A1 Donor recruitment and retention

A 1.1 Barriers to blood donation
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Introduction: Red Cross of Serbia and Monte Negro conducted a survey about attitudes toward blood donation. The objective was to develop social marketing strategy. Among other points, research covered major barriers to donating blood.

Method: Qualitative and quantitative research [face-to-face survey on a national representative sample of 1506 adults].

Results: Non-donors’ reasons for not donating blood, first cited reason: fears related to blood donation (38%), lack of interest (29%), health reasons (21%). Donors’ opinions about reasons for not donating blood: fears (52%), lack of interest (20%). When non-donors were asked if there were any particular reasons that would encourage them to give blood, 81% said ‘yes’. First cited reason: someone close in need (71%); all cited reasons: need of a close person (94%), emergencies (58%), need of a newborn baby (50%). Those that would not give blood under any circumstances cited health issues (54%), fear (11%), and lack of interest (16%) as reasons for not donating. There are no differences between donors and non-donors regarding knowledge and need for information about blood donation, opinions about motives for donation and general life-aims. Donors and non-donors differ significantly regarding the core meaning of blood donation: for donors donation is primarily about giving and helping others, and for non-donors it primarily means risking personal well-being.

Conclusion: National strategy should address barriers in a very subtle way.

A 1.2 Substance abuse in scholars: will it impact on future blood supply?
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The South African National Blood Service, Republic of South Africa

Introduction: An increase in substance abuse, by scholars, has been reported. The link between substance abuse and high-risk behavior has been established. This presentation reports on substance abuse patterns in South African scholars and discusses the possible impact on blood supply.

Materials and methods: Data was taken from the 2003 Medical Research Council (MRC) Study on youth risk behavior.

Results: Region statistics for HIV prevalence in donors <20 years are shown below.

|          | 2000 | 2001 | 2002 | 2003 |
|----------|------|------|------|------|
| HIV prevalence in donors <20 years | 0.190 | 0.126 | 0.105 | 0.092 |
| HIV prevalence in all donors | 0.280 | 0.217 | 0.165 | 0.173 |

Aim: Healthcare professionals attitudes and behaviours to blood donation
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Background: Few studies assess healthcare professionals donation behaviour attitudes to it despite them being the occupational group most aware of its need.

Materials and methods: A cross-sectional questionnaire of doctors and nurses working on the wards in four main hospitals in Birmingham, UK was conducted between March and May 2003. It was designed to establish their donating behaviour and attitudes to donation.

Results: Of 172 healthcare professionals asked, 43 (25%) had donated blood in the last 2 years and are considered blood donors. The biggest reason for not donating blood was lack of time (30%). Doctors were more likely to be aware of an upcoming shortage in donated blood due to new guidelines on CJD than nurses (P = 0.021). Previous donors and people of white ethnicity were significantly more likely to agree that knowledge of a shortfall in donors would increase their likelihood of donating (P = 0.004 and P = 0.015). Doctors less likely to object than nurses on the issues of whether healthcare professionals should be required to donate yearly and whether all blood donors should be financially reimbursed (P = 0.025 and P = 0.036).

Conclusion: Healthcare professionals are more likely to be blood donors than the average member of the general public is. Ethnicity and previous donation behaviour affect attitudes toward blood donation. Donation attitudes between doctors and nurses differ. Further studies need to be done to compare this with the general population.

A 1.4 Review of the blood donations among high school students for 2002/2003
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Aim: We aim to exhibit and compare the results of the blood donation actions at the high schools in Skopje and in Macedonia for 2002 and 2003, and to point to the achievements and difficulties with their realization.

Materials and methods: National Blood Transfusion Institute in Skopje performed blood donation actions among graduating students in 36 high schools in Skopje and 63 in other Macedonian cities. The target group was multiethnic.

Results: In 2002, 41 actions were held at 18 schools in Skopje and 55 at 32 schools in the country. 4085 blood units were collected; 1202 in Skopje (29.42%) and 2883 (70.58%) in other cities. In 2003, 35 actions were held at 18 schools in Skopje and 55 at 31 school in other regions. 4268 blood units were collected; 827 (19.38%) in Skopje and 3441 (80.62%) in other cities.

Discussion: The total number of donations increased for 4.48%. In Skopje it decreased for 11.2%; in the country it increased for 18.35%. The average number of donations per action in Skopje decreased for 24.08%; it increased for 19.34% in the country. In 2003, after a longer period, actions were held in Tetovo (124 Units for 3 days).

Conclusion: Proper and persistent informing of the youth in their mother language (accordingly to their ethnical and cultural background) and precise coordination is crucial. The reasons for such discrepancy in the results shall be presented.

A 1.5 Dry chemistry determination of haemoglobin. Is it suitable for the pre-donation selection?
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Background: The haemoglobin (Hb) determination method used in the pre-donation selection as to be safe, fast, easy to perform and accurate to allow adequate donor selection.

Aim: To evaluate the suitability of two dry chemistry haemoglobinometers for the blood donor Hb screening.

Materials and methods: In the first stage, 10 samples of venous whole blood (VB), collected in EDTA, were tested on a cell counter (Sysmex® SF-3000) and on two different haemoglobinometers (Hemo_Control and HemoCue®). In the second stage, Hb
values of 56 blood donors (13 males; 23 females) obtained with Hemo_Control using capillary blood collected by finger puncture were compared to the Sysmex Hb values obtained with VB collected in EDTA. All data were compared for differences.

Results: First stage data (mean of 10 readings per sample)

|                        | Hb (g/dl) |
|------------------------|-----------|
| Sysmex®                | 10.6      |
| Hemo_Control           | 10.8      |
| HemoCue®               | 10.8      |

In the second stage all the men had Hb values ≥13.5 (max. difference: 2.5) and five of the 23 women had sysmex values <12.5, being the corresponding Hemo_Control values ≥12.5 (max. difference: 1.6). This gives a 0.217 probability of incorrect selection of women as donors.

Conclusions: Hemo_Control had better inter-assay repeatability and lower error values. The difference in Hb values between sysmex® and dry chemistry systems can lead to incorrect selection of donors, especially women. To avoid this, minimum reference Hb value can be raised.

A 1.6 Media in favour of voluntary blood donation

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Voluntary blood donation promotion is practically impossible without the activities and support of both press and electronic media. In 2003, National Blood Transfusion Institute provided higher interest of the media in the follow up and interest in voluntary blood donation (realization of the national program of blood supply) through a qualitatively new approach, in a preplanned and continuous way. Appeal, as a form of addressing the public was avoided, since it was previously demonstrated that it could be counter productive. We initiated realization of the events using marketing tools such as TV spots, billboards, posters, etc. We had thousands of seconds of free TV broadcasts of the spots shot by the professional marketing agencies, both on national and local TV stations throughout Serbia. Our press clipping for only 6 months is over 100 articles in the leading newspapers, both on national and local TV stations throughout Serbia. Our press clipping for only 6 months is over 100 articles in the leading newspapers. In local press there were several hundreds of such articles. We have realized that media require an interesting event as a starting point as way to make their task easier. Well-prepared letter for the journalists is a guarantee that the information will be properly transmitted and the goal achieved. Results reflect decrease of the number of family donors of 5.600 (12%). It represents a significant step towards our main objective, i.e. elimination of the family blood donation as a whole.

A 1.7 Kell blood group: the incidence of KEL1 and KEL2 antigens in Greek blood donors

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Background: The Kell blood group system assigned over 23 different blood group antigens. This polymorphic variation is due to single base mutation in the KEL gene. Some of the Kell antigens are highly immunogenic. The antibodies in the Kell blood group system are mainly developed after a mismatched transfusion or during pregnancy. The commoner Kell antibody is anti-KEL1. In our earlier study the presence of antibodies to other Kell antigens is clinical significant and induces haemolytic transfusion reactions and haemolytic disease of the newborn. The KEL2 (K) antigen is a high-frequency antigen and the blood donors lacking this antigen are quite rare.

Methods: A total of 5363 blood donors were examined for K-antigen by an automatic frequency antigen and the blood donors lacking this antigen are quite rare. Some of the Kell antigens are highly immunogenic. The antibodies in the Kell blood group system are mainly developed after a mismatched transfusion or during pregnancy. The KEL2 (K) antigen is a high-frequency antigen and the blood donors lacking this antigen are quite rare.

Reactions with anti-K  Reactions with anti-k  Phenotype  Blood donors  Frequency %

|    |        |          |        |        |
|----|--------|----------|--------|--------|
| *  | 0      | K-k-     | 7      | 0.13   |
| +  | +      | K-k+     | 266    | 4.96   |
| 0  | +      | K-K+     | 5090   | 94.91  |

### Conclusion:
The incidence of K and k antigen in the Greek population is similar of that of Caucasians. The 'k-negative' donors are rare and their identification and registration (especially in blood group O-, K-positive) would result in a convenient and inexpensive stock of these rare units.

A 1.8 Risk behavior among blood donors who donate for HIV test in Shiraz, BTO in 2003

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Introduction: There has been concern that some individuals may donate blood primarily motivated by the access to (HIV) testing and that such donors may represent a risk to the transfusion service.

Materials and methods: This is a cross sectional study utilizes a questionnaire. The sample was selected using systematic random sampling the questionnaires include.

Personal characteristics of respondents, reason for blood donation, risk factor of HIV if they donate blood for HIV check up. Chi-square, logistic regression and analysis of variance were done for analysis.

Results: Mean age of respondents was 34.6 ± 11.3 SD. 82.4% were male and 69.8% were married the mean number of blood donation was 6.7 ± 0.325. A total of 14.8% respondents reported to have donated blood in order to check up. The tendency for HIV check up was higher in men, single donor and first time blood donor (P < 0.05). There were no correlations between job, education, age and HIV check up (P > 0.05). The risk factor of them for HIV were 38.3% sexual contact, 18.7% contact with person who suspicious to HIV, 3.7% drug use, 2.8% tattoo and 36.5% unknown.

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

A 1.9 Medical personnel and blood donation

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Background: In the need for strategies to recruit and retain donors, ones of the directions that can be considered is the potential of medical personnel for blood donation.

Aim: To investigate blood donation behaviour and motivation of medical personnel.

Materials and methods: An anonymous survey of randomly selected sample of medical personnel from Surgical Hospital, Pediatric Hospital and Institute for Health Protection was conducted to determine donor status, age, education level, motives for donation, non-donation and ceasing blood donation.

Results: Completed questionnaires were returned by 52% (214) of the randomly selected 400 employees. Responses revealed that current donors (CD) represent 20.9%, former donors (FD) 23.2% and non-donors (ND) 56.9%. Among surgical hospital respondents there are 33.3% CD, in comparison to other institutions (7.8%). The educational level in all of the groups corresponds with the same of the sample. Average number of blood donations in the group of CD is 3.4 and 3.3 in the group of FD. Main motive for blood donation is altruism (93.2%). Health issues are the main reason for ceasing blood donation (53.1% of FD) and for non-donating blood (76.3% of ND).

Discussion: The higher participation of surgical hospital employees in blood donation might be due to their awareness of the need of blood and also the existence of a transfusion department that works on promotion of blood donation.

A 1.10 A survey of positive and negative blood donors’ motivation in Shiraz blood transfusion in 2003

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Introduction: To prepare safe and adequate blood to meet patient’s need, it is essential to know of factors that invite people to donate blood and become regular blood donors.

Materials and methods: This is a cross-sectional study. The sample size was determined by review of past surveys. 10% of samples were selected using systematic random sampling. This questionnaire includes personal characteristic, positive and negative motivations. Analysis of variance and chi square method were used to analyze.

