet
Centro de Transfusión de la Comunidad Valenciana. Valencia, Spain

**Background:** The OrbiSac BC System automates PC production process from BCs. After a first validation step with five BCs our aim was to check if the procedure with four BCs could deliver similar PCs as our routine manually-prepared PCs made out of five BCs.

**Material and methods:** In the OrbiSac method, four isogroup BCs and 300 ml Platelet Additive Solution (PAS) were sterile docked on the OrbiSac validation set. Pooling, centrifugation and expression of PC through a leukoreduction filter into the PC storage bag were automatically performed. For the manual method, five isogroup BCs were pooled together with 300 ml PAS. This PAS-BC pool was centrifuged at 455 g during 7 min and the PC was expressed by the plasma extractor through a filter. The collected PC data included pH, reactivity against CD62, platelet and residual leukocyte count, PC volume. Platelet and WBC were performed with an autoanalyzer (ABX micros 60) and with a cytometer (Facscalibur, B-D). Statistical analysis was made with SPSS.

**Results:** Results are shown in the table below.

|            | BCs/PC vol (ml) | Pool (x10^11) | PAS/BC (x10^11) | CD62 second day | pH second day | WBC second day |
|------------|----------------|---------------|-----------------|----------------|--------------|----------------|
| OrbiSac    |                |               |                 |                |              |                |
| n = 18     | 4              | 363           | 3.37            | 0.84           | 20.41        | 51.91          | 7.53           | 7.28           | 0.06*         |
| Manual     |                |               |                 |                |              |                |
| n = 18     | 5              | 354           | 3.24            | 0.64           | 35.33        | 47.54          | 7.22           | 7.11           | 0.02*         |

*P < 0.05

**Conclusion:** The OrbiSac BC system optimized the BC process and therefore increased the yield per BC by 31%. This allowed a reduction in the number of BC units to pool (from 5 to 4) without difference in the absolute platelet number per PC.
Mitochondrial membrane potential in platelets during storage

HA Leaver* and CV Prowse
National Scientific Laboratory, Scottish National Blood Transfusion Service, Ellen's Glen Road, Edinburgh EH17 7QT, UK

Aims: To study the effects of storage and interrupted agitation on platelet mitochondrial membrane potential (MMP).

Methods: During the international BEST study of platelet storage, indices of platelet apoptosis and viability were analysed in four platelet pools, each divided into 6 units with variable agitation and platelet count. Platelets were stored in 30% plasma 70% PAS-IIIM (10 mM citrate, 30 mM acetate, 26 mM phosphate, 115 mM NaCl, 5 mM potassium, 1.5 mM magnesium). The indicator of the intrinsic pathway of platelet apoptosis, mitochondrial membrane permeability, together with its sensitivity to calcium ionophore A23187 were monitored, as these factors have shown sensitivity to platelet storage in vitro.

Results: The JC-1 probe, which displays dual red/green fluorescence, indicated the predicted high polarisation of platelets, even after 7-day storage (87.9 ± 0.82% of platelets with polarized mitochondria on day 1; 81.9 ± 0.96% day 5; 74.8 ± 2.6% day 7, mean ± SE). MMP results were consistent with other indices of platelet function, showing a decline in viability during interrupted agitation in pool 1. A significant effect of storage was detected (P = 0.001 paired t-test 1 day vs. 1 day 5; P = 0.00008 day 1 vs. day 7, n = 18 samples each day) together with divergence in MMP in different treatment (interrupted agitation) groups on days 5 and 7, compared with day 1.

Conclusions: Platelet storage and calcium ionophore affected mitochondrial membrane potential, which may be a useful indicator of platelet function in vitro and in vivo.

P 9.17

Performance of the Mirasol PRT system with buffy coat platelet and apheresis platelet concentrates

J Li, D De Korte†, M Woolum, SD Keil, A Segers and RP Goodrich
Navigant Biotechnologies, Lakevood, CO, USA and 1Sanquin Research at CLB, Amsterdam, The Netherlands

Background: The Mirasol Pathogen Reduction Technology (PRT) has been developed to improve the safety of transfusion of platelets obtained by either buffy coat preparation (BCP) or apheresis procedure (APP).

Material and methods: BCP were prepared with the Compatom G4. APP were obtained by Trima apheresis. After PRT treatment on day 1, both products were stored for 5 days. A panel of cell quality assays was performed. Separate BCP were spiked with viral and bacterial pathogens and the infectivity was measured after PRT treatment.

Results: Cells counts and plasma LDH during storage indicated PRT did not induce significant cell lysis. Decrease in glucose and increase in lactate were observed with no significant difference in the rates between treated BCP and APP, and in the level between treated and control BCP on day 6. The pH remained above 7.0. Platelet morphology was well preserved as determined by swirling and Kunicki scores. P-selectin expression and microparticle formation showed partial platelet activation without significant difference between treated BCP and APP. The JC-1 assay displayed no loss of mitochondrial integrity. The ESC and HSR assays confirmed that treated products had similar response to control BCP. PRT achieved pathogen reduction of >5.15 log for E. coli and 4.02 log for HIVvca.

Conclusion: PRT treatment had a mild effect on the development of the normal platelet storage lesion and achieved significant reduction in infectivity for E.coli and HIVvca.

P 9.18

Polyolefinacetate bags for photodynamic treatment and for storage of platelet concentrates

H Mohr*, F Tolksdorf†, W Walker† and TH Müller†
1Blood Center of the German Red Cross Chapters of NSTOB Institute Springe and 2MacPharma Tourcoing, France and Langen, Germany

Aim: To investigate the suitability of novel plastic bags made of polyolefinacetate (POA) for photodynamic treatment of plasma-reduced platelet concentrates (PC) with thionine/Thyellow light and ultraviolet light B (UVB).

Materials and methods: The POA bags tested and the illumination device used [MacPharma, France] for illumination were from MacPharma, PC in platelet storage medium PAS-IIIM containing approx. 30% plasma were prepared from pools of four buffy coats. Photodynamic treatment of PC with yellow light was in the presence of 1 μmol/l Th. UVB irradiation was on a laboratory device equipped with fluorescent tubes emitting UVB [wavelength range 290–320 nm]. The titre of bacteria and viruses and platelet parameters were estimated by standard procedures.

Results: The POA bags exhibited an almost 100% light transmission in the visible range of the spectrum and a transmission of approx. 70% in the UVB range. For comparison, the UVB transmission of commercially available polyolefin bags was approx. 50%. The kinetics of inactivation of viruses were similar to those in polyolefin bags, the inactivation of bacteria at identical light doses was better. It was also found that POA bags can be used for storage of PC for up to 7 days.

Conclusions: POA bags exhibit high light transmission in the visible and in the ultraviolet range of the spectrum. This makes them suitable for photodynamic treatment of PC with Thyellow light and UVB. In addition, the bags are may be used for storage of PC.

P 9.19

Validation of Intercept treatment on splashed apheresis products

JC Osselers*, N Messe†, M Geoffaux†, MC Vandendaele†, M Van Hooydonk†, E Gossenaerts†, JM Payrat† and A Boddy†
1BTC and 2Department of Haematology, Mont-Godinne University Hospital, 5530 Yvoir, Belgium and 3Baxter-Fenwal, 1050 Brussels, Belgium

Intercept blood system, through the use of Amotosalen and UVA, achieves pathogen inactivation in platelet products, both pooled and single donor and could allow extension of platelet shelf life. Ten double apheresis platelet products were splashed. One bag was treated with Intercept, whereas the concomitant bag was used as control. A product sample was taken before and after Intercept treatment, and consecutively on days 3, 5, 7 and 9. Following tests were performed: platelet count (PLT), mean platelet volume (MPV), pH, lactate, glucose, LDH, hypotonic shock response (HSR). Platelet concentration remained stable throughout the 9-day period. Intercept treatment led to a combined volume and product loss of 7.0 % (±1.1) %. A slight diminution in pH was observed until day 7; pH values tended to be slightly lower in the Intercept group, but were never below 6.7. MPV values increased steadily, without any difference between the two groups. Glucose consumption occurred similarly in the two groups until day 7, with a concomitant increase in lactate levels. The level of LDH increased almost twofold in both groups over the 9-day period. Hypotonic shock response witnessed good platelet viability in all products, at least until day 7. All products tested were within Intercept guardbands until day 9. No significant changes were observed between Intercept treated and control platelets. Shelf-life of platelets, at least according to in vitro parameters, could reasonably be extended to 7 days.

P 9.20

Profiling pathogen reduction

C Prowse†
Scottish National Blood Transfusion Service, Edinburgh, UK

A number of companies are developing pathogen reduction (PR) technologies for single donation cellular and plasma components. For each of these, prior to extensive clinical trialling, manufacturers and users need to be reassured on three areas, apart from any health-economic considerations:

• The level of pathogen reduction obtained for a range of pathogen species.
• The margin of safety in respect of any toxic effects of the new product.
• The impact of PR processing on product quality. Each of these three fields can include a vast amount of data, which may be difficult to easily understand. Here a simple stacked histogram approach is proposed to summarise such data in a standard and compact format for each technology, which allows:
  • An easily understood summary of the range and extent of pathogen reduction for a large number of subjects and with options to include kinetic and ‘reserve capacity’ data.
  • A simple histogram approach to summarising margin of safety data for toxicolgy.
• Graphic representation of the levels of residual coagulation factors (for FFP) or recovery and survival data (for red cells or platelets) as an indication of product quality. The combination of these three profiles on a single sheet should cover the major parameters of interest for PR technologies and allow easy comparison of the different approaches.

P 9.21

Seven-day-old random donor platelets are functional

G Rock*, D Neurath and M Freedman
The Ottawa Hospital, Ottawa, ON, Canada

Introduction: At present platelet concentrates are licensed for use up to 5 days of storage because of concerns for bacterial contamination. Seven days of storage would significantly reduce wastage, which, in Canada averages 25% each year.

Methods: Blood was collected into CP2D and platelets (RDPs) were made. Samples were taken at 3, 5 and 7 days for platelet count, pH, aggregation with ADP and collagen, hypotonic shock response (HSR), Kunicki morphology score (KMS), thromboelastogram (TEG), and pO2, pCO2. Platelet activation (CD62) was measured by flow
cytometry. The CCI was calculated after infusion of 7-day-old platelets into thrombocytopenic patients. The Pall BDS system was used to sample the platelets for bacteria.

**Results:** There was little change in pH or the level of CD62 expression over time. The R-value in the thromboelastogram remains reasonably consistent. The aggregation response decreased over time but were still seen at 7 days. The hypotonic shock response decreased from 77 to 63% and the KMS also showed progressive decrease. No bacteria were detected at 7 days. At 7 days the CCI was acceptable, 18 950 ± 9800 (n = 23).

| Day | pH | Aggregation (ADP,COLL) | HSR % | Rec | KMS | TEG K (mm) | Flow | CD62 |
|-----|----|------------------------|-------|-----|-----|------------|------|------|
| 3   | 7.3 ± 0.1 | 15.6 ± 13 | 65.8 ± 30 | 77 ± 20 | 300 ± 57 | 13.6 ± 2 | 0.4 |
| 5   | 7.2 ± 0.1 | 10.1 ± 10 | 68.2 ± 27 | 77 ± 20 | 231 ± 40 | 14.1 ± 2 | N/A |
| 7   | 7.1 ± 0.1 | 3.2 ± 4   | 48.9 ± 22 | 68.2 ± 25 | 164 ± 38 | 14.3 ± 2 | 0.4 |

**Conclusion:** Seven days storage of RDPs is possible. Seven days platelet resulted in appropriate increments in patients supporting the use of RDPs for at least 7 days.

P 9.22
Platelet concentrates produced by the OrbiSac BC System and stored in different media
E Rombouts 1, P Schoenmakers 1, H Tran 1 and P De Smet 2
1Sanquin Blood Bank, Region Southeast Maastricht, The Netherlands and 2Gambro/BCT, Blood Bank Technology, Zaventem, Belgium

**Background:** In Europe, a predominant part of all platelet concentrates (PCs) are prepared manually using the buffy coat (BC) method. The OrbiSac BC System automates and optimizes this process and was used in this investigation to validate different storage media.

**Materials and methods:** Using the OrbiSac BC System (Gambro BCT) five BCs were pooled with storage medium and a leukoreduced PC was prepared and further tested. Pooling was done with 200 ml of three types of storage medium: T-sol (Baxter), Composol (Fresenius Hemocare) and autologous plasma. Platelet yield, recovery, and quality parameters were determined during a 7-day storage period.

**Results:** All PCs showed normal swirl and pH within 6.8–7.4 during a 7-day storage period.

**Table 1. Results on day 0 of processing (mean ± SD)**

| PC                  | Volume (ml) | WBC count/µl |
|---------------------|-------------|---------------|
| Per BC              | (±1011)     |               |
| Per culture pool    | (±1012)     |               |

- T-Sol
  - (n = 19) 546 ± 11 913 ± 134 4.98 ± 0.73 35.0 ± 14 1134 ± 144 4.00 ± 0.49 81 ± 10 0.11 ± 0.09

- Composol
  - (n = 24) 549 ± 12 775 ± 132 4.25 ± 0.74 362 ± 13 1035 ± 168 1.74 ± 0.61 90 ± 17 0.06 ± 0.04

- Plasma
  - (n = 21) 560 ± 20 969 ± 150 5.42 ± 0.81 360 ± 21 1186 ± 176 4.49 ± 0.59 84 ± 9 0.21 ± 0.53

**Conclusion:** Yield and recovery was high with all storage media tested. WBC counts compiled with the European guidelines for leukoreduction. No deterioration of the tested parameters was observed.

P 9.23
Comparison of thrombelastography and aggregometry for in vitro evaluation of stored platelets
K Schallmosser 1, T Wagner and G Lanzer
Department of Blood Group Serology and Transfusion Medicine, University of Graz, Austria

**Purpose:** Results of rotation thrombelastography (ROTEG®), Pentapharm) and aggregometry (BCT, DB) were compared for testing platelet function in buffy coat derived platelet concentrates (PCs) during storage.

**Methods:** Fifteen PCs were tested on days 0, 1, 3, 5 and 7. Using ROTEG® we analysed Clot Formation Time (CTT), Maximum Clot Firmness (MCF) and Maximum Clot Elasticity (MCE). Using aggregometry, Maximum Aggregation (MA), Maximum Velocity of MA (Vmax) and Time for Vmax were measured. Platelet counts and metabolic markers (Roche Omni) were estimated on days 1 and 5.

**Results:** Concentration of platelets was 2.6 ± 0.1 x 10^11/U; pH was 7.2 ± 0.1, glucose 257.4 ± 20.1 mmol/l and lactate 13.6 ± 2.7 mmol/l. Using ROTEG® by activating the intrinsic pathway, MCF and MCE decreased significantly beginning on day 3 compared with day 0 (77.7 ± 1.9 vs. 70.3 ± 1.1 and 267.6 ± 28.0 vs. 236.6 ± 12.2, respectively, P < 0.05). Using tissue factor as activating agent, no differences in MCF and MCE were observed. Permuting aggregometry, MA (%) decreased significantly on day 3, compared with day 0 (69.8 ± 6.8 vs. 87.8 ± 9.0, P < 0.05). Vmax (mm/s) declined on day 5 compared with day 0 (365.3 ± 86.4 vs. 694.8 ± 167.6, P < 0.05) (values as mean ± SD). Conclusion: In vitro platelet activation and velocity of aggregation decrease significantly beginning on day 3, but using tissue factor as activator of platelets, ability for clotting is well maintained during 7-day storage. Additional studies are necessary to evaluate usefulness of thrombelastography for quality control of PCs.

P 9.24
A novel rapid flow cytometric assay for the simultaneous enumeration of residual WBC and RBC in PC
HP Spengler 1, B Lambrecht, D Hilfig, F Nauwelaers, H Mohr, U Bauerfeind, W Sireis, E Seifried, T Müller and T Tonn
BD Biosciences, Erembodegem, B; German Red Cross NISTOR, Inst. Springe, D; Inst. f. Transf. Med. and Immunohematology, Frankfurt, D Germany

**Purpose:** European recommendations and national guidelines define the quality control (QC) specifications of platelet concentrates (PC), i.e. for leukocyte-depleted PC: rWBC < 10^6/µl, rRBC < 10^6/L.

**Methods:** A rapid one-step staining procedure was developed for absolute counting in a single BD TruCOUNT tube w/o fixation, permeabilizing or washing steps. The PI stains WBC, and the antibody CD235a (anti-glycoprotein-A)-PE labels RBC. Assay validation was based on ICH and NCCCLS guidelines completed over a minimum of 5 days using a BD FACScalibur flow cytometer. Extended validation data was collected by assays performed alongside routine QC of PC (n = 100). Results were compared with Nageotte and Neubauer counting chambers and with the BD LeucoCount assay (WBC).

**Results:** Validation showed no carryover or drift. Unspecific background (mean of n = 21): 0.02 ± 0.00 WBC/µl and 0.3 ± 0.2 RBC/µl. Linear regression analysis and imprecision analysis: WBC, R² = 0.992 [CV<33–12%, 0.6–6.0 WBC/µl]; RBC, R² = 0.999 [CV<12–6%, 800–8,000 RBC/µl]. In QC samples, FACS vs. Neubauer chamber: R² = 0.902 (75–4,900 RBC/µl). Cell counts were below 0.2 WBC/µl in 99% QC samples (97%) by Neubauer. Manual counting gave higher values. In summary, more than 95% of the QC samples were within the specification range.

**Conclusion:** The novel BD flow cytometric test passed validation. This test is a rapid, simple and reliable single tube assay and a potential alternative for the existing manual microscopic counting procedures.

P 9.25
Multi-center evaluation of two flow cytometric methods for counting low levels of white blood cells
MJ Dijkstra-Tiekstra 1, PF van der Meer 1, RNL Pieterse 1, J de Wildt-Eggers 1
1Sanquin Blood Bank North West Region, Amsterdam and 2Sanquin Blood Bank North East Region, Groningen, The Netherlands

**Background:** Flow cytometric (FCM) methods can be used to count WBCs in WBC-reduced blood products, which must contain ≤1 x 10^6 WBCs (~3.3 WBCs/µl). In this study two FCM methods for counting low levels of WBCs under routine conditions at nine laboratories were evaluated.

**Materials and methods:** Panels of red cells, platelets and plasma were prepared containing 33, 10, 3, 3.3, 1.0 and 0.3 WBCs/µl. Samples were counted in 12-fold using the LeucoCOUNT (BD Biosciences; four laboratories) or LeukoSure (Beckman Coulter; five laboratories) FCM method. Requirements were that at the level of 3.3 WBCs/µl, CV was ≤20%, and that accuracy was ≥28%. Quality control (QC) FCM data of WBC-reduced blood products from two laboratories were analyzed.

**Results:** At 3.3 WBC/µl the results of none of the laboratories conformed to the requirements for all three blood products. At this level the overall CV was 21% and the accuracy was 77% (n = 324). The results of the LeucoCOUNT method met requirements at more laboratories than those of the LeukoSure method for red cells (3/5 vs. 1/5) and platelets (4/2 vs. 4/5). The opposite was observed for plasma (1/4 vs. 4/5 laboratories). QC data showed that >99% was below the 95% prediction interval for 3.3 WBCs/µl.

**Conclusions:** None of the laboratories met requirements for accuracy and precision for all three blood products. However, QC data showed that >99% of the products were WBC-reduced conform guidelines, which made both FCM methods suitable for QC.

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P 9.26
The use of INTERCEPT blood system for platelets in a blood transfusion centre
I Van haute*, M Van Vooren, L Noen, Y Benoit, J Flament, N Moerman and B Vandekerckhove
Bloodtransfusioncentrum Oost-Vlaanderen; Ghent Univ. Hospital, Department of Haematology and Department of Pediatrics-Oncology; Baxter, Fenwal, R&D Europe, Belgium
Introduction: The INTERCEPT Blood System for platelet concentrates (PC) is a new method that makes blood products safer for transfusion. The system is based upon the addition of amotosalen to the PC, followed by UVA irradiation. This procedure inactivates viruses, bacteria, protozoa, and residual leukocytes. We evaluated the feasibility of this procedure in our centre and focussed on platelet recovery.
Materials and methods: One hundred and forty-nine PC’s from five buffy coats were prepared. From each PC, two samples were taken before treatment for calculation of the platelet yield and after processing for bacterial screening and platelet yield. The treated PC’s were transfused in trombocytopenic haematological patients.
Results: The platelet yield was 3.74 10^{11} (2.78–4.71). During the procedure, volume loss was 11.9% (7.5–16.3%) or 38.6 ml. Platelet content was reduced 17.4% (7.3–27.5%). Sampling (±15 ml) and platelet retention during fluid transfer (±20 ml) contributed to this reduction. The mean 1 h CCI of 40 transfusions was 8731 (±5842).
Conclusion: The volume loss for INTERCEPT and for bacterial screening are comparable. Because pathogens and leukocytes are inactivated, sampling for bacterial screening may be unnecessary as well as γ irradiation. No difference in CCI was noticed when treated and untreated products were transfused. INTERCEPT Blood System for platelets can be considered as feasible in routine preparation, with a satisfactory clinical outcome, bringing improved safety in transfusion.

P 9.27
Evaluation of buffy coat platelet quality in concentrates produced manually and automatically
A Vetlesen*, MR Mirkashfari, F Edidjani and J Kjeldsen-Kragh
Department of Immunology and Transfusion Medicine, Ulleval University Hospital, Oslo, Norway
Background: The OrthoSac System (Gambro) automates the preparation of platelet concentrates (PC) from pooled buffy coats (BC). We aimed to evaluate platelet (plt) quality for the automated system (A) compared with our manual routine (M).
Materials and methods: After overnight storage at 20–22 °C, 5 BC’s were pooled with 300 ml T-Sol (Baxter). Forty-two PC were produced either manually (n = 21) using the Immugard III S-PF set (Terumo) with integrated soft leukoreduction filter, or by an automated procedure (n = 21) using the OrthoSac Validation BC Set (Gambro) equipped with the LRPS leukoreduction filter (Pall). Quality testing included plt count, WBC count, RBC count, blood gas analyses, glucose and lactate. Activation and residual activation potential were measured as expression of CD42a, CD62P and PAC-1 with and without stimulation by thrombin receptor activation peptide (TRAP).
Results: P1 recovery: A: 79%, M: 68% (P < 0.0001). PLT yield: A: 3.43 × 10^{11}, M: 3.15 × 10^{11} (P < 0.0001), WBC conc. (median): A: 0.1 µL⁻¹, M: 0.0 µL⁻¹ (P < 0.003), RBC content (median): A: 386 × 10^{12}, M: 581 × 10^{12} (P = 0.0004). No statistical significant differences were found in glucose consumption, lactate production or TRAP-induced upregulation of PAC-1. TRAP-induced CD42a downregulation was significantly largest in the A PC, whereas TRAP-induced upregulation of CD62P was significantly highest in M PC.
Conclusion: Preparing plt concentrates by the automated OrthoSac System improves plt yield without reducing plt quality.

P10 Fresh frozen plasma
P 10.1
MB-photoinactivated FFP is effective and safe for treatment of coagulopathies and bleeding disorders
F Equitani*, G Mistretta, M Hortencio de Medeiros, L Mele, U Paladini, V Filoni, A Vetlesen*, MR Mirkashfari, F Ezligini and J Kjeldsen-Kragh
Evaluation of buffy coat platelet quality in concentrates produced manually and automatically
A Vetlesen*, MR Mirkashfari, F Edidjani and J Kjeldsen-Kragh
Department of Immunology and Transfusion Medicine, Ulleval University Hospital, Oslo, Norway
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Conclusion: Preparing plt concentrates by the automated OrthoSac System improves plt yield without reducing plt quality.

P 10.2
The effect of methylene blue inactivation of whole blood plasma on Fc VIII related to storage time
M Baeten*, A Rapalle, D Donnay, M Van der Beek, D Sondag and P Vandekerckhove
Transfusion Centre Antwerp, Transfusion Centre Liege, Red Cross, Belgium
Background: Virus inactivation of plasma can be achieved by a MB photochemical method. This method is known to be effective on viruses with lipid membrane and to have a damaging effect on proteins as clotting factors VIII (Fc VIII), XI and fibrinogen. The method was developed by the Springe Institute and improved by Maco Pharma.
Material and methods: To investigate the moment of inactivation of plasma derived from whole blood in regard to legal, qualitative and organizational aspects, processing of whole blood and MB inactivation (with removal of MB) of the plasma were performed in two centres at different time-intervals from whole blood donation. Prior to inactivation, whole blood and plasma derived from whole blood were stored in the same circumstances at 20 °C. Fc VIII samples were taken after whole blood processing and after inactivation. Fc VIII measuring was done using the one stage APTT clotting assay with Fc VIII deficient plasma. Results were analysed using repeated measures ANOVA.
Results: Results show a significant and constant loss of Fc VIII because of the inactivation process and a significant loss of Fc VIII during storage. There is no difference in the losses of Fc VIII between stored plasma and stored whole blood.
Conclusions: In relation to the Fc VIII activity of plasma, MB inactivation has to be done within a limited time period after whole blood donation. The processing of whole blood can be performed at any time between donation and MB inactivation of the plasma.

P 10.3
Provision of US-sourced fresh frozen plasma for UK infants and children born since 1st January 1996
G Nicholas* and ME Ashford
On behalf of the Non-UK Plasma Project Boards, National Blood Service (NBS), UK
In February 2000, the UK Departments of Health reviewed the potential for blood components to transmit vCJD. A key recommendation was that FFP for infants and children born since 1st January 1996 should be imported and pathogen inactivated. The NBS carried out an options analysis and risk assessment for FFP provision and initiated a project to undertake preparation of specifications, audit of potential suppliers, validation of the delivery system and the procurement process. Key outcomes were the specification of male donors to minimise risk of transfusion-related acute lung injury, assurance that virus test systems met UK standards, skewing blood group mix towards A and AB to maximise clinical flexibility and a decision to use methylene blue (MB) photoinactivation as the pathogen reduction system for the imported plasma. During 2003, the NBS and the chosen US plasma supplier (Biosource Inc.) trailed the importation system end-to-end. This identified specification differences and unacceptable breakthrough levels which have since been resolved. Since January 2004 the NBS has received approximately 1000 U of imported FFP per month. Post-MB treatment testing of the first batch received showed a mean FVIII of 0.93 IU/ml (n = 165) with 91% >0.5 IU/ml thereby complying with UKBS guidelines. The mean component volume was 232 ± 19 ml. The NBS has successfully imported and reprocessed US plasma, achieving conformance to specifications, as a major safety initiative for infants and children.

P 10.4
Inactivation of blood-borne pathogens in apheresis plasma using the INTERCEPT blood system
L Sawyer*, R Mahabangloob, M Propst and A Sampson-Johannes
Cerus Corporation, Concord, CA, USA
Background: The INTERCEPT Blood System for plasma utilizes amotosalen HCl and UVA light to inactivate a wide variety of blood-borne pathogens in plasma intended for 30°C. Serology concerning HCV, HBV, HIV, CMV, EBV, HAV, PVB 19 has been performed, as well as the levels of clotting factors and inhibitors, so the clinical improvement was evaluated.
Results: All units showed a significant decrease of enveloped viruses. The assessment of clotting tests after i.p. revealed no significant changes for aPTT and TT, factor VIII, factor V, vW factor and fibrinogen Clauss were decreased by a mean value of 22.6%, AT III, PC and PS of 10–15%. A total of 133 of 160 pts recovered by a median time of 2.6 days, with a median consumption of 2.4 unit of p.i. FFP; 15 pts, affected by DIC, were not responders, seven after a trauma, one after delivery, four after abdominal surgery and three after cardiothoracic surgery; six pts early died. Last six pts presented a new disease, so they were only median of 12 procedures, by using a median of 102.5 U of plasma.
Conclusions: Our study showed the PMB plasma is effective and safe in arresting any severe bleeding or coagulopathy, with no increased request.
P14 Stem cells

P 14.1
Pre-treatment with bryostatin improves imatinib efficacy against chronic myeloid leukemia stem cells in vitro
E Allan*, T Holyoake, M Elliott, S Harrison, L Richmond and H Jørgensen
SNBTS, University of Glasgow; Cancer Research UK Formulation Unit, and Glasgow Royal Infirmary, UK

Quiescent [G0] Phs+ stem cells persist in chronic myeloid leukemia (CML) following exposure to imatinib (IM; Gleevec®). Resumption of quiescence of the IM-insensitive cells may improve drug efficacy. The protein kinase C (PKC) modulator bryostatin-1 (bryo) was hypothesised to augment IM-mediated Ph+ cell death by inducing cycling of the G0 population. As bryo can temporally up-/down-regulate PKC, drug scheduling may be important. Phs+ K562 cells and primary material from CML at diagnosis were studied. Primary samples enriched to >85% CD45+ and >90% Phs+ by FSHS were used. Cycling cells were distinguished from non-cycling by propidium iodide uptake, and cell death measured by dye exclusion. On exposure to IM (48 h), >70% of surviving K562 cells was in G0/G1. Together with cell kill, there was a relative enrichment for G0/G1 cells with respect to untreated control. However, pre-treatment with bryo (24 h) both reduced the percentage of cells recovered in G0/G1, and increased total cell kill, resulting in a reduction in the absolute number of G0/G1 cells. Although exposure of primary cells to IM (72 h; n = 2) did not significantly increase the percentage of G0/G1 cells, pre-treatment with bryo (24 h) reduced their absolute number following the combination treatment with respect to IM alone (Patient 1: 0.07 ± 105 vs. 1 × 105 G0/G1 cells, combo vs. IM, respectively). Hence, scheduled combination of bryo with IM may improve efficacy against IM-insensitive G0/G1 Phs+ stem cells.

P 14.2
Differentiation of natural killer T cells from cord blood CD34+ cells
B Zhen, H Fan*, Y Liu, L Tao, X Nie, H Lu, F Gao and Y Zhu
Shanghai Blood Center, Laboratory for Blood Engineering, Shanghai, China

Objective: To verify that natural killer T (NKT) cells can be developed from CD34+ stem cells in vitro in the absence of any thymic influence and optimize the culture conditions to induce the differentiation of NKT cells.

Methods: CD34+ cells purified from human cord blood were cultured in the presence of several cytokines and analysed by flow cytometry. The T lymphocytes-free PBMCs from cord blood were added to the culture system. The α-glycosylceramide and dendritic cells were used to expand the NKT cells differentiated from cord blood CD34+ cells.

Results: After 28-day culture, the highest frequency of TCRVα24/41 NKT cells was observed in the presence of IL-15 and SCF. When the T lymphocytes-free PBMCs were added to the culture system, the frequency of TCRVα24/41 NKT cells increased to (2.10 ± 0.87%)%. The NKT cells differentiated from cord blood CD34+ cells could be proliferated by α-Glycosylceramide and DC.

Conclusion: IL-15 in combination with SCF and/or Flt3 can induce the differentiation of extrathymic NKT cells from human cord blood CD34+ cells. The PBMCs that were removed T lymphocytes can support the differentiation of NKT cells in vitro.

P 14.3
Progenitor cells mobilized after myocardial infarction in relation to patient age
W Gebauer*, E Fallgren Gebauer, N Grommes, K Kronberg and F Schunter
Red Cross and Klinikum Oldenburg, Germany

Autologous progenitor cells have been recently used with the intention to support regeneration of cardiac tissue after myocardial ischemia-induced injury. Cells aspirated from bone marrow or circulating progenitor cells have been used. By flow cytometric means we describe progenitor cell populations circulating in patients suffering in age at two points of time after myocardial infarction. At the time of invasive diagnostics analysis of CD34 positive, CD133 positive and CD34 positive vs. CD133 positive populations circulating in the blood of patients differing in age from 37 to 78 years of life were described. This qualitative and quantitative immunological description was again performed 7 days later; high-resolution MRI was used to describe cardiac damage. Comparing results generated at the time of invasive diagnostics to measurements performed 7 days later a relation of progenitor cell concentration to patient age was demonstrable. Knowledge of progenitor cell mobilisation kinetics indicating possible endogenous repair efforts in patients suffering from cardiac ischemia should be collected on the way to cell-based therapies of myocardial infarction.

P 14.4
Suppressive activity of annexin II on immunological responses of cord blood cells
M Kheirandish
Iranian Blood Transfusion, Tehran, Iran

The presence of elevated levels of annexin II in retroplacental serum might indicate not only a role in protecting the fetus from immunological attack by the mother but also a role in producing the microenvironment which can modify, in part, immunological response of cord blood cells. Annexin II belongs to a family of calcium-dependent phospholipid-binding proteins. We have shown that soluble annexin II at 10 mg/ml has an immunosuppressive effect on lymphoproliferation of mononuclear cells. The aim of this study was to determine whether annexin II can modify NK cytotoxicity and T cells cytotoxic profile. The NK cytotoxicity of term, preterm cord blood and peripheral blood mononuclear cells were determined after 3.5 h of incubation in the absence or presence of RFl-2 and 10 mg/ml of annexin II with flow cytometry. For evaluating the intracellular cytokine profile, cord and peripheral blood mononuclear cells were stimulated with PMA and ionomycin in the presence of monensin and annexin II at 10 mg/ml. After incubation, the production of IFN-γ, IL-2, IL-10 and IL-4 were measured by flow cytometry. Results show incubation with RFl-2 for 3 days increased the NK activity but combination of RFl-2 and annexin II producing the lower increase. Annexin II alone has no effect on intracellular cytokine profile of T cells and NK cytotoxicity. Annexin II has immunosuppressive activity and in combination with the other factor(s) may be responsible for reduced function of cord blood cells.

P 14.5
Peripheral blood stem cell yield in malignant lymphoma and multiple myeloma
R Reiner1,2, E Schloe,3 E Filz,3 G Hofinger1, M Moestl1, H Muehlberger1, A Nader4, H Tschelld1, N Wore1 and M Bernhart1
1Third Medical Department, B. Bolzmann Inst. f. Hematology, Leuphana Univ., Celle, Germany; 2Department of Pathology, Hanusch Hospital, and 3Clin. f. Blood Group Serology & Transfusion Medicine, University of Vienna, Austria

Multiple factors (age, mobilisation chemotherapy, cytokine application, previous chemotherapy regimens, blood volume processed) influence the selection of peripheral blood stem cells (PBSC). We investigated the influence of these factors on the yield of CD34+ PBSC in patients with Multiple Myeloma (MM) compared to Non-Hodgkin’s Lymphoma (NHL) and Hodgkin’s Disease (HD), with high dose cyclophosphamide (CPM) and G-CSF for SC mobilisation. From 1993 to 2000, 150 patients (MM 31, NHL 77, HD 42) received CPM 4 g/m2 and G-CSF 5 µg/kg body weight (BW)/day. Pre-harvest chemotherapy regimens: median 1 (1–7). NHL/HD: 66

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male, 53 female, median age 44 years (17–65); MM patients: 15 male, 16 female, age median 56 years (35–65). In 117 patients with lymphoma and in 28 patients with MM aphereses were performed, a median of two collections (1–6) were necessary to achieve >2 × 10^6 CD34+ peripheral blood progenitor cells per kg BW for single transplantation and >5 × 10^6 CD34+ peripheral blood progenitor cells for tandem transplantation. Two patients with MM and two patients with lymphoma, respectively, did not have detectable CD34+ cells in peripheral blood. The median yield of CD34+ PBSC was 5.05 × 10^6 (range: 0.16–37.7) in malignant lymphoma and 8.44 × 10^6 (range: 0.82–48.1) in multiple myeloma. In conclusion, stem cell collection is feasible in the elderly and sufficient yields of blood stem cells for tandem PBSC transplantation can be harvested.

P 14.6
Cell line-specific evaluation of chimerism after allogeneic stem cell transplantation in pediatric patients
U Koehl, O Beck, D Schwabe, T Klingebiehl, E Seifried and C Seidl* Department of Pediatric Oncology, JW Goethe University and Second Department of Transplantation Immunology and Immunogenetics, RCBDS, Frankfurt am Main, Germany

Background: Determination of donor host chimerism in PB or BM by microsatellite (STR) marker provides relevant clinical information about engraftment of donor cells after allogeneic stem cell transplantation (SCT). Lineage-specific chimerism in highly purified leukocyte subsets seems to be even more informative for detection of graft failure or relapse of the disease.

Materials and Methods: We compared a singleplex in-house STR set with two commercially available multiplex STR systems (Profiler and Cofiler, ABI, Germany) in pediatric patients. Chimerism was detected routinely in PB, BM and CD3 selected T-cells. In some cases the cell populations investigated included granulocytes, monocytes, B-cells, stem cells, NK cells and T-cell subsets (CD4+ and CD8+) (all purified cells). In some cases the cell populations investigated included granulocytes, monocytes, B-cells, stem cells, NK cells and T-cell subsets (CD4+ and CD8+) (all purified using microbeads, AutoMacs, Miltenyi Biotec, Germany).

Results: Based on the in house STR system only, calculation of chimerism resulted in 3–9 informative STRs per patient. Standard deviation was <4.2% in PB (n = 297), <5.5% in BM (n = 46) and 6.9% (n = 39) in the purified leukocyte subsets CD34+, CD4+CD14+, CD6+CD15+, CD5+CD19+ and CD4+CD14+ and CD19+. Using both, the in house and the multiplex STR system gave comparable quantitative results.

Conclusions: Our results indicate that molecular assessment of chimerism can be performed with a wide variety of STR loci either by single or multiplex PCR. Quantitative determination of cell line-specific chimerism should facilitate therapeutic interventions.

P 14.7
The use of umbilical vein mesenchymal stem cells for expansion of cord blood hematopoietic stem cell
M Soleimani*, H Mozdarani and M Kadivar T.M.U., Hematology Department, Medical Genetic Department, Pasteur Institute, Tehran, Iran

Ex vivo expansion of human umbilical cord blood cells (HUCBC) is explored by several investigators to enhance the repopulating potential of HUCBC. The proliferation and expansion of human hematopoietic stem cells (HSC) in vitro is desirable to use these cells in clinical settings. For this purpose, umbilical vein mesenchymal stem cells (UVMSCs) are used as a support system for HUCBC. UVMSCs are able to produce hematopoietic colony forming units and support HUCBC proliferation and differentiation. FLT3/Flk2 ligand, SCF, IL-6, IL-3 under serum-free conditions. After 1 week or 2 weeks of incubation, respectively. In addition, LTC-IC assay were expanded more than 2– and 3.8-fold after 1 and 2 weeks of culture, respectively. Additional studies are needed to establish the functional activity of expanded UCB-HSC. This ex vivo expansion system should prove valuable in clinical settings in which UCMSCs are available from recipients or stem cell donors.