Results: The mean age with positive motivation was 32.73 ± 9.6. 92.1% were male, 74.3% married. Positive motivation was help to other people 65.3%, check up of their health 12.9%, supporting a family member 8.9%, positive effect of blood donation in health 12.9%, supporting a family member 8.9%, positive effect of blood donation in

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health 8.9%, their curiosity toward blood donation 4%. The mean age of with negative motivation was 25.24 ± 7.54 years. 22.7% were male, 67.3% single. Negative motivation was fearing of dizziness and faint 45.5%, fear of disease development as a result of it 22.7%, fear of injection and blood 18.2%, fear of getting infection 14.5%, lack of time 4.5%. Negative motivation in women was more (P < 0.05). Age, marital status, education did not correlate with their motivations (P > 0.05).

Conclusion: We have to encourage people to donate blood only for helping others and do not donate for health check up. To remove negative motivation is essential to aware general public about the safety of blood donation procedures and sufficient blood donor care.

A 1.11
The evaluation of the attitude of Iranian women towards blood donation in eight provinces
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The most important goal of IBTO is to prepare safe blood components. It is likely that women population compared with men is at lower risk in regard to high-risk behaviors leading to blood-transmitted infections. However, the donation attempts on part of women compared to men are less frequent. A cross-sectional study was conducted on Iranian female population at the age range of 17-65 in eight provinces of Iran. A questionnaire was prepared. The number of samples was calculated as 12 000 using statistical formulas. The sampling method was multi-stage cluster. Finally, the data were analyzed using spss version 11 statistical software. Out of 12 000 female subjects under study, 40.4% of them afraid of being infected with blood-borne and infectious diseases and 37.7% cause of the lack of sufficient awareness-raising activities were not willing to donate. Generally, 75.2 and 24.8% of them had a negative and positive attitude towards blood donation, respectively. The results show that IBTO information-dissemination system about donation is not appropriate and more efforts are required. Radio and TV can be the best choice to convey the relevant and useful information. Moreover, some strategies should be implemented so that female false implications including the negative impact of blood donation on pregnancy or the possible transmission of infectious diseases through donation are modified through awareness-raising activities.

A 1.12
Voluntary blood donor program: efforts of tertiary hospital blood transfusion services, Kerala, India
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Background: A comprehensive National Blood Policy was overlooked till recently in India. Initiatives and developmental strategies were at the local level. As a result the growth of transfusion services was hospital based, isolated and sporadic. Attempts are being made at STICMIST, an Institute of National Importance to develop a model BTS. Its aim is to provide optimal supply of safe blood, collected from voluntary blood donors of least risk. Patients receive a quality product hassle-free and donors, meticulous care. This is achieved by bringing together the various components of a voluntary blood donor program and by a process of image building. The BTS, set up in 1976 never had a paid donor system, then a prevailing practice in the country. The voluntary donor pool consists of repeat donors, periodical donations from Colleges/Institutions and Panel donors. Donor recruitment programs are planned, organised and implemented through out the year. Unique efforts include development of a Rural Participatory Model of VBD program, Project on Networking for Blood and Direct Recruitment strategy. Studies on donors and patients needing transfusion are undertaken and data utilized to improvise the Services and disseminate information.

Conclusion: The challenge here lies in surmounting problems that plague the BTS of developing nations and build an integrated voluntary blood donor program for the State and as a long term goal, share knowledge and experience for benefit of the country.

A 1.13
PR activities of the National Blood Transfusion Institute in the period of blood lack crisis
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Throughout 1-year period blood donation passes through various stages. Two stages are predominant: period of the lack of blood and period of relatively sufficient blood supply. As of its foundation, i.e. over 50 years, National Blood Transfusion Institute (NBTE) has the problem of the lack of blood in winter (January and February) and in summer (June, July, August and September). Additional efforts are required for the realization of the new activities in order to avoid blood lack. That was the main task of the NBTE PR in the past year. The first important activity was to make a closer contact with blood donors, using mobile teams, in a new and interesting way. In 22 places, including Belgrade, 33 campaigns of voluntary blood donation were organized, and 3.050 blood units were collected from June 11, till July 13, 2003. Marketing preparations of the PR campaign included: motto, leaflets, posters, billboards in four largest places, TV spot of both educational and motivating content, promotion team and organization-realization team, small motivation gifts. Campaign was announced 7 days earlier with a press conference. Activities were transferred to the local level – press conferences on local TV and radio station stations. Key factors of voluntary blood donation on the local level were engaged: municipal Red Cross organizations, local authorities, local media and other authorities related with blood donation, such as blood transfusion services. The final result was there was no lack of blood in the stated period.

A 1.14
Protective antibody against hepatitis B virus infection among Northeast Thai blood donors
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Objective: This study was carried out to determine the situation of anti-HBs, the protective antibody against HBV infection among Northeast Thai blood donors. The information is useful for blood safety and health promotion.

Materials and methods: Sera from 342 HbsAg and anti-HCV negative donors were screened for anti-HBs and anti-HBc by ELISA. The positive samples were reexamined for IgG anti-HBs concentration.

Results: Anti-HBs was detected in 19.8% (22/111) of first time and 36.4% (84/231) of repeated donors. All of 106 anti-HBs positive samples had above 10 mIU/ml, which 74.5% demonstrated over 100 mIU/ml of anti-HBs concentration. Natural immunization (anti-HBs and anti-HBc positive) was found in 14.4% (16/111) and 22.1% (51/231) of first time and repeated donors, respectively. The antibody induced by vaccination (anti-Hbs positive only) was found in 5.4% (6/111) of first time and 14.3% (31/231) of repeated donors.

Conclusions: Only 36% and 20% of repeated and first-time donors with HbsAg and anti-HCV negative ensured no risk of HBV transmission. Repeated donors demonstrated significant higher prevalence of anti-HBs to first time donors (P < 0.005). Retention of regular repeated donors with positive anti-HBs is better to reduce of transfusion risk associated HBV. HBV vaccination advice to individual with negative HBV serological markers should be done for enhancing blood safety and health promotion.

A 1.15
Information, motivation and attitudes toward blood donation: a pilot study
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Aim: This study was designed to determine the blood-donation behavior and attitudes for the purpose of developing promotional and educational approaches to enhance levels of donor participation.

Materials and methods: A questionnaire of 12 questions concerning transferrable diseases, previous donation history, motives, society appreciation, etc. was distributed and answered by randomly selected 311 blood donors, with different level of education, sex, age, occupation.

Results: Men consisted 81% and first-time donors were 23% of the whole group. Repeated donors donated 15 times in average. Students and policemen are informed about the transferrable diseases in 95%, compared to the group of craftsmen and primary education donors in 55%. The students have received written materials in 58% and the other subgroups in 25–40%. Information was understandable for 70% of the donors. Interesting data is that less than 50% have another blood donor in their family, and almost 90% have a blood donor in the close environment. 81% are voluntary non-renumerated blood donors. The desire to help those in need is a motive in 77%. Civic duty is the policeman’s motive in 25%.

Conclusion: Donors are found to vary in their behaviors, attitudes and beliefs, but not hugely. A perception of a lack of information indicates a need for improved education on issues like the transferrable diseases. These data may enable blood centers to optimize recruitment by tailoring a more effective strategy.
A 1.16 Challenges in blood safety in medical center, Strumica
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The quality and safety of blood transfusion therapy is a continuing concern especially when blood for transfusion is collected from unsafe donors. Can we have a cost effective blood system when blood safety is paramount? Many decisions are made in the Blood Transfusion Centre in Medical Centre, Strumica in order to protect the safety of blood: the improvement of a quality system, efficient laboratory procedures in blood group serology and adequate testing of donated blood for transfusion-transmissible diseases. The motivation for our voluntary and non-remunerated donors is the feeling of social pride given by belonging to a self-selected group of citizens. They are citizens endowed with particular feelings of personal, social and moral responsibility who periodically give their blood voluntarily, freely and anonymously. Absolute blood safety and ‘zero risk’ do not exist. Problems can arise from errors in the administration of blood, a lack of access and appropriate use of blood and blood products for patients requiring transfusions. Maximum blood safety could be reached not only with blood testing alone, but also with the education of people in the safety of blood transfusion, which is mostly the result of responsible blood donors and knowledgeable physicians and health care workers. Adequate funding could provide the necessary technology and staff to overcome the challenges to blood safety.

A 1.17 Overview of blood donations in the high schools in Macedonia according to the NITM (2001–2003)
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Background: High school students are very important group of blood donors. In that period, for the first time, they become familiar with blood donation, and from that moment they will maintain their positive or negative attitude towards it. So, the aim of this study is to show how many of them donate blood in the last 3 years, and to compare blood donations in the high schools in Skopje with the others in the country.

Materials and methods: Blood was taken from 17 and 18 years old high school students in Skopje and in the country, most often twice a year, with the mobile teams of NITM from January 2001 till December 2003.

Results: There were 11 215 blood units collected from them, which is ~7.5% of all donated blood. 1144 students donated blood in Skopje in 2001, 1097 in 2002 and 807 students in 2003; and 2179 students from the country donated blood in 2001, 2306 in 2002 and 3482 students in 2003. The total number of country students that donate blood for the last 3 years is 7967 Units (71%) versus Skopje’s students 3248 (29%), which is more than twice.

Conclusion: Although the high school students show great interest in blood donation, the optimal number of blood donation has not been reached yet (we have 7.5% vs. planned ~15%). Country students are more interested in blood donation than Skopje’s students. We need to maintain positive attitude and pay more attention to the education of the youth about voluntary blood donation starting from their earliest age.

A 1.18 Blood collection in the National Institute for Transfusion Medicine, Skopje, Macedonia
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Republic of Macedonia has ~2 000 000 people and ~3% of the people should donate blood. However, we still do not have these 60 000 blood donations per year; yet, it is ~50 000 blood donations. Approximately, 20 000 blood unit per year are collected by the NITM, which is the main institution of this type in our country. The aim of the study is to show how much blood is collected by the NITM, who donate blood, types of donation and how the blood is collected (in NITM, with mobile units, etc.).

Materials and methods: The data is used from the database of NITM in Skopje, from January 2003 till December 2003.

Results: There are 18 738 blood units collected for the mentioned period. 2725 blood units are taken in the NITM (14.5%), of which 1854 (68%) are family donated, and the rest 871 (32%) are voluntary donated. The others 16 013 blood units are taken with the mobile teams of the NBTI 5 046 (26.9%) units in Skopje and 10 967 (58.5%) in the country. Family donated blood makes 9.9%, high school student’s blood donations are 4289 (22.9%) and university students are 1381 (7.3%).

Conclusion: The number of blood collection remains the same as the previous year. More than 30% of the taken units are from the youth, which is good. We still have family donors (~10%) and we should work on turning them to voluntary donors, and voluntary donors to regular donors, that donate blood at least twice a year.

A 1.19 Donorship in Russian Federation
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Backgrounds: The problem of blood donors recruitment is very important in Russia.

Method: A study about the donorship was conducted using the reports from all regions of Russia followed by a computer statistical analysis.

Findings: All the regulations for examination of donors and testing the donors blood are approved by the Russian Ministry of Health. Volume of whole-blood donation is determined by the physician examining the donor. A standard donation must not exceed 450 ml of whole blood. In first time donors, as well as in persons under 20 and over 55, standard donation must not exceed 300 ml. Nowadays an average single unit of blood donated is 393 ml. In 1888 amount of donors was 4 544 775 (voluntary donors: 96%), in 1999, 2 178 813 (voluntary donors: 82.6%); in 2002, 2 097 064 (voluntary donors: 87.5%). In 2002, 1 606 895 l of whole blood was collected, 75% of blood being given by voluntary donors. 4.5% of donors blood was discarded as defective because of HbsAg 16.3%, anti-HCV antibodies 26.7%, syphilis 6.8%, HIV antibodies 0.8%, elevated ALT level 30.5%, other causes 17.1%. Anti-HIV determination does not prove its value, because the rate of the virus infection is low within Russia.

Conclusion: Amount of donors has been decreased for the last 12 years. To provide the hospitals donors blood, components and preparations and to develop alternatives transfusion is actual national problem at present.

A 1.20 The survey of blood donations among the university students in Macedonia
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Background: Students are one of the most important groups of blood donors and we should pay a special attention to them. The aim of this study is to show the number of blood donations among the students, their distribution in the spring and winter school semesters and to point out what might be the best way to recruit them.