P 14.8
Engraftment in NOD/SCID mice of human CD34+ cord blood cells after thrombopoietin ex vivo expansion
F Vulcano*, G Mattia, L Milazzo, P Pascauccio, G Maccioni, A Giampaolo and HJ Hassan Department of Hematology Oncology and Molecular Medicine, Istituto Superiore di Sanita, Italy

Cord blood (CB) enriched with hematopoietic stem and progenitor cells is increasingly used as donor source for transplant into patients receiving high-dose radio

chemotherapy and is associated with delayed platelet recovery. We investigated on the capacity of thrombopoietin (TPO) amplified CD34+ cells from CB to reconstitute human hematopoiesis in NOD/SCID mouse model. 7.5 × 10^6 purified CD34+ cells were expanded in serum free medium in presence of 100 ng/mL of TPO for 7 days and then injected in 6–10 week irradiated NOD/SCID mice. The engraftment was evaluated on bone marrow cells by flow cytometry analysis using human CD45 MoAb staining: short and long term engraftment was observed with a mean value of 0.7% (range: 0.38–1.0%), 21.0% (range: 4.0–40.7%) and 12.0% (range: 1.51–62.0%) of CD45+ cells at 4, 8 and 12 weeks, respectively. Multilineage engraftment was also confirmed by flow cytometry for lineage committed (CD34+ B CD19, T CD34), neutrophilic (CD15) and MK (CD41 and CD61) cell specific markers. Mouse peripheral blood was collected via retro-ortbial bleeding at different time in EDTA-coated tube. Platelet enriched plasma was analyzed by flow cytometry after human CD41a-FITC staining. Low level of human platelets could be detected from 2 to 6 weeks after transplantation, indicating that TPO amplified CD34+ human cells were able to sustain platelet release. Furthermore, TPO contributes to reconstitute human hematopoiesis.
episodes, donors with DO1 alleles were involved. The AHTR's were probably caused by alloanti-Do(a). These antibodies were not identified earlier, because available cell panels do not allow for their identification. Selection of Do(a)-negative units by cross-matching failed, indicating that serologic methods were not reliable in this case. Molecular genotyping methods for DO1/DD2 are useful for patient identification and donor selection.

P 17.29
A patient with weakD type 1 and anti-D: auto or allo?
EAM Beckers*,1, FC Lightart2, MAM Overbeeke2, PA Maaskant2 and DJ van Rhenen1
1Sanquin Bloodbank Southwest Region, Rotterdam, and 2Sanquin Diagnostic Services Amsterdam, Amsterdam, The Netherlands

Weak D phenotypes are considered to result from low expression of complete D antigens. As a consequence alloantibodies to D are not to be expected. We present a patient with anti-D while expressing a weakD phenotype. Three years earlier she was typed by the hospital blood bank as D-neg with polyclonal anti-D serum; 1 year thereafter she was typed with monoclonal anti-D reagent as being D-pos and transfused with 2 units of D-pos RBC’s. Our aim was to determine the nature of the anti-D. Serologically the patient appeared to be weakD-positive. RH DNA and D exon-specific sequencing established the genotype as weakD type 1. Anti-D was detected in serum by PEG-IAT (+1) and papaine Diamed LC (+4). The DAT of the and the acid/ether eluates from the patient’s RBC’s were negative, even in the Diamed papaine LC, indicating alloanti-D. Adsorption studies with bromelain-treated RBC’s (1% D-pos in D-neg, D-neg, weakD type 1, patient’s RBC’s) were performed. Anti-D reactivity in comparable strength was found in the acid eluates of D-pos, weakD type 1, patient’s RBC’s. The acid eluate from the D-neg RBC’s remained negative. In the corresponding adsorbed sera anti-D reactivity was diminished in the D-pos, weakD type 1 and the patient’s RBC’s, when compared with the D-neg control. Indicating autoanti-D. The anti-D in this patient is most probably an autoantibody. The negative DAT and eluates of the patient’s RBC’s might be explained by the low expression of the D antigen on the patient’s RBC’s.

P 17.30
An unusual case of anti-Ge3
LS Carter*, J Banks*, J Poole*, A Wilkes*, M Needs* and N Win1
1NBS-Tooting Centre, and 2IBGRL, Bristol, UK

Background: Anti-Ge3 is a rare antibody that normally reacts serologically with papain-treated antigen-positive cells. There is one case report of severe delayed haemolytic transfusion reaction caused by anti-Ge3. We report the case of a patient with anti-Ge3 with unusual serological characteristics.

Case study: Mrs BH received blood during repair to her aorta in 1999. She now has an alloantibody to a high frequency red cell antigen.

Results: The BH cells were found to have the rare Ge:-2,-3,4 phenotype that was confirmed in immunoblotting experiments using anti-GPC/D monoclonal antibody BGLR 100. Her serum contained a strong antibody that was compatible only with Ge:-2,-3,4 and Ge:-2,-3,-4,-5 cells. The antibody was presumed to be anti-Ge3, but was unusual because the antigenic determinant was papain-sensitive. The Chemiluminescence Test result (opsonic index 7.2) suggested the antibody could be clinically significant.

Transfusion support: Eight Ge:-3 units were needed to cover the operation. There are no Ge:-3 donors in the UK. Two units of autologous blood and 1 unit from the International Rare Donor Panel have been obtained and stored as frozen units at Birmingham Frozen Blood Bank, UK. Three more autologous units will be collected preoperatively.

Conclusion: We report an example of apparent anti-Ge3 that was unusual in being papain-treated. The antibody may be directed towards a novel Ge-related determinant present on Ge:-2,3,4 cells.

P 17.31
H-negative erythrocytes are most strongly agglutinated by the galactose-binding lectins
N Gilboa-Barbera,1 D Sudaklevitz, C Levene and Y Yahalom1
1Faculty of Life Sciences, Bar-Ilan University, Israel, and 2The Blood Services Center, F. aeruginosa

The giant marine snail Aplysia produces a galactose-binding lectin AGL exhibiting anti-I specificity (Transfusion, 1999). The human opportunistic pathogen Pseudomonas aeruginosa also produces a lectin PA-IIL, which binds fucose and interacts with H and Lewis antigens (Vox Sanguinis, 2004). The three lectins with H-positive and H-negative erythrocytes using hemagglutination test showed that while PA-III strongly agglutinated the H-positive cells and weakly the negative ones (resembling Ulex europaeus lectin), AGL and PA-II revealed a reversed pattern. They agglutinated the H-negative cells significantly more strongly than the H-positive ones. The stronger interaction of AGL and PA-II with the H-negative cells could be attributed to the terminal galactose of either the nonfucosylated precursor of the ABH system or to the increased level of the I antigen in those cells resulting from free precursor availability. As both PA-II and PA-III are recognized as potential adhesins of this pathogen to the host cells, it would be interesting to statistically compare the sensitivities of the H-positive and -negative populations to P. aeruginosa infections.

Results of such a study would also provide some information as to the relative contribution of PA-II and PA-III to this bacterium infectivity.

P 17.12
Twenty-five years of rare donor screening at NBS Tooting centre
AR Gray*, ME Needs and N Win
National Blood Service, Tooting, UK

Background: Screening for rare blood donors has been performed for over 25 years at our Blood Centre. The donors’ details are put onto a local database, the NBS computer system (PULSÉ) and the International Donor Panel (IDP), maintained by the IBGRL. Methods and results: The current proven microplate methodology has been described previously. Screening figures and results over three time periods are presented. A survey of the fate of subsequent rare donations, over a 2-year period, has been undertaken and the results tabulated. The current number of active rare donors within the region are presented.

P 17.13
Transfer of cholesterol between intact human red blood cells
L Tse*, TJ Greenwalt, UJ Dumaswala and MMJ O'Leary
Harvard Blood Ctr., OH, USA

Previous studies of the transfer of cholesterol from red blood cells (RBCs) were limited to plasma, small unilamellar vesicles, and to ghosts. Our aim was to see if transfer between intact RBCs was possible. Group A RBCs were labelled with [4-14C]-cholesterol by incubation for 6 h at 37 °C with constant agitation. They were washed and resuspended in Basal Eagles Medium and then mixed with an equal volume of washed unlabelled group 0 RBCs. The mixtures were incubated and mixed for 32 h at 37 °C in a shaking-water bath. The A cells were agglutinated with a monoclonal anti-A reagent. The A cells were then trapped in a glass-head column, and the O cells collected (n = 4). The O cells were shown to have no contaminating A cells. The amount of labelled cholesterol transferred to the O cells was measured in their lipid extracts by liquid scintillation spectrometry.

Results: A total of 38.4% of labelled cholesterol was transferred from one cell population to the other.

Conclusion: Cholesterol will transfer from one intact RBC population to another in an ionic medium by contact alone.
ABO genotyping of 235 cadaveric kidney donors by PCR using sequence-specific primers

J Downing*, L Hammond* and C Darke
Welsh Transplantation and Immunogenetics Laboratory, Welsh Blood Service, Pontycym CF72 9WB, Wales, UK

Kidney transplantation across the ABO barrier generally leads to rapid graft rejection due to natural ABO antibodies. Transplant recipients and their donors are usually ABO grouped by serology. However, we use a PCR-SSP method for typing cadaveric organ donors that can be executed with HLA typing prior to transplantation. Thirteen primers in eight SSP mixtures differentiate the ABO alleles into five groups: [1] (including all A alleles, except A01); [2] A01; [3] O1 (all O alleles, except O03); [4] 003; and [5] B (all B alleles). All ABO alleles [see - www.abc.org.au/abc/blood] are detected. The PCR-SSP 'phenotypes' of all 235 donors were in accord with their serological ABO group. Thirteen of the possible 15 ABO genotypes were found with frequencies (%) of: A1A, 34; A1B, 30; A2B, 26; A1O, 26; A2O, 9; A2-, 9; A2B, 13; A2O1, 60; A2O2, 30; B, 43; B1, 81; B2, 0; D, 30; D-, 30; D2-, 30. These, and the 'phenotype' frequencies, were not significantly different [Fisher's exact P-value [corrected for number of comparisons] < 0.05] from 166 random 'Welsh' blood donors. Our PCR-SSP method is a quick, accurate and simple means of ABO genotyping. It allows simultaneous testing for the two major histocompatibility systems, ABO and HLA, on the same sample of DNA. As cadaveric donor typing is often performed 'on-call' during 'unsociable' hours this is a small, but important, addition to donor typing security.

Protein 4.1R (4.1R)-binding death receptors of the erythrocyte membrane

D Head*, ZE Lee, J Poole, L Kennedy and ND Avent
CRB, UWE, Bristol, UK

Integrin-associated protein (CD47) has been reported to have a diverse repertoire of cellular functions. One is as a death receptor to mediate a form of caspase-independent apoptosis in a variety of nucleated haemopoietic cell lineages. Quantitative RT-PCR (QRT-PCR) was used to study the expression of five CD47 isoforms in erythroblasts. Immunoprecipitation, immunoblot, mass spectrometry and proteomic experiments (QRT-PCR) was used to study the expression of five CD47 isoforms in erythroblasts. Apoptosis in a variety of nucleated haemopoietic cells was studied by quantifying the expression of the high-incidence antigen Tc in a pregnant woman and the expression of Tc in her Black husband. Case study: The serum of a pregnant woman [34 weeks] who was at risk for a premature partus with possible blood loss was screened for the presence of irregular erythrocyte antibodies. Anti-Tc c was present and, in addition, a positive cross-match was found with a Colb(+) donor and with the Colb(+) husband. Because of the rarity of anti-Tc c information on the risk of HDN is antithetical to the high-incidence antigen Tca and the low-incidence antigen Tcb. Until now, Tc c expression has been found only in Caucasians. Here, we describe an example of anti-Tc c in a pregnant woman and the expression of Tc in her Black husband.

Identification of anti-Tc c in a pregnant woman with a Tc(a+c+) Black husband: a case study

PC Ligthart*, J Poole, J Banks, JD Gosting, WM Van der Graafl, MAM Overbeek and CC Follman
Sanquin Diagnostic Services, Amsterdam, The Netherlands, 2BGRL, Bristol, UK, and Ziekenhuis Bernhoven, Veghel, The Netherlands

Background: The Tc c antigen is a low-incidence antigen of the Cromer blood group and is antibalitical to the high-incidence antigen Tc and the low-incidence antigen Tc. Until now, Tc c expression has been found only in Caucasians. Here, we describe an example of anti-Tc c in a pregnant woman and the expression of Tc in her Black husband.

Conclusion: Because of the rarity of anti-Tc c information on the risk on HDN is lacking.
Conclusion: The UK guidelines do not require Cw as part of the antibody screening profile. With no maternal antibody detected, there was no initial requirement to perform a DAT (BCSH guidelines). Non-requested testing revealed the low Hb and blood film abnormalities of the delivery sample. Irrespective of the presence of a maternal antibody, clinical monitoring of the newborn is of the utmost importance. A DAT can provide a simple and rapid diagnostic test in all cases of neonatal jaundice and anemia.

P 17.40
The incidence of red-cell alloantibodies underlying pan-reactive warm autoantibodies
M Maley*, D Bruce, RG Bahb and AW Wells
National Blood Service, Newcastle, UK
Introduction: One of the recognized hazards of transfusing patients with pan-reactive warm autoantibodies is that clinically significant alloantibodies can be masked. Collective data studies have shown the incidence of alloantibodies in such cases to be around 32%. We report on a study of samples from 107 patients referred to the Newcastle NBS Red Cell Immunohaematology laboratory over a period of 18 months. The study concerns patients from the north-east of England, a population from which data has not previously been reported.

Methods: Samples identified as containing pan-reactive warm autoantibodies were subjected to absorption procedures (78 by alloabsorption and 29 by autoabsorption). Absorbed sera were then tested to identify underlying alloantibodies.

Results: Of 107 samples tested, 32 samples (30%) contained a total of 53 red cell alloantibodies. Thirteen samples (12%) contained two or more specificities. Antibodies identified included the following specificities: E (17), D (7), C (5), c (5), Fy (5), Fy (3), Jk (2), K (2), Kp (2), Fy (2), Cw, N, n, f (ce), and e.

Conclusion: This study reinforces the value of absorption studies, whether using autologous or allogeneic reagent red cells, when pan-reactive warm autoantibodies are present. The results of this study also show that it is not appropriate in these cases to simply issue blood which is ‘least incompatible’ of Rh phenotype and k antigen compatible.

P 17.41
Anti-D quantification by flow cytometry
JK Manku*, M Williams and D Armstrong
Leeds Blood Centre, UK
Anti-D quantification is important in the management of haemolytic disease of the newborn (HDN). Continuous flow analysers (CFAs) offer reliable, reproducible results. Disadvantages of CFAs include artificially high results due to nonspecific antibodies to enzyme-treated cells, or IgM antibodies. CFAs are expensive and can only be used for antibody quantification. Anti-D can be quantified by Flow Cytometry (FC). This retrospective study compares results obtained from antenatal samples containing anti-D, by CFA and FC. Information on the severity of HDN was collected in a number of cases.

Method: The FC method was adapted from previously published work. Analysis was performed on an Ortho Cytoron Absolute at 489 nm. A calibration curve was prepared using dilutions of Second British Standard anti-D against the mean peak fluorescence obtained, and patient and EQA samples compared with the curve.

Results:

| Rank severity of HDN | Anti-D level (IU/mL) | CFA | FC |
|----------------------|----------------------|-----|-----|
| 1                    | 21.0                 | 12.5|     |
| 2                    | 8.0                  | 7.3 |     |
| 3                    | 59.0                 | 50.2|     |
| 4                    | 10.0                 | 4.7 |     |
| 5                    | 13.0                 | 7.5 |     |
| 6                    | 46.0                 | 33.5|     |
| 7                    | 4.0                  | 1.7 |     |
| 8                    | 5.0                  | 3.5 |     |
| 9                    | 132.0                | 120.1|    |
| 10                   | 22.0                 | 10.6|    |
| 11                   | 5.0                  | 2.0 |     |
| 12                   | 5.0                  | 1.5 |     |
| 13                   | 4.0                  | 0.9 |     |
| 14                   | 9.0                  | 7.5 |     |
| 15                   | 28.0                 | 21.3|     |
| 16                   | 4.0                  | 1.2 |     |

Conclusion: The FC offers an accurate and reproducible alternative to the CFA for anti-D quantification. FC detects IgG antibodies that can cross the placenta and cause HDN, high values due to IgM anti-D are not obtained as with the CFA. A drawback of FC is that it is a lengthy process requiring plasma titrations. FC is more flexible than the CFA and can be used for many purposes for a similar outlay.

P 17.42
ABO serology and genetics in a family with an Xq subgroup propositus whose parents are blood group O
B Michalewska1,*, A Walaszczyk1, Å Hellberg2, MA Chester1 and ML Olsson1
1Inst. Haematology and Blood Transfusion, 2Blood Transfusion Centre, Warsaw, Poland, 3Department of Transfusion Medicine, Lund University and Blood Centre, Lund, Sweden

Background: Apparent deviation from Mendelian rules in inheritance of blood groups is rarely observed. Patients with blood group O having children expressing weak A subgroups have been described previously but not explained.

Materials and methods: Direct agglutination tube tests and adsorption/elution were performed on fresh EDTA blood with commercially available monoclonal and polyclonal reagents and lectins. Anti-A/A1 in plasma was titrated. The ABO locus was analysed by PCR-ASA1/RELP genotyping and direct DNA sequencing of exons 6–7 and intron 6.

Results: The presence of a weak A antigen was suspected on routine grouping of a woman whose parents were previously grouped as O. The daughter’s RBCs were very weakly agglutinated with monoclonal anti-A but distinctly with polyclonal anti-A-B, i.e. typical reactions for Ax. Anti-B and -A1 were present in serum. Again, reactions typical for group O were observed in her parents’ blood, although the anti-A/A1 titre was lower in the mother. Adsorption/elution demonstrated A antigen on the daughter’s cells only. The ABO genotypes were: daughter A4/02, mother A4/01, father O1v/02. Mother and daughter both had the A4 hybrid allele with a crossing-over breakpoint between A1 and O1v between positions 236 and 445 in intron 6.

Conclusion: ABO genotyping revealed the presence of an A hybrid allele, earlier known to express either weak A or no activity, the expression of which appears to depend on the O allele present in the trans position.

P 17.43
Dramatic anamnestic response in a patient with anti-CHIDO due to transfusion of PCC
EL Park*, RE Broadberry, S Yates, CIG Ghevaert, FE Boulton1 and J Poole1
1Transfusion Dept, Southampton General Hospital, 2NBS, Southampton Centre, and 3IBGRL, UK

Background: Ch/Rg antibodies are directed against C4 epitopes and are considered clinically insignificant with regards to RBC transfusions. However, transfusions of C4-containing plasma products have resulted in severe anaphylactic reactions. In this report administration of Beriplex® prothrombin complex concentrate (PCC) resulted in a dramatic anamnestic response in a patient with Anti-Ch.

Case details/results: A 77-year-old female who had previously undergone cardiac surgery and was on long-term anticoagulation, had a group and antibody screen prior to an emergency colo-rectal procedure. Anti-Ch (titre 4) was detected. FFP was requested to correct the patient’s raised INR but due to the potential risk of anaphylaxis, Beriplex® was substituted. Three days later anti-Ch was no longer detectable, probably due to neutralisation by C4, which was subsequently confirmed to be present in Beriplex®. A further dose of Beriplex® was administered. Two days later the anti-Ch titre had risen to 2048 reaching a maximum of 32 768 on day 9, which continued for several days. Anaphylaxis was not observed.

Discussion: The anamnestic response observed in our patient may indicate that PCCs are not suitable alternatives to FFP in patients with anti-Ch, as was originally hoped. Patients with anti-Ch who require coagulation factor therapy may be managed more appropriately with products that do not contain C4, such as Novoseven® (activated FVIII), thus avoiding the potential risk of anaphylaxis.

P 17.44
Observation of an anti-D after D-positive transfusion in an individual with weak D type–1 phenotype
D Roxby*, M Coloma, WA Hiegel, J Poole, P Martin and R Abbott
Flinders Med. Cen., Bedford Pk, South Australia, Abbott Pathology, South Australia, IKT Ulm und Univ. Ulm, Germany, IBGRL, Bristol, UK

Introduction: In 0.2–1% of Caucasians, D is expressed on red cells in a weak form. The weak D phenotype is caused by many RHD alleles encoding aberrant RhD proteins which express distinct serologic phenotypes. In many countries it is recommended to transfuse carriers of the prevalent weak D types, including type 1 with D-positive red cells.