Materials and methods: In the period from January 2001 till December 2003, blood units were taken from the university students in Skopje, with the mobile units of our Institute. Blood donor actions were organized approximately twice a year in spring and winter.

Results: There are 2761 voluntary blood donations from the students in the last 3 years, which is ~1.8% of all donated blood in Macedonia. In spring 2001 we had 217 donations, in winter 2001, 235; in spring 2002, 398; in winter 2002, 530; in spring 2003, 640 and in winter 2003, 741 blood donations. The number of students blood donations compared with the whole amount of blood donated in 2001 is 0.9%, in 2002 is 1.8% and in 2003 is 2.8%. The total number of the first time blood donors is 939 (34%). There are more donations in the winter period.

Conclusions: The number of voluntary blood donation among the students is increasing in the last year and a half due to our greater effort, but we still need to work a lot on their education and motivation about blood donation to maintain the needed number of 5%. Retention of student donors needs more frequent reminding and other forms of recognition.

A 1.21 Special aspects in blood donor recruitment
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Background: The authors investigate the attitude of blood donors and their motivation to donate blood under the new social circumstances in county Baranya.

Materials and methods: In a month-long study, anonymous questionnaires were distributed among donors. The questions were directed to their families, education, social behavior and blood donation habits. A total of 1133 volunteers gave answers: 59% males and 41% females. Of which 442 (39%) of them were first-time donors (FBD). The evaluation of the answers was performed by age-groups, sex and first or multiple blood donations.

Results: 85% of the volunteers live in an unbroken family compared to 62% in the general population. 54% of FBDs and 68% of multiple blood donors (MBD) sympathies
with the unemployed but near twice as many FBD are uninterested to them or consider them faulty compared to MBDs. 4% of MBDs and 16% of FBDs were persuaded by their friends to give blood. Most of the FBD (52%) heard about blood donation first time in their family, only 4% of them from the Red Cross activists. In case of MBDs, ratio was 40% and 14%. 15% more MBD than FBD gave blood on humanitarian reason. Conclusion: The volunteers themselves recruit best new and young blood donors. To convince MBDs to donate blood regularly, requests to help people, favourable experiences during blood donation and the role of family doctors are very important. Activities of the Red Cross and of the media targeting specific age groups remain to be improved.

A 1.22 The distribution of ABO and Rh-D blood groups among blood donors in the municipality of Strumica
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Introduction: To present the distribution of ABO and Rh-D blood groups and sex in blood donors who have donated blood in Medical Centre, Strumica.

Materials and methods: We analyzed the test results of 3940 blood donors who donated blood from 2001 to 2003. Blood groups ABO and Rh-D were analyzed on a slide in a tube with test sera Biotest, and with micro-agglutination technique in gel cards DuMed ID.

Results: The distribution of blood groups is as follows: blood group A Rh(+) 1257 (31.9%), Blood group A Rh(-) 198 (5.0%), Blood group O Rh(+) 1149 (29.2%), Blood group O Rh(-) 253 (6.4%), Blood group B Rh(+) 637 (16.2%), Blood group B Rh(-) 109 (2.8%), Blood group AB Rh(+) 280 (7.1%) and Blood group AB Rh(-) 43 (1.1%). Out of the blood group A donors, A1 Rh(+) are 1142 (90.8%), A1 Rh(-) 175 (14.8%), A1 Rh(-) 115 (8.9%), A2 Rh(-) 22 (18.5%). Out of the blood group AB donors, A1B Rh(+) are 171 (61.1%), A1B Rh(-) 39 (90.7%), A2B Rh(+) 109 (28.9%) and A2B Rh(-) 4 (9.3%). The total number of Rh(+) is 3337 (84.7%) and Rh(-) 603 (15.3%). 3313 (84.1%) of blood donors are male and 627 (15.3%) are female.

Conclusion: The most frequent group among the blood donors was Blood group A Rh(+), while Blood group AB Rh- was the least frequent. The distribution of blood groups ABO and Rh-D was given in table 1. The results show the necessity of increasing the number of blood donors of group O Rh(+) and group AB Rh(-). Blood group A Rh(+) was the most frequently found in the age group 20–30 years, Blood group O Rh(+) in age group 10–20 years and group AB Rh(-) in age group 60–70 years.

A 1.23 Evaluate the attitude of inhabitants in six provinces having high prevalence in TTIs
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The most significant goal of IBO is to supply and deliver safe and adequate blood and blood components for use in clinics. The best way ensuing to achieve this goal is to have the non-remunerated voluntary, trained and retained donors as regular donors. The research was designed to observe the society's perception about the blood safety and blood donation, whether or not some at risk individuals refer to blood transfusion centers to give their blood and whether they would be detected as ineligible donors by those centers. The analysis of 9000 participants in this research indicates that only 59% of the population under study were confident of services delivered by IBO. It is worth mentioning that 32% of participants considered that blood donation is the best way to know if they have been infected with hepatitis. Notwithstanding this research, the total of 15% of blood donors have shown to have risk factors leading to their deferral from blood donation. These results show that IBO through cooperation and collaboration with influential cultural centers and institutes, has to do more job on public confidence and has to meet new strategies for getting people more familiar with the services of IBO. Due to missing the donation of blood by the 15% at risk groups with the risk of transmission of some blood-borne diseases during window period at the time of blood donation, IBO has to put more efforts in recruiting, training, and retaining non-remunerated donors.

A 1.24 Profile of blood donor in Congolese rural area
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Background: The prevalence of antibodies VIH and HBS is 4.2% of 1995 to 2002 and that of HBS antigen is 11.8 % from November 2002 to January 2003. The actual preval-
A2 Donor care and bedside processing

A 2.1 Questionnaire on autotransfusion programmes affecting number of blood donors
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Introduction: Autotransfusion programmes involve hope that part of autologous donors will thus augment the numbers of blood donors. The aim of the study is to evaluate the information effectiveness about autotransfusion; to find out what was patients' motivation to undergo autotransfusion; to estimate how many autologous donors would become homologous.

Material and methods: The study comprised 382 autotransfusion patients (298 males). The questionnaire included six questions about the motivation factors to undergo autotransfusion, patients' attitude to blood donation and the haemovigilance. The statistical analysis was based on t-test.

Results: The study revealed that, out of 382 autotransfusion patients, 96 (25.1%) were willing to donate blood regularly. Blood had been donated by 20.4% of the patients. The information obtained from a physician was insufficient for 17.8% of the patients, and 6.8% would not have had autotransfusion if there had not been a risk of a infection. Almost half (48.9%) did not express their opinion about the haemovigilance, leaving this to experts.

Conclusions: The analyses show that autotransfusion programmes might influence positively the number of blood donors. The motivating factor about autotransfusion is mainly the information from a physician. Patients seem to be significantly ignorant about the haemovigilance. There is a need for an educational programme on existing risk factors involved in blood transfusion and the methods to increase its safety.

A 2.2 Adverse effects in blood donors: a study based on staff observation and solicited infos
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Aims: Every study about adverse effects (AEs) in blood donors could potentially be used to ameliorate donor comfort and safety.

Materials and methods: During a half-year period (July 2002-December 2002) a total of 1625 whole-blood donors have been observed during the donation procedure and interviewed approximately 7 days after for AEs. The postdonation interview, by phone, was based on a questionnaire about precise general symptoms and arm problems.

Results: Twenty-one percent of the donors had one or more AEs (17% of men vs. 41% of women). Repeat blood donors and occasional blood donors had fewer AEs than first-time blood donors (17% vs. 17% vs. 39%). The most common arm findings were arm soreness (11.5%), numbness (7%) and hematoma (1.7%). The most common systemic AEs were fatigue (6%) and sleepiness (4.2%). However, only few blood donors had severe acute AEs: nausea and vomiting (0.5%) and vasoagal symptoms (0.2%).

Conclusions: The postdonation interview seems to be an attractive supplementary method for searching AEs in blood donors and we believe that its application is feasible and thus avoid VVR.

A 2.3 Prevention is better than cure: avoiding vaso-vagal reaction in new donors. A psychological approach
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Background: One of the significant factors that determine first-time donors from becoming long-term donors is vasoagal reaction (VVR). The initial donation is a crucial and ticky step and it may produce a mild or strong emotional reaction according to how the mind records the experience. The aims of this research are: (1) to understand why VVR happens during first donation; (2) to find an effective method of preventing VVR.

Materials and methods: This research is based on the belief that psychological associations linked with blood donation are the main cause of VVR. In order to combat this it is necessary to create a close doctor-donor relationship based on attentiveness, acceptance and professional competence. Through only one initial interview prior to donation it is possible to interpret the donor's true needs and activate interior resources that allow the donor to cope.

Results: In the experimental group of 63 donors no one had syncope and only seven exhibited sweating and paleness. In the control group of 35 donors, 27 had syncope, six suffered from vertigo-dizziness, sweating and paleness, and only two experienced no discomfort.

Conclusions: Syncope during blood donation is a physical expression of emotions that can no longer be contained on a psychological level and are therefore transferred on to the body. We have demonstrated that a psychological interview prior to donation provides the donor with sound preparation, allowing them to contain their emotions and thus avoid VVR.

A 2.4 Communicating news of patients' death: a study to identify best practice guidelines
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Aim: In 2002, a study was instigated to implement a literature review and identify the most appropriate method of breaking bad news, with the aim of compiling best practice guidelines.

Method: Following a literature review, an ethically approved questionnaire was conducted to two pilot groups, both consisting of ten donors who had donated stem cells during the last 3 years. Group 1 had been informed of patients' death via a letter, and group 2 via the telephone, followed up with a letter.

Results: Of this pilot study demonstrated that 60% of group 1 would have preferred an initial telephone call and 90% of group 2 preferred the personal touch of the contact. Examples of donors' comments were: "receiving a letter appeared cold, personal touch would be preferable", "a phone call would have helped, I could have asked more questions", "found personal touch reassuring". WBMDR best practice guidelines currently include Stem Cell Donor Managers: (1) promoting continuity of care, (2) undertaking a formal counselling training, (3) break bad news via telephone, (4) assess whether face to face contact is required or offer the option of a follow up telephone call in a few days, and (5) Send a follow up letter confirming the information provided. To date 100 donors have completed the questionnaire and full results will be presented.

A 2.5 Do hemapheresis donors exceed the annual allowed RBC loss?
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Introduction: The collection of blood and blood components is in Germany limited to a maximum donation volume for women up to 1000 ml and for men up to 1500 ml erythrocytes. Despite following the guidelines of the BAK, it is possible to exceed the allowed blood loss in case of multiple donations.

Methods: Donor registration and donation procedure of 40 regular donors at the Institute for Transfusion Medicine, were recorded with the Vista Information System (VISTA™). The final donation volumes are typically composed of (1) draw of blood sample for testing (±18 ml RBC), (2) residual volume of the tubing set with (±30 ml RBC) and without blood-return procedure (±95 ml RBC), and (3) donation type and product volume.

Results: Donations volumes and erythrocyte loss:

| Number of donations/year | RBC loss of tubing set/ml | RBC loss blood samples/ml | No. of incomplete procedures |
|--------------------------|---------------------------|---------------------------|-----------------------------|
| Female donors n = 10     |                           |                           |                             |
| Minimum                  | 17                        | 459                       | 306                         | 0                           |
| Maximum                  | 22                        | 782                       | 396                         | 4                           |
| Mean                     | 19.5                      | 642                       | 351                         | 1                           |
| Male donors n=30         |                           |                           |                             |
| Minimum                  | 15                        | 740                       | 270                         | 0                           |
| Maximum                  | 27                        | 1106                      | 486                         | 3                           |
| Mean                     | 23.2                      | 735                       | 417                         | 0.65                        |

Conclusions: With VISTA™ it is possible for the first time, to obtain a watertight computer aided documentation of donation procedures, with inclusion of the corresponding blood-loss volumes, annual donation volumes and donation time frequencies have to be reconsidered, because 30% of the female donors and 9% of the male donors exceeded the allowed erythrocyte volumes.
A 2.6
Multi-component donations considering the safety of the donor
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2Gambro BCT, Zaaranton, Belgium

Introduction: Multi-component donations (MCDs) are defined by obtaining more and different products from one single donor, using flexible and automated collection procedures.