Case report: In October 2000 a 74-year-old Caucasian female (FS) of European background was typed as D negative. Her antibody screen was negative. Later she was typed as D positive (weaker than normal expression) and received D-positive red cells. In May 2001 anti-D was detected by indirect antiglobulin (IA) and gel along with a positive direct antiglobulin test. Anti-D was eluted. In all subsequent transfusions D-negative red cells were used. Anti-D remained detectable in her plasma by IA and gel to
April 2003 (3 months prior to her death) with no evidence of haemolysis. Direct antiglobulin test was negative. Interestingly, in specimens received during May–July 2003 the anti-D had disappeared. Results obtained from polymerase chain reactions (PCR) using DNA extracted from an EDTA sample confirmed her genotype as CcDe D-. Further extensive studies by PCR using sequence-specific priming demonstrated that her red cells were weak D type 1.

Discussion: We report the observation of anti-D after D-positive red cell transfusion in a patient carrying the weak D phenotype (weak D type 1). No evidence of haemolysis or clinical sequelae was reported.

P 17.45

A new hybrid RhD-positive, D-antigen negative allele

CP Shaoa, W Xiong and YY Zhou
Shenzhen Blood Center, P.R. China

Background: To determine a RH/RHCE hybrid allele in Rh-negative individual.

Materials and methods: The Rh factors were serotyped routinely and genotyped with PCR-SSP in a Chinese donor. The intron 2 of RH and the downstream Rhhesus box were also detected through a PCR kit (GHT Company, CA, USA). Moreover, the RH coding region was sequenced by using a published method that amplified all 10 RH exons with 10 pairs of D-specific intron primers.

Results: The donor was determined truly Rh-negative (DO was excluded) with C c + e E e+ phenotypes in both serological tests and genotyping method, which detects exons 3–7 and 9–10 of RH and exons 1, 2 and 5 of RHCE in the sequencing PCR. However, it detected sequences identical with normal RH in exons 1 and 2 while exons 3–10 tested negative. Since RH and RHCE have identical sequence in exon 2 and the lower primer for exon 2 is RHCE-specific in the sequencing method, the intron 2 of RH was further detected with one pair of D-specific primer. A negative result showed that a hybrid RH-CE2(10)-10 was likely in this donor. Another feature of antigen D-negative, RH-positive alleles is the presence of the downstream Rhesus box. It was tested positive.

Conclusion: Our results suggest that a new hybrid RH-CE2(10)-2 allele is present in this D-negative, C-positive Chinese individual.

P 17.46

Review of 67 cases of Fetomaternal Haemorrhage (FMH) with follow-up data

E Simpson*, NA Clark and G Burgess
Department of Red Cell Immunohaematology, National Blood Service, Cambridge Centre, UK

D negative women with D positive babies, showing an FMH >4ml are referred for estimation by flow cytometry (FC) in order that the correct dosage of prophylactic anti-D immunoglobulin may be given. We have reviewed 67 cases (3–125 ml FMH) in which we have data for additional samples tested after prophylaxis, including four cases where subsequent sensitisation to the D antigen has occurred (three following stillbirth). Summary of results:

| No. of cases | FMH range | Clearance | Anti-D given |
|--------------|-----------|-----------|--------------|
| 31           | 3–325 ml  | 100%      | iv* and i.m. |
| 23           | 4–187 ml  | >50%      | i.v. and i.m.|
| 10**         | 6–26 ml   | <50%      | i.m.         |
| 2**          | 7 and 19 ml| 0         | i.m.         |

*iv. anti-D given in most FMH >100 ml
**Of these, at least four did not have free anti-D detected in the patient’s plasma, postprophylaxis.

From our results it appears that mothers receiving i.v. and i.m. anti-D showed clearance of D positive cells, but delayed clearance was seen in some patients receiving only i.m. prophylaxis. Stillbirth must be regarded as a risk factor for sensitisation since it is associated with three of four cases. Expected clearance of FMH, after the appropriate dose of i.m. anti-D has been given, happens in the vast majority of cases (82%) in this series. A few cases (18%) did show poor clearance and this may lead to an increased risk of seroconversion.

P 17.47

Identification of RhCG isoforms in the erythrocyte membrane

LH Skinner*, 1 JD Head, 1 MR Wang2 and ND Aveney1
1CRIB, UWE, Bristol UK, and 2National Laboratory of Molecular Oncology, Beijing, China

The Rh protein family consists of RhD, RhCE, RhAG, RhBG and RhCG. Absence of RhD, RhCE and RhAG expression in nonerythroid tissues suggested them to be erythroid specific proteins. However, the recent identification of RhAG in cesophagial tissue proved otherwise. RhBG and RhCG were described as nonerythroid proteins expressed in kidney, brain and liver. Sequence homology between Rh and MEP/AMP transporters indicated a role for the former as NHE3 transporters. This was further corroborated when RhAG was found to export NH4+ from intact erythrocytes. However, the absence of the Rh complex does not totally eliminate NH4+ transport, thus suggesting the presence of another NHE3 transporter on the surface of red cells. Polynucleotide antibodies directed against a downstream, exoDio 3 and exoD 6 of the published RhCG protein sequence were generated and used to immunolabell erythrocyte ghost membranes which were either untreated or digested with a panel of proteases. Multiple erythroid isoforms of RhCG (103, 76 and 66 kDa) were identified within membrane preparations from both normal and Rhnull erythrocytes. The behaviour of the three proteins when digested with selected proteases suggested that they differed from the published RhCG sequence and each other at the N- and C-termini. The work presented here describes the identification and characterization of novel RhCG protein isoforms, whose presence may explain the existence of NHE3 export within Rhnull erythrocytes.

P 17.48

An unusual case of autoimmune haemolytic anemia (AIH) with a negative DAT and a low-affinity autoantibody

R Stamps*, 1 D Booker1, P Wright1, T Loades1, J Sharpe2 and SG Jones3
1National Blood Service, Sheffield, and 2Kings Mill Hospital, Sutton-in-Ashfield, UK

AIH in which the DAT is negative may be due to low affinity autoantibodies. It has been reported that low affinity autoantibodies can be detected by performing the DAT with cells washed with cold saline or by gel column. We report a case of AIH where the DAT was negative using both these methods. A patient presented with Hb 5.2 g/dl, WBCs 23.7 x 109/l, retics 5.2 x 1012/l, bilirubin 67 µmol/l, and LDH 899U/l. The blood film showed mild lymphocytosis with smear cells and a haemolytic picture. CLL was confirmed by immunophenotyping. Diamond gel was used for antibody ID, Rh phenotyping and a DAT. Tube tests included LISS IAT, saline agglutination and a DAT with patient’s red cells washed in cold PBS. The patient’s C and e-antigen strength was assessed by titrating monoclonal anti-C and anti-e and comparing the patient’s titre score with an R+ control cell. The Rh phenotype was D c+ e E e+.

Diamond IAT and saline agglutination tests, including auto tests, gave strong reactions with evidence of anti-C e specificity, but only weak reactions were obtained using tube LISS IAT. The DAT was consistently negative by both gel and tube technique. The titration study revealed no evidence of antigen suppression. Although cold washing procedures and/or gel column methods are usually sufficient to demonstrate the presence of low-affinity autoantibodies, our case illustrates that this is not always successful thus confirming that a diagnosis of AIH should not be ruled out by a negative DAT.

P 17.49

Cytokine role in autoimmune hemolytic anemia (AIHA)

C Toriani-Terenza*, E Fagiolo and U Pozzetto
Transfusional Centre; Shock Centre, Catholic University of ‘Sacro Cuore’, Rome, Italy

AIHA is a autoimmune disease caused by autoantibodies against red blood cells self-antigens. To identify the immunological factors contributing to the autoimmune onset and maintenance, several murine strain, which spontaneously develop a complex autoimmune syndrome including AIHA, have been extensively studied. In this study, functional abnormalities of PBMC have been investigated, in particular about cells activation and cytokines production. Titrative role of IL-2, IL-4, IFN-g, IL-10 and IL-12 in AIHA have been investigated by examining the spontaneous and mitogen-induced (OKT3 or LPS) production of these cytokines by ELISA methods in PBMC to evaluated if the manipulation of IL-10/IL-12 balance can affect autoimmune diseases incidence. Our results affirmed that AIHA exhibited an increased basal synthesis of IL-4 and decreased levels of IFN-g compared with controls and then IL-12 and IL-10 neutralization may be efficacious in diminishing the clinical pathology associated with Th2 subset prevalence. The treatment with IL-12 could offer a second and independent level of blockade against the consequences of the immune diseases incidence. Our results affirmed that AIHA exhibited an increased basal synthesis of IL-4 and decreased levels of IFN-g compared with controls and then IL-12 and IL-10 neutralization may be efficacious in diminishing the clinical pathology associated with Th2 subset prevalence. The treatment with IL-12 could offer a second and independent level of blockade against the consequences of the over B-cell activation associated with AIHA.

P 17.50

Twenty years of red blood cell (RBC) phenotyping in alloimmune stem–cell transplantation (SCT)

A Wikman*, M Remberger, O Ringdén, E Watz, M Uzunel and A Shanwell
1CRIB, UWE, Bristol UK, and 2National Laboratory of Molecular Oncology, Beijing, China

During the period 1977–2003. The aim of this study was to identify a difference in RBC
phenotype between the recipient and the donor to determine the origin of erythro- poiesis at different time intervals after SCT.

Materials and methods: In 521 cases RBC phenotype were available in the recipients and the donors before SCT and in the recipients after three months, six months and then yearly. The diagnosis were hematologic malignancies 411, solid tumors 14, nonmalignant hematologic diseases 58, metabolic diseases 36 and immunodeficiencies 2. Blood groups (ABO) and RBC phenotypes (Rh, MNSs, Kell, Duffy, Kidd, Lutheran, Lewis and P1) were determined with routine methods.

Results: In 97% of the cases a marker was identified. In 44% an ABO-difference between the recipient and the donor was found. After 3 months 38 %, 6 months 83%, and 1 year 91% of the recipients had the donor RBC phenotype. After one to 16 years 23 (5%) were chimeras with both recipient and donor RBC for two to 15 consecutive years. Of these 14 were treated for nonmalignant and nine for malignant disease.

Conclusion: A marker for the RBC origin could be identified in 97% of the SCT. In long-term follow-up 15% of the patients transplanted for nonmalignant disease and 2% of the patients transplanted for malignant disease became stable RBC chimeras.

**P 17.51**  
Whole exon 5 and intron 5 replaced by RHCE in D^Vs (Hus)  
W Xiong*, CP Shao and YY Zhou  
Shenzhen Blood Center, P.R. China

Background: The D^Vs (Hus) was previously investigated through cDNA analysis, which revealed a RH-CE(5)-D hybrid allele. However the 5′ and 3′ breakpoints remain unknown.

Materials and methods: The gene recombination between the RHD and RHCE alleles was investigated by a combination approach of a sequence-specific primer-PCR and a RHD full-length coding region sequencing method on a Chinese subject with weak D phenotypes. The hybrid Rhesus bus was also investigated through an established PCR-based method.

Results: The sample was serotyped with Rh phenotypes of Decer. By genotyping, a partial D^Vs (Hus) was identified carrying one hybrid RH-CE(5)-D allele. Further sequencing analyses showed that the sequence of intron 4 is identical with RHD, whereas the whole sequence of exon 5 and intron 5 is identical with RHCE except for seven polymorphisms in the intron 5 (EMBL/GenBank/DDBJ AY330698): 23–25(GCA), 986G>A, 205–206InsT, 494–495InsA, 1256–1257InsC, and 1347G>T. Combined with the result of the the RH D zygosity test, this sample was mostly a D^Vs allele genotype assuming the RHCE and RH D in cis.

Conclusion: We may concluded that in the case of this Chinese D^Vs (Hus), the whole exon 5 and complete intron 5 of a total segment of 1801 nucleotides were replaced by RHCE suggesting that the breakpoints of the replaced region are 5′ end of the exon 5 and 3′ end of the intron 5.

**P 17.52**  
Is: a new Gerich blood group antigen located on the GPC and GPD  
R Yake*, M Uchikawa1, H Tuneysama2, K Ogasawara2, T Toyoda1, Y Suzuki2, H Shimizu1, S Uchida and K Nakajima1  
1JRCS Tokyo Western Blood Center Tokyo, Japan, and 2JRCS Tokyo Metropolitan Blood Center, Tokyo, Japan

The Gerich blood group system consists 3 high-incidence (Gez,Ge3,Ge4) and 4 low-incidence antigens (Wb, L*, Dha, An?) that located on the glycosphingolipid C (GPC) and/or D (GPD). We describe here the new low-incidence blood group antigen, IS, in Gerich system. Anti-IS was identified in our laboratory by routine serological tests. By the antiglobulin test using the anti-IS serum, we detected three IS individuals from 32 852 Japanese blood donors (0.009%). In addition, we detected three anti-IS in 5.447 Japanese (0.06%) using the IS RBCs. Family studies showed that IS antigen was inherited by the simple Mendelian manner. The serological tests revealed that IS antigen was affected by the treatment of papain, trypsin and neuraminidase, but was not by chymotrypsin and AET. Immunoblots after SDS-PAGE under the reduced condition, approximately M, 40 000 and 30 000 bands were observed on IS+ RBCs. When monoclonal antibody-specific immobilization of erythrocyte antigens test using murine anti-Ge4 antibody was performed, clearly positive result was observed on IS+ RBCs when compared with IS- RBCs. Nucleotide sequence analysis of GYPCE gene revealed that IS individual had a single nucleotide substitution at position 95 C > A resulting the asparagine amino acid substitutions, Thr32Asn for GPC and Thr11Aam for GPD. These results indicated that the new low-incidence antigen, IS, should belong to the Gerich system, locates on both GPC and GPD, and is created by a 95 C > A nucleotide of GYPCE gene.

**P 17.53**  
Molecular genetic analysis of the Am phenotype  
L-C Yu*, M-J Hou, Y-C Twu and M Lin  
Inst. Biochemical Sciences, National Taiwan Univ., Taiwan; Dept. Medical Res., Mackay Memorial Hospital, Taiwan

A molecular genetic analysis of the samples from a Taiwanese family with the Am phenotype was carried out. The A1 allele with G–A change at nucleotide 664 was identified in the family members with the Am phenotype. The G–A change predicts the amino acid alteration of Val to Met at residues 212 in the encoded product. A PCR-RFLP for NalII was designed to detected the ABO allele with the G664A mutation. A total of 40 randomly selected samples from the general population with the common A1 phenotype were screened by the PCR-RFLP method, and none of them had the allele with the G664A mutation. The transferease activities of the Am plasma and the expressed product from the Am cDNA were examined as H type 2 and also H type 1 structures serving as acceptor substrates. The A transferase activity was not detected in the Am plasma when H type 2, nor when H type 1, using as acceptor substrates. Compared with the expressed A1 transferease, the Am transferease expressed in COS cell showed weak A transferease activity on both H type 2 and H type 1 substrates. The Am transferease identified in this pedigree did not seem to have different substrate specificity between H type 2 and H type 1 acceptors.

**P 18.1**  
Foetal K1 genotyping from maternal plasma  
S Abdullah1, M Moss1,2, M Greiss1,2, S Urbaník1,2 and S Armstrong-Fisher1,2  
1ATMI, University of Aberdeen, UK, 2SNBTS, Aberdeen, UK

Background: Women who have antibodies to K1 and who have K1-heterozygous partners have a 50% chance of carrying a foetus at risk of Kell HDN. As the molecular basis of the K/k (K1/K2) polymorphism has been elucidated, it should be possible to determine the K1 status of a foetus from amplification and KEL genotyping of free foetal DNA present in maternal plasma.

Materials and Methods: Venous blood (5 ml) from 50 K1-negative (kk) pregnant women was drawn into tubes containing EDTA. The range of gestational age at the time of blood sampling was 8–37 weeks. Corresponding delivery and cord blood samples were also collected. DNA was extracted from stored plasma aliquots of these samples using a QIAamp Blood Kit. Real-time PCR assays were performed in triplicate for K1, SRY and β-globin using an ABI 7700.

Results: In total 51 plasma samples were obtained from K1-negative pregnant women. No positive signals were detected in 46 confirmed K1-negative pregnancies. Two fortuities were correctly predicted to be K1-positive by real-time PCR. In the remaining two pregnancies; two of three replicate results gave very weak K1 signals. Later these two fortuities were confirmed to be K1-negative by serology and genomic DNA typing of cord blood cells.

Conclusions: Our study demonstrates the feasibility of KEL genotyping using DNA extracted from plasma. Early, non-invasive foetal genotyping by real-time PCR should facilitate the management of haemolytic disease of the newborn in K1 sensitised pregnant women.

**P 18.2**  
ceRA: a new allele of the RHCE gene, a rare new blood group  
H Ansart-Pirenne1, P Gallion, G Juszcak, C Paterau, C Tournamille, P Rouger and F Noizat-Pirenne  
CNRS, INTS, France; EFS Béziers, France, and EFS Henri Mondor, France

Background: Many altered Rhce alleles have been described in Black individuals. Some of them induce partial e. Here, we describe a new altered Rhce allele.

Methods and Results: RBCs from an Indian weakly expressed the Rhes antigen. The phenotype was ddccee. No antibody was found in the serum, but he was not pre- previously exposed to normal Rhe antigen. Pattern of reactivity (agglutination and flow cytometry) with monoclonal anti-e antibodies showed a reaction with only one antibody among 6. No reactivity was found with anti-RH19 (He) reagent, suggesting
a partial Rhesus antigen. Molecular analysis showed a G→C and a G→C substitution at the homozygous state producing a Trp16Cys and a Gly180Arg substitution in the intramembranous domain of the Rhce polypeptide. Reactivity of c antigen was decreased with only one monoclonal antibody among 5. Then, replacement of glycine by a charged amino-acid in position 180 may account for conformational changes with high consequences on Rho antigenicity and low consequences on Rho antigenicity.

Conclusion: This new RhoC allele is probably a partial antigen since reactivity was negative with anti-RhoC1 reagent and since most of anti-e monoclonal antibodies did not react with this variant. We think that anti-e allo-immunisation could occur against lacking epitopes. Blood donation in the frozen rare Rh blood bank has been strongly advised.

P.18.3 Evaluation of genotyping blood groups in daily transfusion practice

EAM Beckers*, L Warnier, J Drunen van, DJ Van Rhenen and PA Maaskant-Van Wijk
Sanguin Blood Bank, South West Region, Rotterdam, The Netherlands

DNA typing of ABO, RhD, RHEe, RHc, KEL1/2, FY1/2 (including GATA-FY) and JK1/2 is routinely performed in our laboratory when [a] serological results are questioned in recently transfused patients, [b] genotyping results might influence serologically based transfusion advice in case of allo-autoantibodies and [c] typing sera are unreliable. We evaluated genotyping results over the last 2 years. A total of 221 requests for genotyping (7 ABO, 27 RhD, 51 RHc, 34 KE, 26 JK and 11 FY) involving 128 samples were analysed. 93/128 patients had received recent blood transfusions. In 86/128 samples the ‘best guess’ phenotype was confirmed. Adjustments of transfusion advice were made in 7/93 samples (7.5%). Distinctions between auto/allo-anti RhE/e, Jk(a), RhD and Rhc could be made based on PCR results. An allo-anti-Jk(a) and an allo-antiRHE could be confirmed by homozygous JK2 and RhE PCR results. Of one patient no serology could be performed and PCR results. An allo-anti-Jk(a) and an allo-antiRHE could be confirmed by homozygous JK2 and RhE PCR results. Of one patient no serology could be performed and PCR results were used (RhCc, RhE, RhC). In 35/128 samples DNA analysis was performed because of typing problems with ABO (5), RhD (21), RhCc (7), RhE (8), Kell (4) and FY (1). In this group five adjustments of transfusion advice [13.5%] involving a partial D instead of weak D and four times confirmations of weak D were made. In conclusion, blood group genotyping might complete indefinite serological results, contributing to further improvement of transfusion safety in daily practice.

P.18.4 Rapid genotyping of blood group antigens by multiplex PCR and DNA microarray

SHW Beiboru*, T Wieringa-Jelma*, PA Maaskant-Van Wijk*, VE Van Der Sloot*, DR Roos*, JT Den Dunnen* and M De Haas*
1Sanguin Research at CLB & Landsteiner Laboratory, AMC, UVA, NL, 2Sanguin Blood Bank South West Region, NL, and 3Leiden Genome Technology Centre, LUMC, NL

Background: Patients in need of recurrent transfusions are at risk to develop allo-
antibodies. When multiple alloantibodies are formed or when antibodies are directed against high-frequency antigens it is difficult to find matching red cells or platelets.

Complete typing of all donors is laborious and not feasible due to lack of sufficient typing reagents. In contrast, the DNA microarray technology offers an efficient high-throughput method. We are developing a DNA microarray for complete genotyping of red cell and platelet antigens.

Materials and Methods: A multiplex PCR was developed to both amplify and fluor-
escently label gene fragments of 18 antigen systems in one tube. The allele-specific oligohybridisation method (ASO) was used to discriminate between two alleles.

A blind panel of 58 donor samples was genotyped for HPA-1, -2, -3, -4, -5 and -15. Samples were phenotyped for HPA-1, -2, -3, -4 and -5 and genotyped for HPA-15 by TaqMan technology.

Results: After DNA isolation, all donors were tested within 25 h and analysed. One discrepancy was found for HPA-3 typing: HPA-3ab instead of HPA-3b. This made us adjust the scoring criteria and validate the new format.

Conclusion: These results show that typing of all clinically relevant red cell and platelet antigens by microarray provides a fast and reliable typing method for blood supply. It facilitates complete typing of all blood donors to guarantee the availability of typed blood products with a rare phenotype.

P.18.5 Foetal RHD gene detection in plasma samples of RhD-negative pregnant women

A Orzin’ska, K Guz, E Brojer* and B Zapanksa
Institute of Haematology and Blood Transfusion, Warsaw, Poland

Methods: DNA isolation: from 1 ml of plasma (Qiagen Kit) (50 samples) or from 2 ml (NucleiSens Extractor, Biorad) (156 samples), Real-time PCR: [A] PRISM 7700 for RHD exons 10, 7, intron 4 and SRY. All RHD and SRY negative plasma were tested for the paternal marker (7 ins/del polymorphisms) if parents’ genotypes were informative.

Results: 189 plasma samples from 143 RhDneg women in various periods of the pregnancy.

Conclusions: [1] The high input automatic DNA extraction is essential for reliable results of foetal RHD in maternal plasma. [2] The amplification of marker genes confirmed the presence of foetal DNA in 93% pregnancies; thus additional polymorphisms are needed as a control for RhDneg female foetuses.

P.18.6 Different DNA profiles in various tissues after allogeneic haematopoietic stem cell transplantation

EM Dauberr*, G Dorner*, M Mitterbauer*, B Glock*, S Wenda* and WR Mayr*,1,3
1Division of Blood Group Serology, 2Department of Medicine I, Medical University, Vienna and 3Red Cross, Blood Donation Centre, Vienna, Austria

A mixture of the genetic patterns of both twins is expected in blood chimeras, but was also observed to a minor extent in other tissues (e.g. buccal cells and fingernails) in two pairs of chimeric twins. In one of these patients, who represent artificial blood chimeras after successful engraftment, the aim of the study was to find out whether or not donor cells can also be found in tissues other than blood. The samples of different tissues (blood, buccal cells, fingernails, eyebrows) have been taken from five patients at least 5 years after successful BMT and from donor and recipient prior to transplantation. Eleven polymorphic short tandem repeat (STR) loci were tested in all samples. After transplantation the DNA profiles obtained from the blood of all recipients were identical with those of their bone marrow donors. In the buccal cells and fingernails of all recipients, a mixture of the genetic patterns of donor and recipient was found. Only the DNA profiles of the hair samples were identical with the results of the blood sample prior to transplantation in all recipients and free of admixture from the donors. These results confirm the findings in the chimeric twins, i.e. that donor cells originating from haematopoietic tissue can migrate into other tissues. Hair has turned out to be the best source to define the true genotype.

P.18.7 RHD MPX PCR to support serology and for prenatal typing, 5 years experience

PC Lichterr,1,2 LDM Schuitemaker*, J Drunen1, PA Maaskant-Van Wijk1,2
MAM Overbeeke1 and M De Haas1
1Sanguin, Diagnostic Services and 2Sanguin, Bloodbank South West Region, The Netherlands

Since 1999, we use an RHD multiplex (MPX) PCR, amplifying exons 3 to 7 and 9 of RHD, to define serologically aberrant Rhd expression (weak or variant D; n = 157) and the foetal RhdD status with amniotic fluid (n = 107). In 51 cases, the RHD MPX confirmed the presence of an Rhd variant: a DAR (n = 28; serologically difficult to define), DII (n = 19), DIII (n = 3), DIV (n = 1), IVs (n = 6) and DfR (n = 2). In 11 cases, a variant D was not confirmed. Subsequent sequence analysis, with genomic DNA and RHD-exon-specific PCRs, showed DIVII (n = 4), DNII (n = 2) and not yet conclusive results in five cases. In 75 cases, the RHD MPX PCR was
performed to confirm the presence of a normal or weak-D phenotype. Weak D was further proven by specific PCRs. In 10 cases an assumed RhD negativity was confirmed. In case of prenatal screening, in 72 cases RhD positivity was predicted; in 35 cases RhD negativity. In one case, a false-positive normal RhD-antigen was noted after birth. Sequence analysis of RhD of the newborn and the father showed that both were carrier of an RhD gene with a deletion of 328T and 330G in exon 2, leading to a premature stop codon. In 17 cases, RhD typing of foetal DNA present in maternal plasma was performed with an RhD-exon 7-specific quantitative PCR. In conclusion, the RhD MPX is a useful addition for RhD serology. It can be used for prenatal RhD typing, but maybe replaced by non-invasive RhD typing with maternal plasma.

P 18.9
Four new RH-alleles with previously unknown polymorphisms
A Döscher1, B Ludewig1, C Gerdes1, C Das Gupta2, S Gnoth1, FF Wagner2, F Schunert1 and EP Petersen1
1Molecular Diagnostic, Red Cross Blood Transfusion Service N.S.T.O.B. and 2Biotest, Dreieich, Germany

Background: Blood samples were routinely analysed with monoclonal sera for rhesus D antigens (BS 226, BS 232, BS' 'blind serum'; Biotest). After phenotypic characterisation DNA from these samples was extracted with standard methods to determine the RH-D-status (weak or partial) using molecular genetic methods. While genotyping 246 weak D-samples for polymorphisms (PM) with multiplex-PCR methods and sequencing, we found four weak D-samples that could not be classified according to the weak D RhD-nomenclature. Here we present data of four new RH-alleles with so far unassigned polymorphisms.

Methods: DNA was first analysed with a fluorescent multiplex-PCR methods in an ABI 310 (screening for fluorescence and size) for the occurrence of RHD exons 2-7, 9 and 10, weak D polymorphisms of type 1-5, D-VII, D-HMIII and known RHCE-polymorphisms. In certain cases dye-terminator cycle sequencing was performed to find point mutations within RHsD exons 1-10.

Results: PM = polymorphism; range of serologic results: 0 a ` 4+, m = microscopy (1 = BS226 20°C; 2 = BS322 30°C; 3 = BS322 20°C; 4 = B- blend sera 30°C ; Biotest Dreieich). Allele A: PM = exon 6 (BS151); SER (1:2; 1:3; 1:4); Allele B: PM = exon 2 (BS260); SER (1:2; 1:3; 1:4). Allele C: PM = exon 1 (BS300); SER (1:2; 1:3; 1:4; 1:5; 1:6). Allele D: PM = exon 6 (BS420); SER (1:2; 1:3; 1:4; 1:5). All these alleles have been found without any irregular anti-D antibodies.

P 18.9
Analysis on FUT1 and FUT2 gene of 10 para-Bombay individuals
Zh Guo*, D Xiang, Z Zhu, X Liu and J Zhang
SBC, China

Background and Objectives: Study the allele composing of FUT1 and FUT2 gene loci of 10 para-Bombay individuals.

Materials and Methods: 10 samples suspected as para-Bombay phenotype by primary serology tests. We used routine and absorbtion-elution test to identify their AB0 type and applied duplex PCR-RFLP for getting their AB0 genotype. Then through direct DNA sequencing of FUT1 and FUT2 gene, we analysed structures of their H and Se gene loci.

Result: All of their ABO genotypes were consistent with the serological results. Six out 10 have recessive homozygous gene in their H locus. Each phenotype of h1h1 h2h2 and h3h3 have two individuals, moreover, one h3h3 is tested. The rest three are h6 heterozygous individuals: h1h3/h2, h1h2/h3 and h1h2/h1. h1h2/h1h2 and h3h4 have two individuals, moreover, one h3h3 is tested. The rest three are h6 heterozygous individuals: h1h3/h2, h1h2/h3 and h1h2/h1.

Conclusion: We detected four kinds of known h alleles [h1-h4], two kinds of novel no-functional FUT1 alleles, and a novel Se (nt167) polymorphism in Chinese para-Bombay individuals.

P 18.11
Sixteen polymorphic sites in the 5’/3’-UTR of the P1 gene do not correlate with P1/P2 phenotypes
Á Hellberg* Lund University and Blood Center, Sweden

Background: The molecular genetics of the P blood group system and the absence of P1 antigen in the P phenotype are still enigmatic. One theory proposes that the same gene encodes for both P1 and P2 transferrases, but no polymorphisms in the coding region of the P1 gene explain the P2 phenotype. We investigated the potential regulatory regions up- and downstream of the P1 gene.

Materials & Methods: Swedish P1 and P2 samples were analysed by direct sequencing of PCR-amplified 5’- and 3’-untranslated regions (UTR) of the A44GALT (P1) gene. PCR-SSP for screening and linkage of SNPs was also performed.

Results: 1600 bp of 5’-UTR sequence contained an insertion and three substitutions compared to a GenBank sequence (1). In 1075 bp of 5’-UTR an insertion, two deletions and three substitutions were found in addition to the six polymorphisms reported by Iwamura et al. (JBC 2001;278:44429), two of which were postulated as P1 mutations (-550insC,-160A>G). All 16 P2 samples investigated here were homozygous for -550insC,-160A>G. However, only 13 of the 16 P2 samples were investigated by us. Ten samples were also homozygous for -160A>G.

Conclusion: The -550insC and -160A>G polymorphisms found only in P2 samples in a Japanese study were found here in both P1 and P2 donors. Since P1 is the null allele in the P blood group system it is unlikely that these mutations cause the P2 phenotype. None of the novel polymorphisms reported in this study correlated with P1/P2 status and the P1/P2 mystery remains unsolved.

P 18.12
Genetic basis of the A_bantu blood group phenotype
B Hosseini-Maaf*,1, E Smart2, MA Chester1 and ML Olsson1
1Department of Transfusion Medicine, Lund University Blood Centre, University Hospital Lund, Sweden and 2South African National Blood Service, South Africa

Background: Among ABO subgroups, A_bantu has the highest frequency in a specified population. The molecular basis of this phenotype is still unknown.

Material and Methods: Buffy coats were collected in KwaZulu-Natal, South Africa, from eight Black donors earlier categorised as A_bantu. Genomic DNA was extracted from these Buffy coats and sequenced.

Results: In 1600 bp of 3’-UTR sequence four substitutions and three synonymous substitutions were found. Surprisingly, A_bantu has a C-to-T mutation at nucleotide 357, which explains the phenotype. Since this nucleotide mutation is also found in P2 samples, it is not the cause of A_bantu phenotype.

Conclusion: The A_bantu phenotype is caused by an unidentified nucleotide mutation in the 3’-UTR of the A_bantu gene.
P 18.13

DHPLC approach to determine RHD variants molecular epidemiology in Brittany (France)
C Le Marchal*, C Guerry, C Benech, L Buriot, M Delamaire and C Ferec
Etablissement Français du Sang - Bretagne, sites de Brest et Rennes, France

Since the first description of molecular genetic bases of Rhesus D blood phenotypes, more than 90 molecular variants have been described. According to the location of the amino acid change in the protein (extracellular loops vs. transmembrane or intracellular parts), a genotype-phenotype correlation is possible. The aim of this study was to evaluate the molecular epidemiology of RHD variants in Brittany (western France) using DHPLC, a sensitive, rapid and automatical technique. Along year 2003, during blood donor typing at the EFS-Bretagne, 73 samples presenting a discordant Rhesus D serology were analysed at molecular level. Hybrid alleles were typed with an exon-specific PCR whereas single nucleotide variations were screened by DHPLC and identified by sequencing. Among 11 variants encountered, we were able to identify two new variants (IVS1+5G>A and A;4226D) despite the low number of samples analysed. Unexpectedly, half of the partial D were D catV type VII (16%) which was known as a very rare allele. Among seven weak D alleles, type 1 (37%) and 2 (22%) were predominant. DHPLC analysis for the RHD gene would allow to extend the population studied in order to confirm the original molecular epidemiology we found in Brittany. Furthermore, this study highlights the interest for transfusion counselling of molecular typing for samples with ambiguous serological results as we identify weak D variants. DHPLC analysis for the RHD gene would allow to extend the molecular epidemiology for transfusion refractoriness. NAITP is the result of fetomaternal platelet incompatibility. The most frequent antibodies causing NAITP are anti-HPA-1a followed by anti-HPA-5b. In case of severe thrombocytopenia in the presence of HPA alloimmunisation, patients would benefit if compatible platelets are immediately available. This requires typing of large numbers of platelet donors. Therefore, we developed a medium-throughput system for HPA genotyping (HPA-1, -2, -3, -5 and -15) using Pyrosequencing. We selected 430 DNA samples of active platelet pheresis donors and typed for HPA-1. These donors contribute to the Dutch HLA-typed donor file as platelet and/or bonemarrow donor. With the purpose to establish a universal platelet donor file, we selected donors which were homozygous for the HLA-A and/or HLA-B loci. 263 samples typed as HPA-1a, 17 samples were HPA-1b, 123 samples were HPA-1ab and of 27 samples (6.7%) no genotype could be determined. The 17 HPA-1a negative samples were confirmed by SSP and also typed for HPA-3, -3, and -5. 13/17 samples were HPA-5b negative. This medium-throughput genotyping technique enables a rapid procedure to build an HPA typed donor type enabling us to meet the demands of NAITP treatment and the treatment of platelet transfusion refractoriness based on combined HLA/HPA antibodies.

P 18.15

Blood group antigen typing using custom BeadChips™
ME Reid*
New York Blood Center, USA

Background: While blood group antigen typing historically has been done by haemagglutination, DNA analyses can now be used. The aim of this study was to determine if analysis of single nucleotide polymorphisms (SNPs) associated with a variety of blood group antigens could be performed using a microarray.

Method: SNPs were tested by elongation-mediated analysis of polymorphisms (eMAFTM) using allele-specific oligonucleotides with variable 3′-terminal sequences attached to colour-encoded beads. The beads were assembled into arrays of small footprint on semiconductor chips (BeadChip™). Elongation products for SNPs or small deletions/insertions were simultaneously detected by instant imaging of fluorescence signals from the entire array. The array simultaneously assayed the following blood group polymorphisms: FYA/B, FYA/B, DDA/B [m(783)], COA/B, LWA/B, DHA/B, and SC/ICSC.

Results: The design was validated using selected DNA of known type, and 50 samples from people of partially known phenotype. To the extent that phenotype and/or PCR-RFLP results were available, there was complete concordance of results. The design was then used in a small population study by testing DNA from 56 Chinese and 67 Jewish people.

Conclusion: The BeadChip™ platform can be used to analyse DNA for blood group SNPs. The design flexibility and convenience of the BeadChip™ platform and eMAF™ enable reliable high-throughput screening of patients and donors with minimal reagent consumption.
Results: Three rounds of binding, elution and amplification enriched for phage/peptides according to their affinity for the target. Clones were randomly selected from the final round of each biopanning assay for DNA sequence analysis. The amino acid sequences of the phage peptides were deduced and their alignment identified overlapping sequences containing conserved or related amino acids. Each set of selected peptides shared a common motif. However, alignment against the RH-D polypeptide sequence failed to identify significant homologies.

Conclusion: The selection of phage/peptide sequences which failed to match to the primary sequence of the RH-D polypeptide suggests that these anti-D antibodies recognise conformational and not linear epitopes.

P 18.19
Expression study of the ABO variant alleles
K Ogasawara*, H Tsuneyama, M Uchikawa, M Satake and E Nakamura
Japanese Red Cross Tokyo Blood Center

Background: In ABO blood group system, almost 100 different alleles were identified from the individuals with common and variant ABO phenotypes. To understand the phenotype-genotype relationships, we developed a functional assay for the ABO alleles.

Materials and Methods: Coding region of the ABO genes was amplified from buffy-coat mDNA using RT-PCR followed by PCR. The DNA fragments of the ABO genes were cloned into the plasmid pVAX1 Invitrogen, then ABO variant alleles were synthesised by site-directed mutagenesis. The DNA fragments of the ABO alleles, cut off from the ABO-pVAX1 constructs by restriction enzymes, were cloned into the bicistronic expression plasmid, pIRE2-EGFP Contech, which includes green fluorescent protein (GFP) gene as the reporter. HeLa S3 cells were transiently transfected with the ABO-pIRE2-EGFP constructs. Expression of A or B antigens on the GFP-positive transfectedants were analysed by flow cytometer.

Results: The amounts of the A or B antigens expressed on HeLa cells transfected with the constructs of common and variant ABO alleles were almost in order of the original red cell phenotypes. In addition, we identified a new B allele (B\(^{+}\)) with a 547G>A (Asp183Asn) substitution from the weak B individual. The amount of B antigen on the HeLa cells transfected with the B\(^{+}\) construct was 50% of those of the common B construct.

Conclusion: We established functional assay for ABO genes that help to understand the variation of ABO phenotypes.

P 18.20
Molecular genetic analysis of the atypical Bombay and para-Bombay phenotypes
K Ogasawara*, H Tsuneyama, M Uchikawa, M Saitou, M Satake and E Nakamura
Japanese Red Cross Tokyo Blood Center

Background: B antigens are synthesised by two different 1,2-fucosyltransferases encoded by the FUT1(D) and FUT2(SE) genes. We investigated atypical Bombay and para-Bombay phenotypes based on the molecular genetic analyses.

Materials and Methods: Two H-deficient phenotypes were identified by serology including absorption–elution tests. H, SE and ABO alleles were identified by nucleotide sequence analysis. H and SE alleles were cloned into the expression plasmid (pIRE2-EGFP, Contech) and transferred into the COS7 cells. The H antigens expressed on the cells were analysed by flow cytometry.