Material and Methods: Procedure registration was handled with Vistaa, an apheresis management software system and Trimao.

Results: Some of the current apheresis devices succeed in collecting a combination of platelet concentrates (P-C), RBC concentrates (RBC-C) and plasma products (PPs). In contrast with the classical approach, MCD allows considerable increases in flexibility, productivity and an adequate supply of blood products. However, one should consider that not all combinations of MCD are economically attractive. Only collection procedures where at least one P-C is collected seem to be advantageous. MCD challenges more the contrast with the classical approach, MCD allows considerable increases in flexibility, platelet concentrates (P-C), RBC concentrates (RBC-C) and plasma products (PPs). In

A 2.7
Follow-up of donors false-positive in a transfusion microbiology screen assay
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It is now policy in the NBS to write to all donors who are false-positive in any transfusion microbiology screening assay. The letter tells the donor that there is no problem with their health and asks them to give a sample in 6 months time (now changed to 3 months). The previous policy was not contacting the donors but to allow them to continue donation, automatically discard the units and refer a sample for testing. This was considered to be unethical. A recent incident with the syphilis screening assay allowed us to easily assess this method of donor contact. A change in assay meant that in a 6-month period approximately 850 extra false-positives were identified. An increase in the number of ‘follow-up’ samples was seen approximately 6 months after the increase in screen reagents. Unfortunately only an increase of 450 was seen, i.e. approximately half of false-positives appear to have been ‘lost’ to the transfusion service.

A 2.8
Multiple component collections with Cobe Trima: efficiency, safety and donor acceptance
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Trima-automated blood collection system can collect platelets (PLT), plasma and packed red cells (RBC). The aim of this study was to evaluate Cobe Trima in terms of efficiency, donor safety and acceptance. For that reason, healthy donors, preselected with regard to vein quality, completed a collection procedure on Trima. The average blood volume was 5.1 ± 0.6 l, platelet count 304 ± 47 x 1011/l, haematoctrit (HCT) 42.9 ± 2.0%. The following procedure targets were defined: PLT (3-6 x 1011/l), RBC (286 ml), plasma (200 ml). Donor blood counts were performed immediately after the procedure. At the end of each collection, the donors were asked to answer questions designed to evaluate their opinions, side effects and readiness to repeat donation on Trima. Following collections, no significant alterations in donor haematological values were observed, apart from the expected decrease in platelet and HCT values. No serious adverse reactions occurred; minor citrate reactions and vein access problems were observed. Product targets were achieved in 94% of procedures. In answer to our questions, 81% of donors rated their experience as very positive, 12% as positive and 7% were neutral; 94% said they would donate on Trima again, mainly due to the convenient single-needle procedures with short run times. In conclusion, Cobe Trima was found effective in collecting multiple components, as well as safe and accepted by most donors.

A 3.1
HBsAg, anti-HCV, anti-HIV, Treponema pallidium antibodies and anti-CMV in Macedonia blood donors
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Goal: To find the incidence of HBsAg, anti-HCV, anti-HIV, CMV antibodies and Treponema pallidium antibodies in volunteer blood donors in the eastern part of Macedonia.

Material and Methods: In the period 1999–2003 for the presence of HBsAg, anti-HCV, anti-HIV and Treponema pallidium antibodies 13 200 blood donations were tested. In Strip routine testing of donated blood for detection of CMV antibodies is not applied. In the Transfusion department in Stip of Transfusiology in Skopje for detection and confirmation of positive results for HBsAg and anti-HIV in both examined groups ELISA tests from the third generation from the company Organon teknika; for Treponema pallidium antibodies TrepnosticaTM TP-Microelisa system; and for this purpose we used CMV IgG instant test.

Results: From 13 200 blood units the presence of HBsAg at 325 (2.46%) is detected and the presence of anti-HCV at 175 (1.3%) is detected. The presence of anti-HIV and Treponema pallidium antibodies is not detected in any of the blood donations. From 1200 tested blood donations the presence of anti-CMV at 565 (47.08%) is detected. Conclusion: The incidence of HBsAg in the blood donations is 2.46%; of anti-HCV is 1.3%; anti-HIV and Treponema pallidium antibodies is 0% and anti-CMV is from 47.08%.

A 3.2
Prevalence of hepatitis C, B and D infection among patients under chronic haemodialysis
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Objective: To determine the seroprevalence of HCV, HBV HDV infection among patients in haemodialysis centre.

Material and Methods: 64 patients (pts) undergoing haemodialysis. All were transfused with blood. Lab records were used to retrieve the total number of blood transfusions received and serologic study results. The detection of the markers was made by the ELISA technique. Patients with hepatitis B virus were tested for anti-delta antibody.

Results: HCV was confirmed to be present in 20 of pts [31.3%] while HBV was confirmed to be present in 17 of pts [26.6%]. The prevalence of delta infection was 3/17 [17.6%]. Coinfection was found in four of the pts who resulted infected with HBV, HCV and HDV [6.25%] and eight pts presented coinfection with HBV and HCV [12.5%].

Discussion: The prevalence of HBV and HCV infections did not correlate with the age and the sex of the patients and depended on the quantity of transfused blood. Coinfection was found in the pts that had the longest mean duration of haemodialysis therapy. The correlation between the duration of the haemodialysis and the prevalence of the HBV and/or HCV infection suggested nosocomial transmission.

Conclusions: It exists a high incidence of HCV and HBV infection in our dialysis units playing a pathogenic role in liver disease in haemodialysed pts. This high prevalence is related to multiple blood transfusions. Every effort must be made for the successful control of this infection.

A 3.3
Evolution of the incidence of HCV-antibodies in blood collected units between 1995 and 2003
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Aims: The mandatory screening for anti-HCV has been introduced since 1995 for all the collected blood units. The study presents a retrospective evaluation of the incidence of anti-HCV positive blood units.

Materials: The authors analysed the data gathered from the compulsory evidence records of the TTI laboratory.

Methods: All the collected units were tested for anti-HCV using third-generation ELISA tests. The repeatable reactive samples were referred to the TTI National Reference Laboratory for confirmation. There, the samples were tested in all currently used ELISAs;
confirmed reactive samples were tested in immunoblot (Decispan or RBIA 3.0). Positive confirmed samples led to the donor’s deferral and medical counselling. Negative, indeterminate and weak positive results led to the temporary deferral and retesting by EIA+/– immunoblot during the follow-up of minimum 6 months.

Results: There have been screened 125,156 blood units out of which 1552 were repeatable reactive (1.20%). During 1995–1999 the incidence decreased constantly from 2.16 to 0.84%; afterwards the incidence maintained between 0.68 and 0.80%.

Conclusion: The introduction of anti-HCV mandatory testing led to the permanent deferral of a significant number of donors (initial, occasional and regular). After 4 years of mandatory testing, the HCV-Ab incidence was less than 0.80%, determined by positive blood units collected from initial (8%) and occasional donors (11%).

A 3.4 Seeking-test donors have a high risk of transfusion-transmitted viral infections in Brazil

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Background: The residual risk for transfusion-transmitted diseases is high in Brazil, although the incidence of AIDS in general population do not differ from other developed countries. In order to evaluate if seeking-test donors contribute to increase this risk, we conducted a short survey in a regional blood centre.

Methods: After the medical interview, prospective donors were asked if they preferred do not donate but being submitted to the screening for infectious disease. If they chose this option, only the samples were collected.

Results: During the study period, 9024 prospective donors were accepted for donation. From these donors, 65 (0.72%) have had only the tests. Nine out of these 65 (13.8%) presented a reactive test result. There were three positive confirmed results for HIV (4.61%), two for HCV (3.07%) and two for anti-HBc (3.07%) and one for syphilis (1.54%). The global prevalence rate in blood donors was 4.8%; for HIV, this prevalence was 0.15%, for HCV 0.18%, for HBsAg 0.17%, for anti-HBc 2.8% and for syphilis 0.6%. There was a significant difference between the ‘seeking-test’ and ‘non-seeking-test’ donors concerning the general and specific (HIV, HCV, HBV, syphilis) virus prevalences (P < 0.001).

Conclusions: The elevated proportion of high-risk seeking-test donors among the Brazilian donors pool indicates that more sites for anonymous and non-remunerated tests for HIV and other infectious diseases are necessary in Brazil.

A 3.5 Prevalences of transfusion transmissible infection (TTI) markers in donors over a 6-year period

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Objective: To investigate if there are any changes in prevalences of TTI markers in donors.

Material and Methods: The data of HBsAg, anti-HCV, anti-HIV and syphilis (Sy) screenings of 123,778 blood donors who applied to our blood bank in 1998–2003 were evaluated. HBsAg, anti-HCV, anti-HIV screening was performed by ELISA (Abbott-Axsym and Vitros-Ortho-Clinical Diagnostics), and Sy by RPR. For HBsAg and anti-HCV, repeated reactivity was accepted as positive. Anti-HIV was confirmed by immunoblot and Sy by TPHA.

Results: We had no donor with confirmed Sy or HIV infection in this period. The results of HBsAg and anti-HCV screenings are given in percentages in the table.

| Year | n  | HBsAg | Anti-HCV |
|------|----|-------|----------|
| 1998 | 11201 | 3.53 | 0.74 |
| 1999 | 20200 | 3.67 | 0.72 |
| 2000 | 23812 | 2.3 | 0.71 |
| 2001 | 17466 | 2.78 | 0.8 |
| 2002 | 22169 | 3.26 | 0.65 |
| 2003 | 17090 | 2.2 | 0.8 |

Discussion/conclusion: The seroprevalence of anti-HCV is low and didn’t change, but there is a slight decrease in HBsAg (chi-square test P < 0.0001). One explanation could be the education of our blood bank staff and the increased attention in donor selection. The big increase in hepatitis B vaccination in recent years could effect it also, but this opinion have to be investigated further.

A 3.6 Hepatitis C infection in a blood donor population

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Background: Our goal is to determine the prevalence of hepatitis C virus (HCV) among blood donors in our hospital and to identify possible ways of infection.

Materials and Methods: All donors who gave blood at the Hospital S. José between 01 January 1993 and 31 December 2003. Those anti-HCV positive were called to repeat analysis for hepatitis C virus and be submitted to a medical interview to identify risk factors associated with hepatitis C infection.

Results: 82,414 blood donations were studied; 584 (0.07%) were found to be anti-HCV positive. Just 382 blood donors (65.4%) answered our call; 99 (26%) were found to be false-positive; 22 (3.7%) blood donors had a seroconversion since their last donation in our blood bank. There was no identified risk factor in 26.5% of those donors confirmed to be anti-HCV positive; 27.2% underwent a surgery, 22.3% had a history of drug addiction, 10.6% received a blood transfusion before 1990, 3.5% had tattoos or piercings, 5.6% confessed a risky sexual behaviour and 4.2% had a professional or family risk. In most cases of the seroconversion group, we could not identify any risk factor.

Discussion/Conclusion: Today, intravenous drug addiction and high-risk sexual behaviour are the most frequently identified risk factors associated with HCV infection. In our study we observed a large number of cases without risk factors identified: could it be that there are hidden factors relevant to this, or that there are more risk factors than we are currently aware of?

A 3.7 Prevalence of HTLV I/II in blood donor population from Centenario hospital - Rosario (Argentina)

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Objective: The study's aim was to ascertain the prevalence of HTLV I/II in blood donors from the Centenario University Hospital (Rosario).

Materials and Methods: BIOKIT Western blot assay was used for both confirmation and differentiation of HTLV-I and HTLV-II seroreactivities. The possible serological profiles defined were the following: HTLV-I seropositive, HTLV-II seropositive, HTLV-I seronegative and indeterminate.