Results: In one individual, ABH antigens on red cells and in saliva were not detected. He had anti-A and anti-H but not anti-B in the serum, and had homozygote of h6 with 462C>A (Tyr154Stop) nonsense mutation, Se\(^{+}\)Se\(^{-}\) and B0. Expression of H antigens on the COS7 cells transfected with h6 construct were not observed. However, the cells transfected with the Se\(^{+}\) construct expressed 14% of H antigen of those transfected with the Se construct. Thus, a few amount of B antigen created by both Se\(^{+}\) and B-transferrase may prevent the anti-B production. In another individual, the ABH antigens on her red cells were not detected. She had homozygote of h allele with 547-552delAG frame-shift mutation, SeSe and A0. H antigen expressed on the COS7 cells transfected with the h construct was not detected.

Conclusion: The combination of H and SE genes causes the creation of atypical H-deficient phenotypes.

P 18.21
Non-deletional 0 alleles express weak blood group A phenotypes
A Selsam*, C Das Gupta, F Wagner and R Blasczyk
Department of Transfusion Medicine, Hannover Medical School; Biotest AG, Dreieich and Blood Transfusion Service of German Red Cross, Inst. Springer, Germany

Background: In contrast to the frequent deletional 0 alleles (95% of all 0 alleles), which encode for a truncated and catalytically inactive AB0 glycosyltransferase due to a single-base deletion, the less frequent non-deletional 0 alleles (5%), such as O03, lack this polymorphism but possess non-synonymous mutations that have been thought to abolish the protein’s enzyme activity.

Methods: Healthy blood donors diagnosed as having weak anti-A isoagglutinins and relatives of them as well as blood group O donors selected for the presence of O03 were subjected to extended AB0 phenotyping. The ABO genotype was determined by PCR-SSP and sequence analysis. Transient transfection experiments into HeLa cells were performed using various ABO expression plasmids.

Results: All donors, including the relatives, who were homozygote (n = 2) or heterozygote (n = 11) for O03 and the rare O03-like allele Aw08, respectively, showed weak A antigen expression only detectable by adsorption-elution technique (n = 12) or typing with monoclonal anti-A (n = 1). The serum of most donors (n = 10) contained weak anti-A or anti-A1, whereas in the remaining three donors reactivity of anti-A isoagglutinins was in normal range. Transfection studies revealed weak A antigen expression on HeLa cells transfected with plasmids containing O03 or Aw08 cDNA expression constructs.

Conclusion: The data provide in vivo and in vitro evidence that non-deletional 03-like alleles produce detectable amounts of A antigens.

P 18.22
High-throughput genotyping of blood donors for minor blood group and major platelet antigens
M St-Louis*, A Monpetit*, MS Phillips* and R Lemieux
1Héma-Quebec, RH&D, Sainte-Foy, Canada, and McGill University and Genome Quebec Innovation Centre, Montreal, Canada

The need to determine minor blood group and platelet antigens to find compatible blood can be costly, labour-intensive and often difficult because of lack of reagents. To overcome this problem, we have developed assays that will allow us to genotype regular donors for several minor blood cell antigens using the high-throughput GenomeLab. A panel of single nucleotide polymorphisms was compiled for the analysis of these blood group and platelet antigens: C(e), C(e), Ty(t), Jk(a), Jk(b), K(a), K(b), Kp(a), M/N, S/n, Lu(a)Lu(b), HPA-1a/1b, HPA-2a/2b and HPA-3a/5b. To ensure genotyping accuracy, all assays were done in duplicate on both strands. DNA from 100 phenotyped blood samples was first isolated and then amplified in a 12-plex PCR reaction. This was followed by genotyping reactions that were performed by single base primer extension reactions. The computer-generated genotypes were compared to the known blood groups of the donors. We observed a 100% concordance with the results of a PCR-ELISA method and an average 98.2% concordance with the blood group phenotypes. Phenotyping of donors using this technology has many advantages such as very small amount of DNA and reaction volumes, and automated high-throughput capacity suitable for eGMP operations. The integration of this genotyping strategy in blood banks could greatly facilitate the cost-effective screening of compatible red cells and platelets for frequently transfused or previously immunised patients.

P 18.23
The highly variable RH locus in non-whites highly hamper RH zygosity determination
Marine GHM Tax1,2, PA Maaskant-Van Wijk1,2, J Van Drunen2 and CE Van Der Schoot1
1Sanquin Research at CLB, Amsterdam, and 2Sanquin Blood Bank South West Region, Rotterdam, The Netherlands

Knowledge about paternal RH hemi- or homozygosity is of clinical interest in alloimmunised pregnant women. RH-determination in whites is usually caused by deletion of the RHD gene. Recently, the physical structure of the RH locus and the mechanism causing the deletion of the RHD gene have been explored. RH-D zygosity determination is possible in whites by specific detection of the RHD-negative genotype (based on the presence of a hybrid Rhesus box). The present study shows that in non-Whites several deviant Rhesus boxes [both upstream and downstream] exist that hamper zygosity determination by detection of the RHD negative locus. The mutated Rhesus boxes in RH-D-negative RHD homozygous Blacks have a frequency of 0.24, whereas in Whites no mutated Rhesus boxes were encountered so far. Due to the high frequency of the mutated Rhesus boxes, zygosity determination by detection of the RH-D negative locus is not feasible in non-Whites. Therefore a real-time quantitative PCR assay was developed, specific for RHD exon 7, combined with an assay on a reference gene, for determination of RH-D zygosity. RH-D zygosity determination directly on the RHD gene appears to be the most reliable method. Furthermore, the cosegregation of variant RHD genes (RHdy and C(c)) with specific deviant Rhesus boxes renders more insight into the evolutionary events concerning variant RHD genes and deviant Rhesus boxes.
P 18.24
Real-time PCR of biallelic insertion/deletion polymorphisms as positive control for foetal plasma DNA
R Dee, RJP Rijnders, B Busser, M De Haas, LGCM Christiaens and CE Van Der Schoot*
Sanguin Research at CLB, Amsterdam; Univ. Med. Center, Utrecht, the Netherlands

Cell-free foetal DNA in maternal plasma can be used for prenatal genotyping, including foetal RHD status. Its diagnostic application is hampered by the lack of a generic control marker for circulating foetal DNA. We applied a set of real-time quantitative PCR assay [Blood 2002;99:4618] as a positive control on the presence of foetal DNA in maternal plasma. These PCRs are allele-specific for 10 biallelic insertion/deletion polymorphisms and can specifically detect one foetal DNA sequence in the background of large amounts of maternal DNA sequences. From the observed distribution of the different alleles in 280 Caucasians it was calculated that at least one informative allele is present in about 95% of pregnancies. The approach was tested in 20 pregnancies. In 13 couples the father was homozygously positive for a marker for which the mother was negative. The mean number of possible informative alleles was 3.1 (range 1–5). The mean observed number of informative systems after birth was 2.35 (range 1–3). All 20 maternal plasma were tested in the different allele-specific PCR assays. The results of 80 different PCRs on maternal plasma were compared with the genotyping results of the 20 newborns. The positive-predictive and negative-predictive values of the test are both 100% (95% CI: 88.4–100% and 92.9–100%, respectively). The availability of this control assay can lead to wider implementation of prenatal genotyping assays based on cell-free foetal DNA.

P 18.25
Comprehensive analysis of Rhesus box variety
FF Wagner1,2* and WA Flegel1
1IKT, Ulm, Germany, and2Zentralinstitut Springe, DRK BSD NSTOB, Springe, Germany

Background: The two Rhesus boxes (RBs) flank the RHD gene. The RHD deletion occurred by a recombination between upstream and downstream RB within the 1463 bp identity region. The resulting hybrid RB may be specifically detected by, e.g., PCR-RFLP, enabling testing for RHD zygosity. RBs harbouring aberrant nucleotide sequences confound this testing approach. A systematic and comprehensive analysis of the RB variety was lacking.

Methods: DNA segments of about 5000 bp representative of both RBs were sequenced in a comprehensive set of alleles covering all four D clusters: Ccde, DIII type 4, DIVa, weak D type 4.0 to type 4.2, RhDP, DAU-0 to DAU-4, DVI type 1 and type 3, DIV type 3, weak D type 1 and type 2.

Results: The differences between upstream and downstream RBs were confirmed. However, these known differences were partially restricted to defined subsets of RHD alleles. More than 40 new polymorphisms were identified. Gene conversions often started within the identity region. They occurred in the downstream RB of Ccde, weak D type 4.1, type 4.2 and DAU-0 and in the upstream RB of RhDP, DAU-1 and DAU-3.

Conclusion: The variability of the RB was considerable and caused by point mutations and gene conversions. The identity region may have triggered the gene conversions. Our results explained the limitations for the detection of the hybrid RB that were previously observed by several laboratories. Our comprehensive data set may guide the development of improved testing approaches.

P 18.26
Mistyping of a new weak D Allele as weak D type 1 by PCR
FF Wagner1*, A Doscher, U Bauerfeind and E Petersen
Zentralinstitut Springe and Institut Oldenburg, DRK Bhaktivedendienst NSTOB, Springe and Oldenburg, Germany

Background: The vast majority of weak D samples is caused by a few weak D types that are often identified by PCR. Usually, a D positive transfusion strategy is advocated if weak D type 1 to type 3 are detected, because no allo-anti-D immunisations have been reported for these most frequent weak D types.

Methods: For blood donor typing, samples with known ‘weak D’ phenotype (including known partial D) were re-checked in a Gel card system (DiaMed AB0/D). Samples that did not meet the usual phenotype of frequent weak D types were further investigated by RHD PCR, weak D PCR or sequencing.

Results: A sample differing from the frequent weak D types was scheduled for molecular typing, because in the routine DiaMed AB0/D typing card, both the monoclonal and the polyclonal anti-D was negative. Surprisingly, without knowledge of serology, the sample was initially molecularly typed as weak D type 1 based on weak D PCR. When the discrepancy between the presumed molecular type and the phenotype was detected, the full RHD coding sequence was established by sequencing of genomic DNA. An RHD1L18V, V270G allele was detected.

Conclusion: Molecular typing of weak D samples should consider serology to avoid mistyping of alleles like RHD1L18V, V270G as frequent weak D types by PCR. A typing strategy exclusively based on weak D PCR may result in erroneous phenotype predictions and, possibly, inappropriate transfusion strategies.

P22 Managing the blood supply

P 22.1
Implementation, use and further development of the ISBT 128 component code system in Sweden
O Akermalm*, S Larsson, N Norda, A-L Strömberg, A-M Svard-Nilsson and S Thyme
1Blood Centres of Uppsala, University Hospital, Uppsala, 2Land University Hospital, Linköping, 3Karolinska University Hospital/Huddinge, Stockholm, and 4University Hospital, Umeå, Sweden

Background: The national component coding system from 1965, modernised in 1983, was applied differently by blood centres in the absence of co-ordination and strict rules for usage. In 1997, the ISBT 128 system, offering a new opportunity, was recommended for nation-wide implementation.

Aim of report: To describe the strategy to implement, maintain and further develop the Swedish application of the ISBT 128 system.

Methods: A working group was formed to design, maintain and further develop a Swedish standard, compatible with the international ISBT 128 system. Blood components were defined by code-strings reflecting preparation details and put on a national list. Modifiers and attributes were printed in eye-readable text on the label only when providing clinical information essential for the end user.

Results: In Sweden, 92% of the blood components are labelled according to ISBT 128. The national list is revised every year and is published on the national website; the latest version contains 252 component codes. The working group has met once every year and has had several conferences by telephone. The process has highlighted the need for standardisation of blood components and their labelling.

Discussion/conclusion: The coding system allows a unique identification of blood components. Essential steps of their preparation are fully traceable. By limiting the eye-readable text on the label to clinically important information, clinicians readily accept the ISBT 128 system.

P 22.2
Kuwait Central Blood Bank process control using ISBT-128 labeling system as a traceability tool
M Aljafza*
Kuwait Central Blood Bank

Background: Bar coding is a method of automated data collection, which helps in gathering information efficiently, accurately and rapidly. ISBT has recommended to replace the current ABC codabar system with ISBT 128 because it codes more data into a smaller space, easily handles alpha-numeric data, provides for internal scanning error checks, and supports concatenation.

Method: Kuwait Central Blood Bank (KCBB) has used the ISBT 128 labeling system for the past 5 years. It supports the Quality System Essentials of process control and traceability by: (a) a unique donation number electronically printed at the point of use; (b) electronic capture of blood pack manufacturer information; and (c) product labeling using international coding.

Results: ISBT 128 provides unique identification of any donation worldwide. In addition, it allows the individual donation barcodes in a number set to be discretely identified thus supporting process control. ISBT 128 also provides a comprehensive and highly flexible system for assigning product codes and an international database allowing a product to be accurately described and traced in any country.

Conclusion: ISBT 128 has supported the international usage of blood products, language independence and the capability to encode an expanded data file on the label. KCBB are proud to have introduced the system in such an exceptional transition time and to be one of the first countries in the world to implement ISBT 128.

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P 22.3  
**Blood utilization management in tertiary care hospital**  
I Bojanic, B Golubic, F Plenkovic, M Lukic, Z Ivanovcic, S Mazic, N Dekic, I Vukicevic, Z Bencic and J Lacko  
Department of Transfusion Medicine, Clinical Hospital Center, Zagreb, Croatia  

**Objective:** To analyze quality indicators of blood utilization in tertiary care hospital.  

**Materials and methods:** The computer records of blood utilization in 3-year period in our 1600-bed hospital were retrospectively evaluated. We analyzed rate of blood units that were not transfused which included: number of wasted units (unit discarded prior to its expiration date), expired units and units returned to transfusion service.  

**Results:** In evaluated period 0.88% of units received from our supplier Croatian Institute of Transfusion Medicine were not utilized [10.18% of received whole blood units, 1.20% of red blood cell (RBC) units, 1.05% of fresh-frozen plasma units and 0.32% of platelet concentrates]. The expiration rate was 0.20%, and only RBC units were discarded because their maximum shelf life was reached. The wastage rate was 0.68%, mostly because unused units were not return to laboratory before their temperature exceeded allowable limits, over ordering of blood products with short shelf life or bag breakage. The rate of returned units was high: 3.29% of issued units were not transfused and were returned at transfusion service. As much as 15% of RBC units issued to surgical patients were returned unused.  

**Discussion:** The rate of blood units that were not utilized is important indicator of blood stock management. It should be regularly evaluated to improve utilization of blood products and minimize wastages. It could also facilitate policy-making and financial planning.

P 22.4  
**Journey to accreditation**  
L Bust  
Western Province Blood Transfusion Service, South Africa  

**Introduction:** In the mid-1990s when Western Province Blood Transfusion Service made the decision to move away from accreditation by the American Association of Blood Banks (AABB) and look for an alternate system, we began an upward climb with the goal of reaching new heights.  

**Materials and methods:** We initially looked at ISO certification but this did not meet the needs of our organization. We therefore decided on accreditation and developed a standardized, three-part system incorporating elements of ISO 9000, the AABB technical parameters and ISO 17025 for the laboratories. Together with the South African National Accreditation System (SANAS) and the other blood transfusion services in the country, a national accreditation checklist was written.  

**Results:** Western Province Blood Transfusion Service successfully underwent full initial accreditation of all facilities in July 2003. Follow-up accreditation was successfully performed in March 2003. The other services are to follow suit.  

**Discussion:** This presentation outlines the path followed leading up to full accreditation and some of the pitfalls and obstacles encountered on the way. It also outlines some of the benefits to the organization of having accreditation.  

**Conclusion:** Although a national accreditation system is now in place and successful accreditation has been achieved, the journey does not end as we strive for continuous improvement. We briefly outline possible directions for the future.

P 22.5  
**ABO and RhD red cell mismatching**  
JF Chapman, R Hick and C Hyam  
Blood Stocks Management Scheme, London, UK  

**Introduction:** The British Committee for Standards in Haematology compatibility guidelines state that red cell components of the same ABO and Rh group should be selected whenever possible. Mismatching can cause a disproportionate demand pressure on a specific blood group inventory. The Blood Stocks Management Scheme carried out a survey to identify the level of mismatching taking place in hospitals.  

**Method:** Hospitals were asked to collect data on ABO and Rh matching over a 4-week period during February and March 2003. A questionnaire was devised which covered the level of mismatching by patient and donor group and the possible reasons for mismatching out of a list of 10.  

**Results:** Ninety-nine hospitals agreed to participate and returned the questionnaire. These accounted for 44.6% of NBS issues during the survey period. A total of 1028 patients were cross-matched with 3105 mismatched units. Of 3105, 352 [11%] were because of insufficient stock available and 963 [31%] were because the unit was time expiring. Nine hundred and ninety-two 0 negative units were mismatched and of these 79% were transfused; 623 [21%] of mismatched units were for group AB patients.  

**Conclusion:** There was extensive use of mismatching (5% of issues) during the survey period. The main reasons were to prevent the time expiry of group O negative units and because insufficient stock was available. The latter applied mainly to red cell units for AB patients. Hospitals have been recommended to review their O negative and AB stock holding.

P 22.6  
**Towards an optimization of the link between transfusion resources and needs using VISTA**  
M Dehest1, MJ Boucaud1, D Rigal1 and F Hofman2  
1EFS Rhone-Alpes, Lyon, France, 2GambroBCT, Zoestern, Belgium  

**Introduction:** VISTA is a software that allows to configure donation procedures, allowing to respond to the prescriber’s requests, whilst optimizing procedures through multi-product donations.  

**Materials and methods:** VISTA was connected to four workstations. Four TRIMA systems were connected to VISTA via a dedicated network.  

**Results:** VISTA increases considerably the possibilities of procedure selection by elaborating the procedures list. The preferred procedure is uploaded into the TRIMA cell separator. Accurate stock management is required because of the nature of the platelet transfusion itself. Respect of recommendations on dosages, blood groups, CMV status, etc., all lead to the necessity of optimizing stock management. The flexibility offered by VISTA, to adapt each individual donation, has allowed us to improve the ratio response/request. In parallel, we have increased our plasma production. VISTA allows also the follow-up of apheresis donations. Multiple reports as alarms summary, procedure time exceptions and incomplete procedures are quality indicators for the use of the cell separators and the performance of the users. This can lead to specific training on a particular point of an apheresis procedure, or in the increased surveillance of this procedure. The productivity, the production and the product verification summary are significant elements in the management of an apheresis service.  

**Conclusion:** With VISTA, it is now possible to align the resources and the needs in a transfusion center.

P 22.7  
**Blood component preparation – indicator for the development of the transfusion service**  
K Dimitrovski, O Danevksa Todorovska and V Stefanovska  
Institute for Transfusion Medicine, Skopje, Republic of Macedonia  

**Aim:** A retrospective study to investigate the general blood production/usage trend was carried out to identify any possible parameters helpful in projecting the future usage.  

**Methodology:** Usage of various components was demonstrated by plotting the time trend and calculating the coefficient of variance of each component. The ratio of mean RBC-PLT-FFP was derived to determine the proportionate usage of these three blood components and the usage of the haemostatic components was found by determining the ratio of mean PLT-FFP-Cryo. Ratio of mean RBC vs. WBC usage was calculated and compared with international standard.

| Year | RBC | PL | FFP | Alb | PL | FFP | Cryo | RBC | WB |
|------|-----|----|-----|-----|----|-----|------|-----|----|
| 1999 | 3.3 | 0.25 | 1 | 0.08 | 1 | 3 | 1 | 55 | 1 |
| 2000 | 2.5 | 0.33 | 1 | 0.13 | 1 | 2 | 0.83 | 44 | 1 |
| 2001 | 2.6 | 0.35 | 1 | 0.08 | 1 | 2 | 0.85 | 43 | 1 |
| 2002 | 2.14 | 0.32 | 1 | 0.07 | 1 | 2 | 0.78 | 30 | 1 |
| 2003 | 2 | 0.41 | 1 | 0.12 | 1 | 1.7 | 0.86 | 28 | 1 |

**Results:** The ratio of mean RBC-PLT-FFP, PL-FFP-Cryo and RBC-WB is as follows.  

**Discussion:** The time trend as well as CV of component usage showed an almost consistent production of all four components during the investigated period and confirmed the consistent usage, with a slight increasing trend the last years. As the production/usage is consistent, the derived RBC usage is a good projection indicator to guide the production of RBC/FFP/PLT. Besides, the ratio of FFP/Cryo/Alb is also a relevant indicator for the appropriate apportionment of various plasma components production. But anyhow, the component preparation and its use are derived by the availability of the source - voluntarily-donated blood.