Results: Confirmatory test was performed on 15 repeatedly reactive samples for HTLV I/II antibodies screening tests (immunoassay) which had been found during the last 12 months (2966 donations). Reactivity pattern is shown in Table 1.

| Donor | WB BIOKIT | BİOKIT HTLV type |
|-------|-----------|------------------|
| K0339 | rgg46(I), rgg46(II), p53, p36, p24, p19, rpg21 | I/II |
| K0645 | rgg46(I), rgg46(II), p53, p36, p24, p19, rpg21 | I/II |
| K0712 | rgg46(I), rgg46(II), p36, p24, p19, rpg21 | I/II |
| K0737 | rgg46(I), p36, p24,p19, rpg21 | II |
| K0765 | rgg46(I), rgg46(II), p53, p19, rpg21 | I/II |
| K0854 | rgg46(I), rgg46(II), p36 | Indeterminate |
| K0904 | rgg46(I), p36, p24,p19, rpg21 | I |
| K1568 | rgg46(I), p24,p19, rpg21 | I |
| K1071 | rgg46(I), rgg46(II), p36, p24,p19, rpg21 | I/II |
| K1265 | p19, p36 | Indeterminate |
| K0046 | rgg46(I), p53, p36, p24, rpg21 | II |
| K0003 | rgg46(I), rgg46(II), p36,p19, rpg21 | I/II |
| K0646 | rgg46(I), rgg46(II), p19, rpg21 | Indeterminate |
| K1015 | p36, p19, rpg21, | Indeterminate |
| K1110 | rpg21 | Indeterminate |

Six donors were typed as HTLV III, two as HTLV II, two as HTLV I and five resulted indeterminate.

Conclusion: We have established for our donor population an HTLV prevalence of 0.38%.

A 3.8 Serorepidemiology of cytomegalovirus and hepatitis B virus in blood donors of young adults

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Aim: The prevalence of CMV and HBV infection in blood donors of age below 35 years.

© ISBT 2004 Blackwell Publishing Ltd. Vox Sanguinis (2004) 87 (Suppl. 3), S93–S145
Material and Methods: The blood samples were collected at the time of blood donation. Tests were done by Abbott Asym system.

Results and Discussion: Blood samples were collected from donors between the ages of 17 and 35. Donations from this group accounted for more than 80% of whole donations. We had 134 samples from first donors and 35 samples from repeat donors. 114 first donors (85.1%) and 310 repeat donors (87.3%) were CMV IgG reactive. CMV could cause serious morbidity and mortality in premature infants and immunocompromised patients. It was reported that CMV infection incidence was about 17% for children if the transfused seropositive blood has been stored for more than 24 h [J Med Virol, 1992]. As CMV-screened blood components are not available in blood centres here, leucocyte-depleted blood components which can significantly reduce post-transfusion CMV are recommended for those patients. For first donors, anti-HBs and HBSAg seropositive rates were 64.3% and 9%, respectively. For repeat donors, 70.4% of them were anti-HBs positive and no one was HBsAg reactive. The HBsAg-seropositive rate of first donors of this group was not significantly different from that of first donors of all ages. Whereas, the prevalence of HBV infection in this group is around 21% and it is lower than the announced data that 90% of adults were infected by HBV and 15–20% were carriers in Taiwan.

A 3.9

NAT screening on blood donations in Italy: results of two years of experience
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Background: The use of NAT technology to screen blood donors became mandatory in Italy in July 2001, but the methodology had been introduced in a trial form during the previous year. In the same period, an EIA test to detect HCV-coreAg had also been introduced. The study was focused to collect epidemiological data on new direct HCV markers and to review the transfusion-transmitted viral residual risk.

Materials and Methods: ‘Italian Group for the Study of Transfusion-transmissible Diseases’ collected data from 219 transfusion services (TSL) relating to the years 2001 and 2002. Data were collected for HCV overall on 2 756 734 units of blood components: 1 906 659 of them were examined with NAT techniques (Roche Diagnostics and Ortho). 888 506 units were tested also for HIV RNA.

Results: 6/1 906 659 cases were found only NAT positive for HCV RNA (incidence = 3.1/101) and 1/888 506 case for HIV RNA (incidence = 1.1/101).

Discussion: Considering that in Italy, 2.5 million of haemocomponents are collected per year the introduction of NAT testing allows 10 units potentially infectious for HCV or HIV to be identified in 1 year and reduces the residual risk of transmitting HCV or HIV via transfusion to 0.5 and 1 units per million, respectively. In addition there was an initial reorganisation of biological validation of blood units due to centralisation of NAT testing in a smaller number of TS.

A 3.10

Fight against HIV transmission by blood safety in a difficult context, in Congo Democratic (DRC)
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Background: In Congo Democratic, the context of wars in which the country lived increased vulnerability of the population to the propagation of the HIV. A project was implemented to fight against HIV transmission.

Aims: To contribute to the reduction of HIV transmission by the blood transfusion and to improve the accessibility of population to a safe blood.

Implementation: With the World Bank funds, the project was carried out from January 2002 to January 2003 by GTZ and the Health Ministry. The interventions were focused on: providing the blood safety supplies, recruitment of voluntary blood donors and training.

Results: The capacity of the CNTS was reinforced to make it able to ensure the coordination of blood safety activities, production and storage of safe blood products. Blood safety supplies were provided to cover 135 000 safe blood transfusions. This increased by one third the number able to give safe blood to the patients from 13 to 40%. The proportion of the voluntary donors in the country increased from 8.2 to 26%. The HIV seroprevalence in blood donors was 5.0%. The financial accessibility of the population to the safe blood was improved.

Conclusion: The improvement of the quality of the transfusions by the intervention of the project allowed the prevention of 6615 HIV blood transfusion infection.

A 3.11

Parovirus B19 – serological status of plasma donors
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Aim: Parovirus B19 (B19) is a human-specific, single-stranded, non-enveloped DNA virus. As a result of the relative stability of virus protein shell, transmission via blood products is also possible. Current inactivation methods fail to kill non-enveloped viruses, even though plasma donor units are not screened for B19. B19 replicates primarily in erythrocytic precursor cells, causing cell lysis. Depending on the immunologic competence, more or less pronounced anaemia can occur. Virus multiplication is limited because of the formation of specific antibodies. Therefore the infection causes no serious effects. Patients with chronic haemolytic anemias, immunocompromised persons and pregnant woman are group of risk. Epidemiology studies have shown that 50–80% of adults have immunity. We estimated the prevalence of B19 in plasma donor population and determined the clinical outcomes of transfusion recipients.

Materials and Methods: Donor units were screened for VP2 IgG antibody by ELISA Ab test and for IgM by m-capture test, twice in period of 2 weeks.

Results:

| IgG | IgM |
|-----|-----|
| neg | lgG |
| poz | lgM |
| < 1 i.U./ml | > cut off | < cut off |
| < 1 i.U./ml | > 0 | 0 |
| 0 | After 2 weeks | 90 (100%) | 0 |

Conclusion: The high prevalence (81%) of donors with only IgG antibody indicates an infection in the past and probably aviremic state. 19% of donors have not been in contact with B19, they have not produced neither IgG nor IgM. Plasma products contain adequate concentration of neutralising antibodies.

A 3.12

Prevalence of viral markers for hepatitis viruses among blood donors in Slovenia
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Introduction: The aim of the study was to present data on the prevalence of hepatitis viruses among blood donors tested at the Blood Transfusion Centre of Slovenia in the period between 1993 and 2002.

Methods: Every blood donation is tested for HBsAg and anti-HCV using EIA. NAT testing of mini-pools for HCV RNA was implemented in March 2000. Viral markers for hepatitis A virus (HAV) and hepatitis E virus (HEV) as well as other HBV markers (anti-HBs and anti-HBc) were determined on a selected number of blood donors.

Results: 512 472 blood donations were tested for anti-HCV. 147 (0.028 or 0.12%) of first-time donors) anti-HCV positive donors were detected. 66.6% of these were found to have HCV virus present. 315 298 donations were tested for HbsAg, 174 (0.035 or 0.39%) of first-time donors were found to be HbsAg positive. 1000 donors were tested for HAV and HEV. Positive results were obtained in 553 (5.3%) and 5 (5%) cases, respectively. 2000 HbsAg negative donors were tested for anti-HBs and anti-HBc. 25 (2.5%) were found to be anti-HBs only positive and 1 (1.1%) anti-HBc only positive. 27 (0.2%) donors were positive for anti-HBs, as well as for anti-HBc.

Conclusions: The results of this study rank Slovenia among regions with a low prevalence of HBV and HCV in the blood donor population. The prevalence of HAV in the same group proved to be within the expected limits. The prevalence of HEV was found to be a bit above the values expected for the Central European region.

A 3.13

The prevalence of hepatitis B markers in volunteer blood donors in different regions in Iran
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Introduction: Iran is a country with an intermediate prevalence of hepatitis B virus (HBV) infection. HBsAg as the first viral marker and anti-HBc as past or current infection marker of HBV infection can be detected. HBsAb is evidence of immunity to HBV due to natural infection or successful vaccination. The anti-HBc and HBsAb usually persist for life. Epidemiology studies have shown that 65–70% of adults have immunity. We estimated the prevalence of HBV in the blood donor population and determined the clinical outcomes of transfusion recipients.

Materials and Methods: 450, 400, 422 and 425 HbsAg-negative blood donors from four different cities including Mashad (northeast), Tabriz (northwest), Esfahan (center) and Zahedan (southeast) of Iran, respectively, were tested for anti-HBs and HbsAb by approved assay.
Results: The anti-HBC prevalence in Masaud, Tabriz, Esfahan and Zahedan were S.1, 3, 1.18 and 11.52% and the HBsAb prevalence in anti-HBc positive blood donors estimated 43.4, 16.6, 0 and 46.9%, respectively. The HBsAb prevalence in blood donors estimated 7.1, 8.25, 5.45, and 14.11, respectively.

Discussion: HBV infection prevalence in different parts in Iran is dissimilar (HBsAg: 0.98, 0.92, 0.58 and 1.25, respectively). These results illustrate that significant difference in prevalence and epidemiology of current or past HBV infection exists among different regions of Iran and related to geographical and social differences and migration status from other countries.

A 3.14
Knowledge on HCV/HBV in blood donors with currently diagnosed hepatitis B or C infection
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Background: HCV or HBV infection may cause little symptoms in infected persons. As early diagnosis is beneficial for therapy and prevention of viral transmission, accidental diagnosis through blood donation may be of advantage.

Methods/Results: We investigated the outcome of blood donors who have been diagnosed being HCV or HBV PCR positive in 2002 by a questionnaire. Analysis of responding donors showed that most of the HBV/HCV positive donors describe their health status as being good or very good. Most donors knew that hepatitis B or C can be transmitted though blood transfusion (24/30), sex (24/30) or i.v. drug abuse (16/30). Although blood transfusion is no longer a frequent way of infection, it is still the most known way of HCV/HBV transmission. One of our donors thought, HBV/HCV could be transmitted by handshake, and three out of 30 donors thought using public toilets could transmit Hepatitis B or C. Only 25 out of our 30 HCV/HBV positive donors did tell their physician about their Hepatitis B or C. This is probably the main reason why only few donors got sonography (12/30), determination of viral load (18/30) and determination of ALT (22/30) after diagnosis. Only five donors felt well informed about their disease.

Conclusion: The lack of knowledge about the way hepatitis B or C can be transmitted and about possible specific therapy is very unsatisfying from an epidemiological point of view. Donor information and co-operation with physicians has to be intensified.

A 3.15
Epidemiology characteristics of HIV prevalence – new diagnostic approach
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Objective: To point out significant differences in HIV prevalence between different population categories.

Methods: Retrospective analysis of all HIV tests performed at the transfusion unit of the general hospital within the 3-year period. Initial tests and repeated tests were performed with BioMerieux (Organon), Boikti and Ortho. All RR samples were sent to the referent laboratory for confirmation.

Results: In this study, we implemented three counties, with an estimated population of 222 000. During the mentioned period, a total of 25 966 samples were tested for the HIV antibodies. All samples were divided into three categories: (1) Low-risk population-blood donors. (2) Moderate-risk population-patients from all departments from the general hospital. (3) High-risk population-person who come once per year, on the 1 of December when is traditionally HIV testing anonymous and free of charge. In the general hospital, (1) Low-risk population (2) Moderate-risk population-blood donors. (3) High-risk population-person who come once per year, on the 1 of December when is traditionally HIV testing anonymous and free of charge. In the general hospital.

Discussion: Encouraging fact is that we don’t have a registered HIV positive VBD in our municipality. The reducing and the absence of the percentage of the positive VBD of the transmissive disease is an result of the good selection of the donors, absence of family donations and the obligatory fulfilment of the questionnaire of self-elimation which is performed in our institution during the last years. But, there is still necessity of introducing SOP for the service and implementation of the national informative system as only way to secure transfusion.

A 3.17
Long-term surveillance on viral diseases markers in a large blood transfusion centre in Greece
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The aim of this study is to present a summary of serological screening tests for the transfusion transmitted viruses HBV (1991–2001, n = 567 762), HCV (1992–2001, n = 528 577), HIV (1986–2002, n = 825 793). The study population consisted from military donors, recruits and enlisted personnel, replacement and regular donors.

Methods: EIA third generation was used for HBV, HCV and HIV testing.

Results HBV: The overall prevalence rates in military recruits [0.61%, 803/131 430] are lower from those of the enlisted military personnel [0.69%, 198/183 200] and both are also lower from replacement donors [0.76%, 1201/158 606]. An overall significantly lower prevalence (0.28%, 268/94 626) in regular volunteer blood donors was detected.

HIV: A homogeneously distributed moderate prevalence (EIA+, Ribase+ and indeterminate) was revealed in recruits [0.26%, 306/116 966] and enlisted personnel [0.28%, 493/172 817] which both are significantly lower from replacement donors [0.42%, 626/144 850]. An overall significantly lower prevalence was detected [0.23%, 212/89 934] in regular donors.

HCV: The overall prevalence rates was: recruits [0.014%, 30/207 471], enlisted [0.0099%, 27/282 679], replacement [0.016%, 35/212 616] and regular donors [0.0065%, 8/123 027].

Discussion: Amelioration of living conditions and consistent preventative behaviour of the population at large with regard to HBV and HIV transmission interpret our findings. HBV seroprevalence remained constant within the sporadic and regular donors.

A 3.18
Prevalence of HCV infection and related risk factors among patients on haemodialysis in Qazvin
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Background: Hepatitis C virus (HCV) infection is common among patients undergoing haemodialysis, and liver disease is an important cause of morbidity and mortality in this population. Management of HCV-related liver disease is a major health concern in patients with end-stage renal disease (ESRD) undergoing haemodialysis.

Objective: We conducted this study to investigate the prevalence of HCV infection and associated risk factors among patients on haemodialysis in Qazvin province.

Methods: In this series study 68 patients on haemodialysis in Qazvin were selected randomly and all were checked for anti-HCV antibodies, using ELISA second and confirmed using RIBA second.

Findings: 16 patients (23.9%) were infected. Patients’ sex, educational level, history of upper GI endoscopy and previous renal transplantation had no impact on HCV infection rate. Blood transfusion was an important risk factor for HCV infection (P = 0.02). The more units transfused, the greater the rate of HCV infection (P = 0.03). Moreover, mean time of haemodialysis was significantly longer in HCV Ab positive cases (P = 0.07).

Conclusion: It seems that early transplantation and avoidance of blood transfusion as much as possible, for example by using erythropoetin, are the two most important practical interventions to reduce HCV exposure rate in patients rate in patients on haemodialysis.

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A 3.19
HIV seroprevalence in Albania blood donors
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Background: The aim of this retrospective study is to assess the seroprevalence of HIV in blood donors between 1993 and 2003 in Albania.

Material and Methods: Blood transfusion centre report every year the number of donations and number of seropositive donors for anti-HIV. For each one, type of donors, date of positive donation and previous negative donation, sex, age, etc. are collected. 191 489 samples are tested for anti-HIV by ELISA (ABBOTT third generation) and confirmed with WB method. Chi-square tests were used for statistical analysis.

Result: The prevalence of anti-HIV in blood donors is 0.1%. To compare between them paid blood donors (PD) and unpaid blood donors (UPD) the anti-HIV prevalence were, respectively, 0.07 and 0.14%, \(P < 0.005\). Among 191 489 donations between 1993 and 2003, 20 donors were found to be anti-HIV positive. Among them 12 were from PD and eight are from UPD (seven are family donors and one voluntary blood donor). According to sex from positive donors 19 are men and one is female. The age of positive donors ranged from 24 to 40. Over the 10 years period, the prevalence was variable. So, the prevalence was increased from 0.09% in 1993 to 0.11%, in 1996 and decreased in zero in 1999, but increased in 0.65%, in 2000 and decreased again in 2003.

Conclusion: The prevalence of anti-HIV in blood donors is low. The prevalence was higher in unpaid blood donors than regular blood donors (which are PD). Among seropositive donors most of them are men.

A 3.20
Seroprevalence of human herpesvirus 8 in healthy blood donors
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Background: The seroprevalence of human herpesvirus 8 (HHV-8) is increased in groups at risk for Kaposis’ Sarcoma. The reported seroprevalence of HHV-8 in healthy blood donor population ranged from 0 to 29%. To determine the HHV-8 seroprevalence in our blood donors we tested 857 specimens that were negative for the mandated transfusion-transmitted viruses (HIV, HCV, HBV and HTLV).

Methods: Sera from 857 blood donors were tested using the HHV-8 latent antibody ELISA designed to detect IgG antibody to latent protein ORF-73 (ORF) [Advanced Biotechnologies, Inc., Columbia, MD]. Testing results were correlated with the donor’s epidemiological data.

Result: Of the 857 donors 437 (51%) were males; 638 (74.4%) were white, 219 (25.6%) black or oriental; age ranged from 17 to 87 (average 40, median 40). HHV-8 serology was positive in 52 of the donors (6.1%, 95% CI: 0.04-0.07). Of the HHV-8 seropositive group 31 (59.6%) were males; 36 (69.2%) were white, 16 (30.8%) black; age ranged from 17 to 71 (average 39.5, median 41). There were no significant differences in gender (\(P = 0.5\)), race (\(P = 0.2\)), or age distribution (\(P = 0.07\)) between seropositive and seronegative donors.

Conclusion: In our healthy blood donor population, the seroprevalence of HHV-8 is 6.1% using a HHV-8 latent antibody assay. No significant associations of HHV-8 seropositivity with gender, age or race were found.

A 3.21
Incidence of HBV infection among young students in Greece
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Aim: The aim of this study is to estimate the HBV incidence in the young population from schools and universities and to determine the effect of immigration on HBV prevalence within this group.

Materials and Methods: From January 2002 to December 2003, blood samples were collected from technical schools and universities. Samples were tested for HbsAg using Abbott AxSYM. From September 2003 to December 2003, samples were also tested for HbsAb and HbcAb, using AxSYM AUSAB and Core kits.

Result: From 2002 to 2003, 5014 blood donations were collected from schools and universities. Among students samples, 20 were positive for HbsAg (0.56%). 75% were immigrants, mainly from Albania. Among university students, only one was positive for HBV. From 449 blood samples, six samples tested positive for HbsAg and 28 samples were positive for HbcAb, 85.7% were immigrants, mainly from Albania. 50.2% of the students had no protective HbsAbs, rendering them at a potential risk of infection.

49.2% of this group showed protective immunity against HBV. Among university students, only one sample was positive for HbsAg and three were positive for HbcAb. 68.5% of university students showed protective immunity against HBV.

Conclusion: Technical school students showed increased HBV prevalence as well as low protective immunity against HBV infection, mainly due to the growing number of immigrants. An increasing effort must be undertaken in order to raise awareness within the young population towards viral infections.

A 3.22
Investigation of acute hepatitis B virus infection in Scottish blood donors
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Background: An increase in the number of southeast Scottish blood donors with apparent acute hepatitis B virus infections was noted at the end of 2002.

Aim: Lothian Health Board Public Health Department requested an investigation into these cases since the donors did not report any risk factors on counselling and were from the same geographical area.

Methods: HBV DNA was amplified by PCR using primers in the preS1 gene. Three recent HBV positive cases living in the same area and also from a randomly selected cohort of Scottish blood donors who had previously been shown to be HBV PCR positive were examined. Phylogenetic sequence analysis was carried out and comparison made with the Scottish cohort and with published sequences.

Result: HBV DNA sequences from the three donors grouped in separate clades but along with other sequences from Scottish blood donors from Edinburgh and Glasgow.

Conclusion: The sequencing results obtained established that the HBV infection in the three donors was not from a common source and were not related to each other. The study demonstrates the value of molecular biological techniques in examining routes of viral transmission.

A 3.23
Risk factors in anti-HCV ELISA positive blood donors
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Objectives: The presence of risk factors for HCV infection in voluntary blood donors who test repeatedly reactive (rr) in HCV antibody tests is relatively unknown. We evaluated the frequency of risk factors in donors testing rr in the anti-HCV ELISA.

Methods: All donations with rr results in the anti-HCV ELISA (Ortho Diagnostics) were tested in the anti-HCV immunoblot (RIBA II). Donors testing RIBA –ve in subsequent donations or with ind or +ve reactivities in the RIBA were recalled to the blood bank to collect fresh blood samples (anti-HCV ELISA, RIBA and cDNA-PCR test) and to obtain a standardised interview.

Result: In a 3-year period (1995-1997), 255 of 254 000 donations (0.1%) tested rr in HCV antibody tests. We evaluated the frequency of risk factors in donors testing rr in the anti-HCV ELISA.

Conclusion: The sequencing results obtained established that the HBV infection in the three donors was not from a common source and were not related to each other. The study demonstrates the value of molecular biological techniques in examining routes of viral transmission.

A 3.24
Emerging viruses – yet another threat to blood transfusion?
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Background: Anonymous archived of non-remunerated blood donors for epidemiological screening are rarely collected by blood banks. Even when blood samples are stored it is often the lack of stored cells that has hampered attempts to establish the
frequency of active infection with cell-associated viruses, such as human herpes virus 8 (HHV-8). The SNBTS is collecting samples to enable a quick response to the discovery of transfusion-associated risk of novel parentally transmissible agents and for making realistic assessments of the frequency of transmission of known viruses for which screening is not carried out.

Methods and Results: Plasma, cells and DNA have been collected from anonymous blood donors. Species-specific real-time PCR assays and other serological and molecular techniques have been used to estimate the frequency of HHV-8 (0.5%), Borna virus (0%), human parvovirus B19 (0.07%) and TTV/TTVM (8%) in the blood donor population.

Discussion: New molecular techniques are likely to lead to the discovery of new human viruses, many of which may exist in a commensal relationship with their host. The ability to screen and explore their epidemiology at an early stage will provide valuable information in assessing the threat that they may pose for the safety of blood transfusion. Viruses with no obvious clinical significance for blood donors such as B19 and TTV can be assessed without the unnecessary potential identification of actively infected individuals.

A 3.25
A simple, rapid and low-cost assay for HCV sero-epidemiology
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Objective: To develop a rapid, inexpensive and sensitive method for anonymised HCV sero-epidemiology.

Materials and Methods: A commercially available HCV gelatin particle agglutination test (GPA) was modified by using diluted particles and centrifuging the test plate (MGPA). A positive reaction was shown by a distinct button of agglutinated particles and a negative reaction as a streak of unagglutinated particles. Total test time was 30 min. For validation:

• Kit positive and negative controls were titrated in parallel using the modified and standard test procedure.
• 416 HCV ELISA negative samples were screened using the modified technique.
• 12 confirmed HCV ELISA and RIBA positive sera were titrated in parallel on three different days to test reproducibility.
• 35 sera, confirmed anti-HCV positive, were titrated in parallel using the modified and standard methods.