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P 22.8
Current trends and utilization of autologous blood donation in Denver, Colorado, USA
D Feng,1,2 A Casebeer,1,3 D Factor,1 D Ambrosio1 and M Orton1
1Bonfils Blood Center, 2Pathology, University of Colorado Health Science Center (UCHSC), 3Internal Medicine, UCHSC, 4Pediatrics, UCHSC, USA

Aim: The aim of this paper was to determine the number of autologous units collected, the per cent discarded, and the patients’ final transfusion status at a regional blood bank.

Materials and methods: After IRB approval, consecutive autologous donors (103–12/03) were asked to complete a survey regarding their perceptions and experiences with autologous donation. All study participants’ donated units were tagged, processed and sent to the hospital for use. Final disposition of the blood was transmitted back to the blood centre.

Results: Of the 134 final respondents with disposition data, the median age was 59 years and 57.5% (77) were female. Nearly, 83% of respondents were donating for orthopedic procedures; 49.3% (66) reported that they requested autologous donation and 70.1% (94) reported that it had been recommended by their physician. Of the 190, 100 (53%) autologous blood units collected during the study period were discarded. Discarded units were much more likely to be for non-orthopedic surgery (78.3% vs. 50.5%, P = 0.01). Eleven (8.2%) of the autologous donors required additional allogeneic blood.

Conclusion: This ongoing study demonstrates that the majority of autologous donations are for orthopedic surgery, are recommended by the physician, and results in substantial discard rates of blood. The use of autologous donation with its high discard rates and the surprising requirements for additional allogeneic blood calls for a re-evaluation of current preoperative autologous donation practices.

P 22.9
Best transfusion practice across Scotland
F Fergusson*, J Spence and M Hart
Better Blood Transfusion Programme, NHS Scotland, Edinburgh, UK

The Better Blood Transfusion Programme (BBTP) in Scotland represents a significant investment by NHS Scotland Acute Trusts and SNBTS to deliver, safer transfusion practice and a 10% reduction in red cell usage by March 2006. The three key areas are: education in safe practice in ordering and administering blood components; focusing on the findings of the SSHT scheme; efficient management of blood components in hospitals through identification and development of quality systems and processes; and effective use of blood components through evidence-based clinical best practice. Establishment and delivery of programme activities is a collaboration between the programme team, BBTP Transfusion Practitioners (15 WTE) based across NHS Scotland, and a Lead Person from each Acute Hospital Trust. A detailed training needs analysis was undertaken to assist Trusts prioritise and plan the delivery of safe transfusion practice education across all staff groups by appropriately qualified trainers. BBTP Project Management is based on the PRINCE 2 methodology using Project Briefs and Plans, a Risks Register and ‘RAG’ matrix to structure, plan, monitor and execute projects. All of the >98 blood saving initiatives identified by Trust staff report progress at local hospital, Trust and NHS Scotland level. Initiatives include audits of blood ordering and transfusion triggers, cell salvage, cross-match to transfusion ratios and the use of satellite refrigerators.

P 22.10
The Virtual Museum of Transfusion Medicine: on-line history resource for blood transfusion
I Franklin*, J Shepherd, N Arnold, A Hyde and J Devine
Academic Transfusion Medicine Unit and the Hunterian Museum, University of Glasgow and the Virtual Museum of Transfusion Medicine, UK

Introduction: The Virtual Museum of Transfusion Medicine (VMTM) has been developed as a web-based resource for the study of the history of transfusion medicine. It will go live fully on 1 July 2004. Being ‘virtual,’ there is no need to own or keep items, only to record them using state of the art imaging techniques. Three aspects of transfusion medicine have been developed.

Poster collection: The VMTM has a substantial collection of donor promotional materials including posters and other promotional media from the 1940s onwards. The collection will be organised into ‘galleries’ of like posters or into donor campaigns. Transfusion artefacts: The VMTM is building a library of images of items of interest in transfusion medicine. So far most have been unearthed in Edinburgh – some have been photographed in Birmingham and Bristol. They include glass apparatus for collecting and transfusing blood, and an early plastic blood bag.

History of rhesus disease: The VMTM has conducted a number of interviews with key figures in the development of anti-D. These include Professor Ronald Finn, Professor Charles Whitfield, and interviews with three previous donors of anti-D. All three donors had Rhesus babies and the interview gives a rich insight into the heartbreak caused by this condition. It is planned to recruit museum curators to develop new areas of special interest, to provide expert interpretation and commentary on items, and to build a comprehensive resource for this field of medicine.

P 22.11
Initial experiences with blood track as a tool to improve safety in the transfusion chain
TJ Haigh*
Lancashire Teaching Hospitals, NHS Trust, UK
Six months use of Blood Track1 to audit removal of blood from blood issue fridges for transfusion to patients is described. The system is interfaced to the Blood Bank computer2 enabling transfer of recipient and donor unit data. The compatibility report is modified to include patient demographics in a PDF barcode, readable by the Blood track scanner, and allowing collation with the blood unit data on collection from the issue fridge. The system is installed in two hospitals and >1000 staff trained to use it.

An early high error rate in collecting blood reduced the ability to track blood removal. The reasons for this and a possible solution are discussed.

Results: Total transactions were 15 099 (100%). Correct transactions 2134 (80%); incorrect transactions removing blood 1879 (2%); Incorrect transactions not removing blood 1096 (8%). Previously audited failure to manually sign for blood and collected from issue fridge: 30%; Reduction in ‘error’ rate when removing blood: 73%. Average monthly blood wastage: 2%. Units per month outside controlled temperature: 80.3%. The Blood Track, blood bank computer interface increase safety in the blood chain, discouraging the removal of blood for a patient other than for whom the presented compatibility report indicates, and flags units outside controlled temperature.

References: 1. Olympus UK Ltd. 2. Bank Clerk: IBG Immucor Ltd.

P 22.12
An audit of the use of FFP, platelets and the impact of a self-educating transfusion request form
CII Hui*, J Williams and K Davis
Transfusion Medicine Unit, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia

Aims: To assess the use of FFP and platelets in a teaching hospital and impact of a self-educating blood component request form.

Methods: The Australasian Guidelines for the use of components was published in 2001. We introduced a new transfusion request form in January 2003, featuring tick boxes for indication(s) against the Guidelines and prompts for laboratory results. We reviewed the data for 988 FFP, 1014 doses platelets from 1097 transfusion episodes in November–December 2003 and compared with the same period in 2002.

Results: Haematology consumed two of three of all platelets while ICU consumed one of FFP. Marrow failure remained the major indication (84%) for platelets and the demand was increasing. Reversal of warfarinisation emerged as the major indication (34%) for FFP. Contrary to what we thought, FFP and platelets were generally appropriately used. A higher incidence of inappropriate FFP requests was seen in haematology; that of platelets by surgical wards. Intensivists tended to order both products well. The introduction of a self-educating request form had contributed only a modest improvement in the appropriate use of FFP (72–76%) and platelets (88–93%) but a reduction in equivocal cases. The compliance was met with a fair success.

Conclusions: Our audit process helped identify the pattern of usage and areas of improvement. The optimal management of warfarin reversal should be targeted. A self-educating request form is a useful tool for transfusionists. It promotes best practices and facilitates auditing.

P 22.13
Platelet stock management in the National Blood Service
M Jelley*, C Harris and K Beard
National Blood Service (NBS), UK

Effective management and co-ordination of national platelet stocks is essential to ensure full provision of components whilst minimising discard due to outdated. National and local stock levels were set by reviewing historical data and platelet issue activity proportional to other centres. Analysis of stocks was performed using returned standard databases. Variance from national targets was addressed immediately. Preuctive management was achieved by either increasing production across all centres or pairing low production and high production centres. Standing orders were established. The interface between Processing and Issue Departments was strengthened. Communication, awareness, speed of action and local ownership was key to improved national performance. The system was implemented in September 2002. For the period
January-December 2001, prior to implementation and January-December 2002, platelet outdate discard rates were mean 10.7 and 10.0% of production. Requests that were not issued as a result of lack of stock availability were 1485 and 1345, being 0.69 and 0.57% of all requests received. For the post implementation period of January-December 2003, platelet outdates were 9.6% and requests not issued were 621, 0.28% of all requests received. The tools developed to help manage platelet stocks mean that supply continuity was further assured and waste was reduced. This process is iterative and it is anticipated that incremental changes and improvements will be made to the process, delivering further benefits.

P 22.14 Quality improvement project for appropriate clinical use of platelets
R Jovanovic*, S Rakic and I Siladji
Blood Transfusion Institute Novi Sad, Serbia and Montenegro

Background: Blood Transfusion Institute (BTI) Novi Sad is essential part of health care system in Vojvodina. Our customers are institutes and clinics. The number of blood units donated annually is 5.21 per 100 inhabitants. In order to improve safety and efficiency of the blood supply in the region, the institute adjusted its practice to requirements of ISO 9001:2000 and ISO 14001 standards.

Goal: The primary goal of BTI is to ensure safe and cost-effective supply of blood and products to fulfill the needs of patients, and to assist hospitals.

Method: We estimated that needs for platelets in chemotherapy increased 30% compared with the last few years. As we have insufficient number of PCs our patients have inadequate therapy. It is very difficult to respond to daily demands for PCs. Obtaining optimal number of compatible platelets for optimal use can be delayed for many reasons demonstrated in Fishbone analysis. We performed SWOT and Stakeholder analyses to understand our strengths, weaknesses, opportunities and threats. Based on this, we defined following elements and objectives of Project: team work improvement, quality assurance documentation improvement and increase of PCs production.

Conclusion: Transfusion practice cannot be safe and efficient, nor can the donated blood be used optimally, unless there is close cooperation between the BTI and hospitals. Still we have a little or no influence to the clinical use of blood and blood components. This Project is our attempt to resolve described problem.

P 22.15 MSF/B intervention on blood transfusion in Bié provincial hospital
RK Kazadi*, S Simons, A Verwulgen and T Fasil
Medecins Sans Frontieres Belgique, Mission, Angola

Background: In 2002, around 2051 anaemic people needed blood transfusion in the Bié provincial hospital. For 92% of them, malaria was the cause and 8% were children under 5 years old. For 2003, technical and material support continued by MSF in order to contribute in prevention of HIV blood transmission and fight against severe anaemia.

Materials and methods: Since May 2003, a transfusion expert closely supervises the project; coordination is under the responsibility of Medical coordinator and Head of mission. Technical support (training and quality insurance) started in July. A first evaluation was carried out in December.

Results: Fourteen people participated to four training sessions on blood transfusion practice. A monthly report and quality system implementation was established; six quality controls on HIV tests were performed. Through monthly quality control of HIV test, discordance went from 40% (July) to 0.8% (December). From 531 donors received, 69 were excluded and 484 tested. Eighteen (3.7%) HIV+, 55 (11.3%) HBs+ and 2 (0.4%) co-infections of both HIV and HBs.

Comments: This intervention allows the prevention of HIV blood transmission. As a perspective, MSF would like to help in elaboration of a national policy in order to organise blood transfusion services in Angola.

P 22.16 ISBT 128 codes for haematopoietic stem cells – Swedish experience
S Larsson*, Ö Akerman, R Norda, A-L Stro¨mba¨ck, A-M Swa¨rd-Nilsson and S Thyme
ISBT 128 codes for haematopoietic stem cells – Swedish experience

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100% depending on the teams. As it is mandatory in France since 1998, blood and platelet units are leukodepleted. To assess our practice, we analyzed allo-immunization in hematologic patients.

**Patients and methods:** A total of 184 adult patients were retrospectively studied during 9 months. Main diagnoses were: leukemia (46), marrow graft (51), lymphoma (38), and myelodysplastic syndrome (14). Patient sera were screened for RHC and HLA antibodies. According to RBC supply, two groups of patients were identified: in group A, 43 patients were previously sensitized or were women under 50 years old. They received RHD-match RBCs. In group B, 141 patients received only ABO/D matched RBCs.

**Results:** The overall RBC allo-immunization rate was 2.2%. Clinically significant antibodies were anti-E and anti-Lu in group A (two cases), anti-Jka and anti-Kpa in group B (two cases). Most antibodies were transient. No correlation with number of transfusions, age or HLA antibodies (5.8% sensitization rate) was enhanced. No hemolysis was encountered.

**Conclusion:** In this study, the low rate of allo-immunization is similar to reported data. In immunosuppressed patients with hematologic disorders, RHD-match RBC units offer no additional benefit in terms of allo-immunization. The role of leukodepletion will be discussed.

P 22.20

**New concepts of forming a QMS in blood banking following EN ISO 9001, EN ISO 13845 and EN ISO 15189**

W Sireis, C Seidl, S Fendhammer, G Soeldel, M Stähle and E Seifried

**Institute of Transfusion Medicine and Immunohematology, RCBDS Baden-Württemberg-Hessen, Frankfurt, Germany**

**Background:** Quality safety and in particular quality management systems (QMS) have gained considerable importance in controlling safety in modern transfusion medicine. In addition, there is an increasing socio-economic demand in optimising blood supply.

**Method and results:** We have therefore established a novel improved QMS for blood banks combining certification of a QMS according to EN ISO 9001/13485 and accreditation of laboratory test parameters according to EN ISO 17025/15189. The quality management handbook and the accompanying 25 procedures provide in detail the process control in blood component production, laboratory testing and autologous and homologous blood transfusion. Based on the ISO 9001 quality elements it fulfills high demands in the control of producing blood components and diagnostic results by risk assessment as well as the control of pre-analytic conditions of samples. The system further includes measurements of GMP and GLP standards as well as several legal regulations and guideline requirements. The system has been recently extended in order to manage to reunion of 13 regional blood banks producing in total c. 1.6 Mio blood components in Germany.

**Conclusions:** Modern concepts of QMS are needed in order to fulfill the increasing socio-economic demands in transfusion medicine. These systems are ideally suited to ensure a high-quality level for blood components, diagnostic procedures and tests provided to hospitals and patients.

P 22.21

**Audit of clinical transfusion practice: a multi-centre study**

J Skodlar*

**Magdalena Hospital, Croatia**

**Objective:** To audit the currently applied clinical transfusion practice and to evaluate the quality indicators for related activities.

**Study design and methods:** In a retrospective, multi-centre study in 11 hospital-based blood transfusion centres over a 3-month period, 4922 transfusion requests accounting for a total of 9491 blood component units and data related to quality indicators were evaluated.

**Results:** During the study period, 7.5% of hospitalised patients were transfused. More commonly used elements of quality indicators were documentation regarding blood request, handling and administration of blood components and documentation on adverse reactions, used in more than 65% of participants. The less commonly used elements were monitoring and evaluation of transfusion therapy; used by less than 20% of the participants. Patient identification was incomplete in 8.8% of blood transfusion requests; 2% of blood transfusion requests were not signed by the requesting physician. The reason for using blood components (laboratory or clinical data) was not stated in 17% of RBC, 21% of platelet and 44.5% of FFP requests. A total of 15.6% of the requests were assessed as inappropriate. According to medical specialties, 25% of such requests were issued from surgery department, 24% from internal medicine, 9.5% from gynecology/obstetrics, and 44% from ICUs.

**Discussion:** Improvement in pretransfusion documentation and clinical decision-making process, education of hospital staff, and a monitoring system are necessary.

P 22.22

**Blood tracking – closing the audit loop with iSOFT – a three year review**

KR Smith*

**Walshall Hospitals NHS Trust, UK**

The SHOT report 1999–2000 suggests the use of ‘novel systems’ to log the movement, issue and transfusion of blood products, i.e. use computers. It also noted that 57% of all wrong blood to wrong patient errors occurred in the area of collection and administration. Commercial systems for the above tracking are available and perform well they most secure and effective in operation. However, at Walshall Hospitals NHS Trust we have with the aid of a member of the iSOFT support team developed software which carries out the SHOT suggestions for a modest sum and without the need to interface equipment manufactured by other suppliers. The system developed allows for electronic recording of collection, movement and administration of blood and products from unmanned remote sites, along with a site-wide enquiry system. All transactions are printed out live on an ‘in-lab’ printer. The printout not only records the correct transactions but also any errors made such as the selection of incorrect units, or the attempted return of units out of correct storage for an unacceptable time. This allows for much tighter stock control and the monitoring of blood product usage across the hospital site by the laboratory. This poster reviews the advantages and disadvantages of such a system, and the problems associated with setting up and running it and the training of nursing staff and others whose computer skills vary from nonexistent to expert, and the lessons learned over more than 3 years of use.

P 22.23

**Blood transfusion practices in relation to DRG at a tertiary university hospital**

BG Solheim1,2, KS Kjellesla1,3, G Rongen1, C Sontum1 and A Krogh4

1Rikshospitalet University Hospital, 2ConCentric, 3McKinsey, and 4The Oslo Blood Bank, Ullevål University Hospital, Oslo, Norway

**Background:** Blood transfusion practices were analysed in relation to DRG, diagnosis (ICD-10) and procedure.

**Methods:** In Norway, the citizen ID number is the common denominator used for tracking information. Merging data from the database of our blood bank with our hospital information systems gave a wider data set that could be used to analyse all blood products and immune-haematological services by patient, department, DRG, diagnosis and procedure.

**Results:** Over half of the consumption of blood products could be traced to three particular DRGs for each department. For the Medical, Cardiovascular and General Surgery departments, 70, 60 and 58% of the expenses could be traced to three particular DRGs. Similar results were found by diagnosis and procedure. Particular consumption profiles were observed with different DRGs, diagnoses and procedures.

**Conclusion:** Analysing blood consumption by department in relation to DRG, diagnosis and procedure gives valuable insight into blood transfusion practices in individual hospitals. An internal departmental reporting system has been introduced to track and optimise consumption practices and improve the quality of care provided at our hospital. One ensuing step is to benchmark our transfusion practices with other major hospitals in Scandinavia. This will add insight into the general average requirements for transfusion by DRG, and the resulting statistical distributions will be evaluated in light of cultural and procedural differences.

P 22.24

**Multitransfused patients (>25 units) utilises 23% of the blood supply at a university hospital**

K Tilsted1, J Georgsen2

1County of Funen Transfusion Service, Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

**Aims:** As a part of our continuous monitoring of transfusion practices we have studied the influence of multitransfused patients on the total blood consumption.

**Materials and methods:** We have developed a procedure for monitoring transfusion practices based exclusively on collection of data from existing computerised registers (blood transfusion register, diagnosis and procedure register, and clinical biochemistry register) in all nine hospitals in a county (Funen, Denmark). Multitransfused patient were defined as patients receiving >25 transfusions during hospitalisation.

**Results:** For the eight local hospitals, only 5% of transfusions were given to multitransfused patients during 1997–2003. At the university hospital, however, as much as 23% (48 909) of all transfusions were given to the 1018 multitransfused patients (2% of the transfused patients; mean RBC 26, FFP 15, PLT 12). Multitransfused patients were mainly found in CV surgery (134 patients, 1 713 transfusions), surgery (293 patients,
P 22.25
Development of provincial quality manual for blood banks
I Wilkinson*, S Turnbull and C Benuer
Blood Programs Office, Manitoba Health, Manitoba, Canada

Objectives: There was no consistent, comprehensive and up to date Blood Bank procedures manual in use by the Manitoba Regional Health Authorities. The development of a Blood Bank Quality Manual was an essential adjunct to the Manitoba Adverse Event Reporting System (AERS) which is part of a National Transfusion Transmitted Infections Surveillance System (TTISS) sponsored by Health Canada.