Results: Of the 416 serum samples screened, 413 were screened negative and three screened positive. All confirmed positive samples were positive by GPA and MGPA and titres by MGPA were reproducible within one dilution factor. The parallel titrations showed that the modified method was more sensitive than the standard.

Conclusions: The modified method is specific, rapid and more sensitive than the standard test procedure and reduces the cost of each test by 90%. Further work is planned to investigate the distribution of positive titres and analyse samples reported as indeterminate.

A 3.26
Parvovirus B19: antigen or genome research for screening in blood donors?
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Parvovirus B19 (B19), an isocaudal, non-enveloped, single-stranded DNA virus replicates in active dividing cells such as erythroid precursors. B19 can be transmitted by transplacental passage, by respiratory route and by transfusion (because its resistant to viral inactivation procedures). 4656 blood donors were screened for B19 antigen research, by a haemagglutination assay. Positive samples were tested for viral genome research by polymerase chain reaction (PCR). 37 donors (0.79%) resulted reactive for B19 antigen, but only six of these (16.2%) were also positive at PCR. Our results suggest that the haemagglutination test has not a sufficient specificity; on the contrary, the contemporary positivity for DNA and antigen allow us to identify subjects affected with acute infection. Considering all donors, the acute infection had a frequency of 0.12% (=equal to 1:776 donations). Nevertheless it’s reported that B19 infection is common (5-50% of general population shows anti-B19 antibodies), even if its frequency in the infectious disease phase is lower, therefore an economical evaluation of the test usable for screening is required. Although parvovirus infections are usually benign and self-limiting, symptomatic forms may be observed and it’s important avoiding the viral transmission to pregnant woman, thalassemic or immunocompromised patients. In our opinion, a B19 antigen test could be proposed for a rapid, easy and not expensive screening, supported by PCR only on positive units.

A 3.27
Prevalence of hepatitis B virus in blood donors
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Background: The aim of this study is to determine the prevalence of HBsAg in blood donors.

Materials and Methods: In this study are tested 8015 donors. From them 23.34% (1781 donors) have been voluntary blood donors (VDBs), 73.99% (5929) family replacement blood donors (FDBs) and 2.68% (215) first-time blood donors (FTDBs). The samples are tested in NBTS for HBsAg by ELISA method (ABBOTT third generation). The data are collected from individual sheet. Chi-square tests are used for statistical analysis.

The result: The prevalence of HBsAg in blood donors is 7.5%. In voluntary donor the HBsAg prevalence is 6.9%, FDB 7.6%, FTDBs 9.9%(P > 0.10). Over the 3-year periods in voluntary donors the HBsAg prevalence ranged from 4.6 to 9%, in family replacement donors from 6.9 to 8%, first-time donors from 3.1 to 14.2%. According to sex the HBsAg prevalence in men is 8.8% and women 4.6%(P < 0.005). Over the 3-year periods the prevalence in men has a decline tendency (9.7–8.3%) and increasing tendency in women (3.5–5.3%). According to age group the HBsAg is 11.4% in 17–29 age group, 8.2% in 30–39 age group, 6% in 40–49 age group, 4.6% in 50–60 age group.

Conclusion: The higher HBsAg prevalence in blood donors explain with higher prevalence in general population. The decreasing HBsAg in blood donors explain with improved blood donors screening and selection method. Considering the quality of blood we always recommend for voluntary blood donations.

A 3.28
The risk of transfusion transmissible viral infections in Nigeria
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Background: This study was undertaken to determine the risk of transfusion transmissible viral infections through transfusion of unscreened blood and blood products.

Materials and Methods: Human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (anti-HCVC) screening was performed on 1500 consecutive blood donors.

Results: Of the 1500 sera tested, 17/1500 (1%) and 7/1500 (0.5%) of donors had HIV, HBsAg and anti-HCVC, respectively. 12/1500 (0.8%) had HIV infection, 2/1500 (0.1%) had HIV-2 while 1/1500 (0.07%) had dual HIV 1 and 2 infection. 2/1500 (0.1%) had co-infection of HIV and HCV. Male accounted for the highest infection burden for HIV 17/1481 (1.1%) and anti-HCVC 7/1481 (0.5%) while female donors had the highest HIV prevalence 1/19 (5.3%). Commercial remunerated donors showed the highest HIV HBsAg and anti-HCVC infection rates 9/561 (1.4%), 11/561 (1.7%) and 5/561 (0.8%), respectively.

Conclusion: This study confirms a high prevalence of transfusion transmissible viral infections among blood donors. This calls for the immediate implementation of a mandatory universal donor screening policy for the exclusion of blood donors with surrogate markers for HIV, HBsAg and HCV infection, the setting up of a national blood transfusion service, run on the basis of voluntary non remunerated, low risk blood donors.

A 3.29
Correlation between anti-cytomegalovirus IgM antibody and HIV positivity
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Iranian Blood Transfusion Research Center, Immunology Lab and Tarbiat Modarres University

About 50% of the general population and 90% of people infected with HIV carry CMV. In order CMV can attack several parts of the body in patients with weakened immune defence by HIV or another diseases, we evaluated IgM, IgG anti-CMV antibodies against CMV in HIV patients. We detected serumic IgM, IgG anti-CMV antibodies by enzyme immunoassay method (ELISA) in 30 HIV positive patients included 24 (80%) males and 6 (20%) females, aged between 20 and 53; 23 (76.6%) of them (all male) were drug addicted and 30 healthy blood donor HIV negative as control included 24 (80%) males and 6 (20%) females aged between 18 and 55 were selected. IgM anti-CMV antibody was presented in 7 (23.3%) patients, one of them
(3.3%) was borderline (doubtful) and 22 (73.4%) were negative. IgM anti-CMV antibody was detected in all the patients and controls. IgM antibody was not detected in controls. Our results showed a significant difference \( P = 0.00 \) in IgM anti-CMV antibody between patients and controls. There was a correlation between IgM anti-CMV antibodies and HIV positivity (\( P = 0.074, P = 0.00 \)). CMV is an important co-infection in patients with HIV infections. As CMV disease can damage many parts of the body, including GI tract, lungs and eyes, if it not treated; so detection of CMV antibody appears to be necessary in HIV positive patients.

A 3.30 Heterogeneous distribution of HTLV-I/II prevalence rates in blood donors from urban areas in Brazil
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HTLV-I/II is highly prevalent in Brazil and screening of blood donors for HTLV-I/II is mandatory in the country since 1993. The public haemocentre network is the largest and most important system of blood collection and the procedures are uniform, following guidelines of the Ministry of Health. An ecological study was conducted to determine the geographical distribution of HTLV-I/II serologic screening prevalence rates in blood donors from large urban areas, from 1995 to 2000.

Methods: Our data refer to the largest urban areas in each State (n = 27). About half of the Brazilian population live in these metropolitan areas. After passing predonation screening questionnaire and clinical examination, eligible donors are tested for HTLV-I/II, HIV-1/2, HBV, HCV, T. cruzi and T. pallidum. In Brazil, law forbids any reimbursement for blood donation. For HTLV-I/II, a donor is considered seropositive if his/her serum sample is positive on EIA and confirmed by Western blot.

Results and Conclusion: From 1995 to 2000, 6 218 619 donors were EIA tested. There was important geographic heterogeneity between the mean HTLV-I/II prevalence rates, ranging from 0.4/1000 in South to a rate 25 times higher, 10.0/1000 in the Northeast. On average, the EIA prevalence rates are lower in the South with an increasing trend towards the North and Northeast. Further studies are needed to better understand the reason for the spatial heterogeneity of HTLV-I/II seroprevalence among blood donors in Brazil.

A4 Viral diagnostics

A 4.2 Detection of hepatitis B virus DNA (PCR) in HBsAg negative blood donors
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Iranian Blood Transfusion Organization (IBTO)

The incidence of post-transfusion hepatitis has been reduced by blood donor screening for hepatitis B surface antigen (HBsAg), but the HBV infection is still responsible for a certain cases of post-transfusion hepatitis. HBV DNA screening of blood donors would be able to reduce the preseroconversion window period. An extra sample was collected from 1000 HBsAg, anti-HCV, anti-HIV and RPR negative and three HBsAg positive blood donors. Every four samples were pooled and HBV DNA detected by PCR method. The sensitivity of the assay was estimated 300 geq/ml according to VQC proficiency panel. The specificity of the primer was characterised by alignment with Blast program. HBV DNA (PCR) was detected in 7 out of 1000 HBsAg negative and 3 HBsAg positive samples. Three out of seven were positive and four of them were negative for anti-HBc. All three anti-HBc positive and two anti-HBc negative donors were recalled after 1 year and they were negative for HBV DNA, HBsAg and anti-HBc. Two other anti-HBc negative donors were HBsAg negative after 6 months. So HBV infection was excluded in 1000 HBsAg negative blood donors. Iran is a country with an intermediate prevalence of hepatitis B virus (HBV) infection. With considering the lab design, test procedure and repeatedly positive samples, contamination in the lab (in this study) was lower than contamination in the sample collection step. Follow-up the HBsAg negative and HBV DNA (PCR) positive blood donors and sample collection precaution strongly recommended.

A 4.3 Evaluation of an HCVcoreAg test system in a blood donor and individual patient population
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Aim: To evaluate the sensitivity and specificity of an HCVcoreAg ELISA test system among a healthy blood donor population, specimens from individual patients and a seroconversion panel of five samples given by ORTHO.

Materials and Methods: 15 109 blood donations and 1240 individual patient samples were tested between April 2002 and December 2003 for the presence of HCVcore antigen using the ORTHO antibody to HCVcore Ag ELISA test system.

Results: Among donor and patient population we detected 15 IR (initially reactive) samples. 7/15 (0.04%) samples were RR (repeatedly reactive) giving a specificity of 99.96%. When the neutralising HCVcoreAg test was performed, 3/7 RR samples were confirmed. These samples were also found to be reactive for anti-HCV antibodies. We were able to follow-up 2/3 confirmed samples (one donor and one patient sample) during the early seroconversion phase until HCVcoreAg be completely undetectable. The blood donor HCVcoreAg appearance was consistently declined during 2 months period (three follow-up samples) until it became completely undetectable. Regarding the patient sample, disappearance of detectable HCVcoreAg was observed after 4 months (four follow-up samples). The seroconversion panel investigation showed a high sensitivity rate for low viral load samples compared to the reference molecular technology.

Conclusion: The HCVcoreAg test (ORTHO) showed a high specificity and sensitivity and it could be suitable for routine testing of blood donations.

A 4.4 Evaluation of the Roche COBAS Ampliprep HIV-1 PCR assay: mini-pools versus single testing
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Introduction: South Africa has a high, growing HIV prevalence. NAT testing has not yet been implemented in South Africa due to the high cost. The aim of this study was to determine whether, using current screening technology, sample pooling could reduce costs while maintaining sensitivity.

Materials and Methods: Four HIV seropositive donors where tested by a quantitative PCR to determine viral loads. These were diluted with negative pooled plasma to obtain viral loads from 25 to 1000 copies/ml. Each dilution was tested using a standard single test with Ampliscript HIV-1 (ST), made into a pool of 5 (SP) and 16 (16P) with negative plasma and tested using the ultrasensitive Ampliprep Two commercially available seroconversion panels were also included and tested neat as ST, 5P and 16P. Viral load was determined, using a quantitative PCR, on an FFP banked unit from a donor who subsequently tested positive for HIV antibodies. This sample was then tested using ST, 5P and 16P.
We could demonstrate that the new method yielded comparable or better results than the old method. The new method is cost-effective, because we have shown that the new method is as sensitive as single sample testing. In addition, the new method is more convenient, because there is no longer a need for manually transferring the supernatant from the pool precentrifugation. Second, the new method may improve safety, because there is no longer a need for manually transferring the supernatant from the pool precentrifugation. Third, the new method is more accurate, because there is no longer a need for manually transferring the supernatant from the pool precentrifugation. (3) All indeterminate SIA results with NS5 band show a clear negative PCR result.