Method: (1) Creation of Manitoba Health Quality Improvement Working Group. (2) Two-day multistakeholder workshop to begin work on adapting British Columbia manual template for Manitoba. (3) Creation of 12-person writing group. (4) Feedback, improvements and/or error corrections.

Results: Creation and distribution of hard copy, CD and electronic versions of the manual. Facilitation of blood bank technologist’s quality network. Decrease in number of queries to experts on procedure-related problems.

Conclusions: This process used to create this highly successful manual was very effective and efficient and will be used to create a similar Quality Manual for Transfusion Medicine Nursing.

P 22.26
West Nile virus and informed consent for transfusion of blood and blood products
O Koester and I Wilkinson*
Blood Programs Office, Manitoba Health, Manitoba, Canada

Objectives: West Nile virus (WNV) can be spread via transfused blood. Screening of donated blood for WNV was not available until after the WNV season had begun in Manitoba in 2003. This made implementation of informed consent essential. Operational and technical constraints limited WNV screening to newly donated blood resulting in ‘dual-inventory’ of blood (WNV screened and unscreened) necessitating physician guidelines on which patients should preferentially receive the WNV screened blood.

Methods: (1) Creation of multistakeholder committee. (2) Creation of Physician Information Package including physician information sheet on risks, patient information pamphlet, sample consent form and frequently asked questions (FAQ) sheet. (3) The process of prioritizing the use of WNV-screened blood and blood products was addressed by a special meeting of medical, legal and ethics experts.

Results: Distribution of Information Package to all licensed physicians. Legal/medical/ethical experts’ consensus meeting on dual-inventory: in the event that there was insufficient WNV-screened blood available, the following prioritization was recommended. Neonates would have the highest priority for WNV-screened blood. All other patients would receive WNV-screened blood on a first come, first served basis.

Conclusions: Implementation of informed consent was complicated by medical, legal and ethical questions. The WNV outbreak served as a challenge and a catalyst to timely implementation.

P 24.1
Efficacy and safety of phlebotomy to reduce transfusional iron overload in acute leukemia patients
G Aprili*, M Franchini and G Gandini
Servizio di Immunonematologia e Trasfusione, Azienda Ospedaliera di Verona, Verona, Italy

Introduction: Transfusional iron overload is a frequent finding in long-term survivors of acute leukemia (AL). Only a few studies have reported the results of iron depletion therapy in this category of patients.

Patients and methods: Between January 1996 and July 2003, 26 consecutive adult patients (median age, 44.8 years) who achieved complete remission of acute leukemia and developed transfusional iron overload underwent a weekly phlebotomy program at our transfusion center. Serum ferritin levels and transferrin saturation were monitored during the iron depletion therapy and the follow-up period. We also checked these AL patients for the presence of 12 hereditary hemochromatosis (HH) gene mutations. 

Results and discussion: After a mean follow-up of 57.8 months, therapeutic phlebotomy [mean number of units collected: 36.6] was effective in reducing mean ferritin concentration and transferrin saturation from 1726.9 to 93.0 µg/l and from 54.7 to 23.3%, respectively. The presence of a HH gene mutation did not influence initial iron status or response to treatment. The phlebotomy program was well tolerated and no adverse events were recorded during or after collection. In three cases the time between phlebotomies was increased because of patient’s poor compliance or low hemoglobin levels.

Our study shows that phlebotomies are a safe and effective method for reducing iron overload in multiply transfused long-term AL survivors with secondary hemochromatosis.

P 24.2
Transfusion hazards locked behind BARS
I Bromilow*, V Dunsmord, T Cave, J Jewell, L Meaney and S Redfearn
DiaMed AG, Fordman Systems, Ysbytv Gwynedd, Weston s. M. Gen. Hosp., St Thomas’ NHS Trust and Poole NHS Trust

The UK Haemovigilance scheme, SHOT, has been in existence since 1996. There is evidence that near miss incidents, not systematically reported, are more numerous than recorded adverse events. Electronic aids to improve safety should be assessed in clinical environments to demonstrate their value. DiaMed BARS (Blood Audit and Release System) comprises a fridge lock and full computer control of blood destined for recipients. Four UK hospitals have submitted statistics. Hospital 1. Units Removed (November/December) 1644. Near miss 8 (0.49%) Hospital 2. Units Removed 794. Near miss 2 (0.25%) Hospital 3. Units Removed 1368. Near miss 30 (0.8%) Hospital 4. Units Removed 1768. Near miss 17 (0.96%). The data show that there is up to a 1/100 possibility of error during removal of units for transfusion. Not all would remain undetected prior to transfusion, nor would all ‘wrong patient/blood’ incidents provoke a serious adverse event if transfused. However, they are ‘near miss’ events, with the potential for causing major morbidity. BARS is easy to use and all responders plan to extend the system to include PBARS for bedside checking. BARS can improve transfusion safety and provide complete audit trails of all activities within the transfusion process.

P 24.4
Transfusion transmitted infections in the UK: microbial highlights of SHOT
KL Davison1,2,3, BC Dow1 and JA Barbary1
1 NBS Colindale, London, 2 Health Protection Agency Communicable Disease Surveillance Centre, London and 3 Scottish National Blood Service, Glasgow, UK

Background: The risk of acquiring an infection through blood transfusion in the UK is very low. A surveillance scheme to monitor transfusion transmitted infections (TTIs) in the UK has been in place since 1995/1998 in Scotland and contributes to Serious Hazards of Transfusion (SHOT) – the UK haemovigilance scheme.

Methods: UK blood centres report incidents of TTI. In addition, suspected transfusion related infections are examined through review of laboratory/clinical reports of blood borne infections made to the Health Protection Agency or the Scottish Diagnostic Virology Laboratories.

Results: Between October 1995 and December 2003, 45 incidents of TTIs involving 48 recipients were identified – averaging 6/year. In most incidents the infection was bacterial (27, 60%). The remainder included 15 viral [nine HBV (in eight the donor had an acute HBV infection), 2 HIV, 2 HCV, 2 MVI, 2 HIV, 2 MVI], one malarial infection and two HIV infections. Both HIV/TTIs occurred prior to leucodepletion and routine anti-HTLV testing. Additionally, a single incident still under investigation was possibly a result of transfusion transmitted vCD – the first such report.

Conclusion: TTIs are rare in the UK. However, asymptomatic infections are unlikely to be ascertained by this form of haemovigilance. Anti-Hbc screening of all donations would not have prevented the majority of the HBV transmissions. Avoidance of unnecessary transfusions and investigation of potential TTIs in recipients remains important.

P 24.5
Canadian transfusion adverse event reporting system: analysis of data 2001/2002
A Giulivi*, N McCombie, J Egan, M Cator and the Members of the National TTISS Working Group
Blood Safety Surveillance and Health Care Acquired Infections Division, Health Canada, Canada

Background: The Transfusion Transmitted Injuries Surveillance System (TTISS) was implemented in Canada to improve reporting of adverse events (AEs) to transfusion. This started with four pilot provinces in 1999 and has expanded to a national system.
Aims of the report: To describe the AEs captured by the TTISS from April 1, 2001 to December 31, 2002. Materials data was collected using standardized definitions; data elements and reporting protocols.

Methods: Moderate and severe events related to blood components and plasma derivatives were transferred to the TTISS, after provincial/territorial validation, for analysis at the national level.

Results: In 2001 (April 1-December 31), 90 AEs were reported by four sites participating in the project. In 2002, 157 AEs were reported by eight sites. For 2001/2002, AEs were categorized for fresh blood components as Major Allergic (30%/36%), Circulatory Overload (10%/13%), Bacterial Contamination (12%/13%), Acute Hemolysis (12%/7%), ABO Incompatibility (10%/6%), TRALI (6%/12%) and Others (9.7%/13%). Over these 2 years, there were, nine cases of death definitely or probably related to transfusion: four related to platelets, three to red blood cells, one to IVIG and one to multiple products.

Conclusion: Voluntary reporting of AEs has increased since implementation of TTISS. To further enhance the system, denominator data will be collected for estimation of risks, and methods to capture viral infections will be addressed.

**P 24.8**

Building the Brazilian hemovigilance system: from the conception to the first results

A. Abib, MED Lopes*, M. Fernandes, AB Pfoetti, M. Felga, Y. Yuni, L. Faggioni and B. MacDowell

Background: Hemovigilance is a new tool for monitoring and preventing adverse effects of blood transfusion. We describe the conception, the implementation and the first results of Brazilian hemovigilance system.

Methods: The Brazilian Hemovigilance system is under the control of the National Agency for Sanitary Vigilance (ANVISA). For initiating the system, a network of 100 tertiary hospitals, called sentinel hospitals, was created. These hospitals had a risk manager, who was in charge of detecting, investigating and notifying transfusion reactions. The notifications are done by software that integrates the ANVISA and the sentinel hospitals, and the reports were analyzed and discussed at local and central level.

Results: From April 2002 through June 2003, 16 hospitals sent notifications to ANVISA. These hospitals performed a total of 403 566 transfusions. The overall rate of transfusion reactions and incidents was 0.55/1000 transfusions. The table below shows the main type of adverse effects.

| Type of complication               | Number of cases | RATE (/1000 transfusions) |
|------------------------------------|-----------------|---------------------------|
| Febrile nonhemolytic transfusion    | 116             | 0.3                       |
| Reactions (FNHTR)                  |                 |                           |
| Anaphylactic reaction              | 3               | 0.007                     |
| FNHTR + circulatory overload       | 1               | 0.0025                    |
| Hemolytic reactions                | 2               | 0.0005                    |
| Circulatory overload               | 3               | 0.007                     |
| Bacterial contamination            | 1               | 0.0025                    |
| Total                              | 224             | 0.55                      |

Conclusions: The implementation of hemovigilance is feasible in developing countries, and it can significantly contribute to blood safety.

**P 24.9**

Development of the Transfusion Transmitted Injuries Surveillance System (TTISS) in Canada

N. McCombie*, M. Cator, J. Egan, M. Wotherspoon, N. Karistiotis, A. Giulivi and the Members of the National TTISS Working Group

Background: Blood surveillance activities and reporting of adverse transfusion events in Canada became a priority due to the contamination of the blood supply in the 1970s/1980s.

Aim: Describe the implementation of a national hemovigilance system for reporting and monitoring adverse transfusion events.

Materials: A standardized reporting form; a user's manual with standardized definitions; agreements on data elements; conditions for reporting data; and a database to record adverse events and produce reports.

Methods: Data is collected from hospitals and voluntarily reported to provinces/territories. Non-nominal data is transferred quarterly for analysis; national reporting and recommendations for risk management. A working group consisting of provinces/territories, federal regulators and blood manufacturers provides recommendations regarding operations. An external review group evaluates data and advises regarding current/emerging transfusion issues.

Results: Since 1999, TTISS has been implemented in eight provinces and two territories, the remaining three provinces/territories expected in 2004/2005. From April 2001 to June 2003, data was received related to 40–100% of blood components transfused in provinces/territories. Data from April 1, 2001 to March 31, 2003 has been published.

Conclusion: National surveillance of transfusion transmitted injuries can successfully describe adverse outcomes and guide policy development.

**P 24.10**

Adverse transfusion reactions in the Quebec hemovigilance system in 2000–2002

P. Roybaird*, L. L., NK Itaji, K. Jochem and N. Garneau

*Quebec Public Health Institute, McGill University, Quebec Health Ministry and Montreal Public Health Department

Objective: A hemovigilance system was implemented in 2000 in Quebec, Canada to monitor adverse transfusion reactions (ATR). Incidence of ATR reported from 2000 to 2002 is presented.
P 24.11
No relevance of ALT testing in blood donor screening in Germany
WK Roth, K Hoorfar, E Seifert and M Schmidt
German Red Cross, Institute Frankfurt, Germany

Background: Analysis of ALT enzyme activity, as a surrogate marker of a hepatitis infection is recommended or even mandatory in many countries for blood donor screening. If ALT activity is above an established threshold (cut-off stage) blood donations are excluded. Since 2003 the threshold is determined with the IFCC reference method for men at 134 IU/l and for women at 89 IU/l in Germany.

Methods: 100 000 donations were examined for ALT, HBsAg, HCV-antibody as well as for HBV and HCV PCR.

Results: Two hundred and forty-four of 100 000 donor samples (0.25%; 133 men and 121 women) were above the threshold. 76 of 100 000 samples were positive for HBsAg but 0 of those (0.00%) had elevated ALT above the threshold. One hundred and eighty-four of 100 000 were positive for HCV antibody but only four of those (0.15%) had elevated ALT above the threshold. 98.43% of samples with elevated ALT above the threshold were negative for HBV and HCV screening parameters.

Conclusion: General screening of blood donations for ALT led to an exclusion of 0.25% of blood donors, however only 1.57% of those were positive for transfusion relevant parameters. Donors with elevated ALT did not shown any clinical symptoms. ALT declined to normal values for the next donation. Neither donor specific look backs nor recipients specific look back examinations yielded a transfusion related infection. Based on our data a general examination for ALT cannot be recommended for blood donations. Therefore it was justified to discontinue ALT testing in Germany.

P 24.12
Serious hazards of transfusion in children – analysis of 6 years’ data from SHOT
B Gibson, D Stainsby*, AAM Todd, H Jones and H Cohen
Serious Hazards of Transfusion (SHOT) Steering Group

Of 1630 events reported to SHOT from 1996–2002, 141 (8.6%) related to patients under 18 years. Sixty–one of 141 (43%) of these were infants under 1 year and 35 of 53 (66%) were under 4 weeks of age. One hundred and thirteen of 141 (80.1%) events were of incorrect blood component transfused. Errors arose because of patient misidentification at the time of blood sampling and at administration. Namecheck bands are especially important in younger children who cannot verbally confirm their identity. Fourteen errors were because of lack of awareness of special transfusion requirements of infants; eight such errors arose in the laboratory. Ninety–one of 141 patients were unharmed; 14 suffered major morbidity of whom 10 were under 6 years old. These included two patients who developed intravascular haemolysis and 4 RhD negative girls exposed to RhD positive red cells. Six patients died from their underlying condition and in two the outcome was unknown. Various immunological reactions were reported including 15 acute transfusion reactions of which seven occurred in infants under 1 year, nine cases of transfusion related acute lung injury (TRALI) of whom three died, and two cases of graft-vs.-host disease, both fatal. Delayed haemolytic transfusion reactions are not common in childhood, the single case reported was in a multi-transfused 17-year-old with sickle cell disease. Post-transfusion purpura was not seen. Paediatricians should be encouraged to report transfusion-related adverse events and to disseminate lessons learned from SHOT to their staff.

P 24.14
A voluntary system of haemovigilance in Denmark – DART
E Taaning*,1 and J Jorgensen2
1Blood Transfusion Centers, Herlev Hospital, University of Copenhagen and2Aarhus University Hospital, Aarhus, Denmark

The DAnish Registration of Transfusion-risks (DART) is a part of the Danish haemovigilance system that covers registration of collected, produced and transfused blood components, and complications in connection with transfusion. DART was initiated in 1998 as an organization under the Danish Society of Clinical Immunology. DART is run on a voluntary and confidential basis similar to the Serious Hazards of Transfusion (SHOT) in the UK. During the first 5 years, DART received 104 reports about a severe risk. In Denmark, approximately 450 000 blood components are transfused per year. Thus, the report rate (ratio) was 5/100 000 components transfused. Half of the severe events concerned the transfusion of an incorrect blood component (ratio 2.4) and nearly all the rest an immunological complication (ratio 2.1). Only a very few concerned a transmitted infection (ratio 0.2). The immunological complications were delayed haemolytic reactions (38%), acute anaphylactic reactions (22%), transfusion-related acute lung injuries (10%), and acute haemolytic reaction (16%). Post-transfusion purpura and transfusion-associated graft-vs.-host disease were reported once (ratio 0.04). Four patients died (ratio 0.2) and 29 (ratio 1.3) had a severe reaction. Conclusion: The results obtained by DART have given rise to initiatives and recommendations aimed at reducing the transfusion risk.

P 24.15
Fourteen Years' transfusion reaction reports: analysis by cause, severity and outcome
G Walther-Wenke1, D Myrtil1 and N Mueller2
1Inst. for Transfusion Medicine, Red Cross Donor Service West, Mnester and2Inst. for Transfusion Medicine, University Clinics, Essen, Germany

Objective: The objective of this study is a systematic review of a register for spontaneous reports of transfusion reactions (TR).

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Methods: Transfusion safety officers investigated and reported ATR to the Quebec Health Ministry. ATR possibly, probably or definitely associated with transfusion were analysed. Rates were calculated based on components transfused as per hospital reports.

Results: For 2000–2002, 2927 ATR were reported by 42 hospitals which transfused 833 970 units. Rates per 10 000 transfused red blood cell (RBC) and platelet (PLT) units were:

|          | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 |
|----------|------|------|------|------|------|------|------|------|------|
| ATR      | 21.5 | 18.3 | 17.8 | 20.3 | 18.2 | 17.4 | 22.2 | 20.3 | 18.8 |
| Minor allergic | 20.1 | 18.0 | 17.5 | 20.3 | 18.2 | 17.3 | 22.1 | 20.3 | 18.7 |
| Major allergic | 1.4  | 0.8  | 1.0  | 2.0  | 1.8  | 2.5  | 2.6  | 2.4  | 2.5  |
| Febrile nonhemolytic | 11.4 | 19.8 | 26.3 | 13.1 | 15.2 | 24.6 | 29.7 | 49.1 |
| ABO incompatibility | 0.7  | 0.2  | 0.3  | 3.7  | 3.7  | 3.7  | 4.5  | 4.5  | 4.5  |
| Acute hemolytic (AHTR) | 0.8  | 0.5  | 0.6  | 3.7  | 3.7  | 3.7  | 4.5  | 4.5  | 4.5  |
| Delayed hemolytic (DHTR) | 1.2  | 1.1  | 1.4  | 6.5  | 6.5  | 6.5  | 7.0  | 7.0  | 7.0  |
| Bacterial contamination (BC) | 0.6  | 0.5  | 0.5  | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  |
| TRALI    | 0.07 | 0.07 | 0.07 | 1.4  | 1.4  | 1.4  | 1.6  | 1.6  | 1.6  |
| Circulatory overload | 1.7  | 3.1  | 2.5  | 0.6  | 0.6  | 0.6  | 1.8  | 1.8  | 1.8  |

*5 Units. There were five deaths definitely associated with transfusion, three to RBC (two AHTR, one DHTR), two to PLT (BC).
Methods: 1276 case reports from 1988 to 2001 were examined for completeness and plausibility and analysed (Access, Microsoft).

Results: Type of reactions: Febrile, nonhaemolytic 837, allergic 167, acute haemolytic 33, delayed haemolytic 22, anaphylactic 18, suspected TRALI 8, septic 9, Graft-vs.-Host reaction 1, haemolysed product 10, incomplete details 72, not caused by transfusion 99, Total: 1276. One hundred and fifty-four (12%) of the cases were classified as severe; three patients died (two septic, one haemolytic). 1190 (93.3%) of the patients recovered without complications.

Conclusions: Assuming that around 4 million blood components were transfused over the 14-year study period, one serious complication must be expected in every 26 500 transfusions. The rate of fatal complications was 1:1.3 million transfusions. Errors in handling the blood components and in blood group serology caused 3.3% of the TRs registered in the study. This is in remarkable contrast to other studies which report considerably higher error rates. The study shows that the TR reporting system must be optimized.