Conclusion: A pool of five with input volume of 200 ml per individual sample followed by ultracentrifugation is at least as sensitive as single sample testing. A pool of 16 with input volume of 67 ml per sample followed by ultracentrifugation is not as sensitive as single sample testing.

A 4.5 Correlational study between SIA HCV and HCV RNA PCR among selected donor sample in NBC, Malaysia
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Screening for HCV was first performed at The National Blood Centre in 1990. Enzyme immunoassay (EIA) kits was used as the first line screening and all samples that were repeatedly reactive on EIA were then tested on strip immunoblot assay (SIA) to determine the presence of true antibody to HCV. The objective of this study was to correlate the SIA results with HCV RNA PCR, where all samples that were tested on SIA were also tested using PCR for HCV RNA detection. This is to assist in providing a clearer picture of the HCV status among these samples. A total of 675 samples were included in this study.

Results: (1) From a total of 279 samples with RIBA-3 positive result, 251 (90%) were PCR positive. (2) Only 17 (1.12%) samples out of 152 samples of RIBA-3 indeterminate were PCR positive. (3) All 244 samples that were RIBA-3 negative (no bands) are also PCR negative, giving a 100% correlation between the two tests.

Conclusion: (1) SIA and HCV RNA PCR show excellent correlation for all negative samples. This allows us to use SIA as a conclusive test for HCV status when the result is negative. (2) For all indeterminate SIA results, testing with PCR may assist in clarifying the HCV status of these samples especially when there are Core and NS3 bands present. (3) All indeterminate SIA results with NS5 band show a clear negative PCR result. This may lead us to question the value of NS5 band inclusion in the SIA test kit and in confirming the HCV status of the sample.

A 4.6 Improvement of in-house PCR testing procedure
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Background: Our present in-house PCR testing method consists of centrifugation of primary samples at 3500 x g, pooling with Tecan pipetting robot, pool precentrifugation at 6000 x g to remove cellular debris, transfer of samples into new centrifugation tubes, high-speed centrifugation [1 h at 48 000 x g] for virus enrichment, virus extraction and real-time PCR.

Material and Methods: Centrifugation force for primary samples was increased to 6000 x g thereby making pool precentrifugation superfluous. 100 pools were spiked and analysed for five viruses with both preparation methods. Results: Cycle threshold (Ct) of real-time PCR for HCV, HBV, HAV and Parvo virus B19 (P < 0.05) was significantly reduced in pools prepared with the new method (without pool precentrifugation) compared with the previous method. For HIV we could demonstrate a slightly (not significant) reduced Ct with the new method.

Conclusion: We could demonstrate that the new method yielded comparable or better Ct values for all measured five viruses. The new method is cost-effective, because we saved time and material for the pool precentrifugation. Second, the new method may improve safety, because there is no longer a need for manually transferring the supernatants into new tubes, which may lead to mixing up of samples in very rare instances.

A 4.7 Australasia’s ‘NATSCREEN’ quality control programme for blood service laboratories
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Introduction: The NRL provides quality assurance programmes for laboratories in Australasia. The ‘NATSCREEN’ quality control (QC) programme is run for donor screening laboratories in Australia and New Zealand performing nucleic acid screening with the Chiron TMA Multiplex assay.

Materials and Methods: Participants were supplied with two commercial QC samples (VQC, The Netherlands). The positive QCs are secondary working reagents calibrated to the WHO International Standards for HIV and HCV. Samples were tested in every assay run and the results sent to the NRL through the NRL’s on-line QC interface, EDChNet (www.nrlqa.net). Data were analysed and summary statistics presented in reports, accessed through the NRL website.

Results: Between 01 September 2001 and 31 December 2003 participants submitted ~29 000 QC results. Analysis of the data from the QC sample batch 004 [n = 11 772] showed that from 561 assay runs, 13 (0.23%) were invalid in terms of the basis of a nonreactive QC sample. Interlaboratory precision ranged from 7.92 to 13.87% (HCV-SPY04) and 9.00 to 16.99% (HIVSPY04). Result accuracy was estimated by calculating bias, which highlighted luminometer-specific trends that were demonstrated graphically by EDCNet.

Discussion: Analysis of the results of testing two commercial QC samples in the Chiron TMA Multiplex assay demonstrated acceptable precision and accuracy. Changes and trends in results allowed potential problems, such as the need to monitor luminometer performance, to be identified.

A 4.8 Two years experience with NAT screening: incidence and prevalence in Piedmont (Italy)
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Background: In Italy NAT is mandatory for HCV screening of all blood units since 29 June 2002 by Circ. Min. No. 14 (19 December 2001). Only Piedmont introduced NAT screening for HCV-RNA in routine since 1 November 2001, according to Circ. Min. No. 17 (30 October 2000) and to DGR 28-3449 (09 July 2001). An epidemiological study was conducted among donor’s population in Piedmont by SIT Sovrazionale.

Methods: In Piedmont NAT screening is performed with two different technologies: Chiron Procleix TMA technology and Roche Ampliscreen. Incidence and prevalence for HCV and HIV infections in Piedmont and residual risk (RR) after NAT introduction were calculated.

Results: In 2 years of NAT routine screening in Piedmont over 450 000 blood units were tested for HCV-RNA, of whom 300 000 for HIV-RNA. Prevalence for HCV was 183.4/100 000 donations (2002) and 139.4 (2003). Prevalence for HIV was 10.3/100 000 donations (2002) and 13.9 (2003). Incidence for HCV infection among periodic blood donors was 1.86/100 000 donations (2002) and 1.44 (2003). Incidence for HIV was 0.83/100 000 donations (2002) vs 1.28 (2003). RR calculated for HCV was 2.54 million donations (2002) vs 1.87 (2003); RR calculated for HIV was 0.96/million donation vs 0.8.

Conclusions: Please provide author and affiliations.This is the first study reporting a real evaluation of RR to transfuse HCV/HIV-infected blood units after 2 years NAT screening in Piedmont. Incidence and prevalence rates in blood donors in Piedmont are comparable to those registered in northern Italy.

A 4.9 A single extraction for HBV, HCV, HIV NAT screening with the BIOMERIEUX – ROCHE diagnostics system
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EFS Bretagne, EFS Rhone Alpes, EFS – DMS, EFS Alpes Mediterranee, France

In France, HCV and HIV-1 NAT screening has been performed since July 2001 using two systems: BIOMERIEUX – ROCHE diagnostics and Chiron blood testing. HBV NAT assays are now available for these two systems. However, routine Nuclisens extraction procedure, performed from 2 ml of pool sample allows only two Ampliscreen assays on the same eluate. A modification of Nuclisens extraction procedure should be necessary to allow three amplifications simultaneously. We decided last year to evaluate the impact of the implementation of HBV NAT screening on the BIOMERIEUX – ROCHE diagnostics system. At first the output of the Nuclisens Extractor for HBV DNA was studied. Our results showed that the extraction output isn’t proportional to the HBV DNA concentration, in particular for concentrations higher than 10^5 units/ml. Nevertheless, this is not a problem for HBV NAT testing as no false-negative result was observed. Next, pool volume used for extraction was increased from 2 ml to 3 and 4 ml, respectively, and the eluate volume from 60 to 90 l. Our inter-sites results showed that the pool volume can be increased to 3 or 4 ml without any technical problem and the eluate volume can be increased to 90 l without significant loss of sensitivity. In conclusion on the BIOMERIEUX – ROCHE diagnostics NAT system, modification of the extraction allows the implementation of HBV NAT assay in addition to HCV, and HIV-1, from a single extraction.
A 4.11

Comparative study between NAT assay Procleix and hepatitis C antibodies tests

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Background: NAT HCV/HIV1 (GenProbe/Chiron) is performed routinely in blood and tissue donors.

Methods: NAT HCV/HIV1 (GenProbe/Chiron) is performed routinely in blood and tissue donors.

Results: A 61-year-old patient was enrolled to collect samples for auto-transplantation in June 2002. The patient was HCV antibodies negative, but NAT HCV reactive for both Chiron Procleix and Roche HCV Amplicor assays. Procleix discriminatory assay confirmed the HCV RNA reactivity. During a follow-up of 10 months we collected five samples and a previous sample was found in our haematological patient’s seroteca: results are summarised in the table. Patient died in May 2003.

| Methods | 19 June | 24 June | 20 July | 26 September | 22 March |
|---------|---------|---------|---------|--------------|---------|
|       | July 1998 | 2002 | 2002 | 2002 | 2003 |
| Chiron Procleix | Positive | Positive | Positive | Positive | Positive |
| Roche Amplicor | Positive | Positive | Positive | Positive | Positive |
| HCV | Positive | Positive | Positive | Positive | Positive |
| INNO-LiPa HCV | Genotype2 | Positive | Positive | Positive | Positive |
| Ortho HCV 3.0 | Negative | Negative | Negative | Negative | Negative |
| ALT (IU/L) | 37 | 11 | 16 | 25 | 29 |

Conclusions: During 5 years, from 1998 to 2003, patient never developed antibodies anti-HCV probably due to his haematological disease and therapy. Bibliography support that, rarely, haematological diseases may not develop immunological response (National Institutes of Health Consensus Development Conference Statement: Management of Hepatitis C, 2002; J.H. Hoofnagle-Course and Outcome of Hepatitis C, 2002).

A 4.12

Improved reproducible results for anti-CMV testing on the fully automated Tecan genesis RMP

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Aim: Tecan genesis robotic microplate processor (RMP) is a fully automated ELISA system. Before implementation for anti-CMV ELISA, the RMP was validated for correct sample and microplate identification, volume accuracy and precision, ease of use and producing correct results.

Materials and Methods: The RMP consists of a robotic sampler with on board 37 °C incubator, washer and reader. Microplates are moved around the worktable by the RoMa arm. Anti-CMV ELISA is currently performed using the Tecan RSP, Dynatech MRW plate washer and SLT plate reader with Medusa 2000 software (RSP). Parallel testing using the RSP and RMP and reproducibility studies to determine the inter- and intra-assay variation were performed.

Results: Concordant CMV results were obtained with 654 samples on the RMP and RSP with an S/CO correlation of 0.85. Correlation for duplicate CMV testing on the RMP was 0.977 (n = 178) and 0.907 (n = 96) on the RSP. There was no sample misreads or failure. The accuracy and precision (CV%) for 10 μl were 0.75 and 2.068 and that for 100 μl were 1.009 and 0.347, respectively. The inter- and intra-assay variation (CV) for the in-house control tested 5 times in one microlitre over 5 days was 10.52% and 5.77.

Conclusions: The RMP is reliable with better reproducible results for anti-CMV testing. The user is trained to set up test protocols and once set up, the system is not difficult to use. It is a batch processor and the software is not flexible to allow continuous processing.

A 4.13

An improved hepatitis C antibody immunoassay using conformational and genotype-specific antigens

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Background: Currently, the licensed hepatitis C ELISAs use recombinant proteins purified under denaturing conditions. There is evidence that conformational HCV epitopes are more immunoreactive and would assist in improving test sensitivity.

Methods: We have designed an HCV antibody assay using a conformational protein NS3/4a and linear fusion proteins MEFA 7.1 and MEFA 7.2 that incorporate all immuno-dominant epitopes of HCV [core, E1, E2, NS3, NS4 and NS5]. Results: NS3/4a purified under denaturing conditions retains enzymatic activity and can detect early seroconversion conformational antibodies better than the c33c antigen. The NS3/4a protein also cross-reacts with different genotype samples better than the c33c antigen. The combination of NS3/4a and MEFA 7.1 detected c33c and c22-3 early seroconversion panels 2.7 and 4.6 days earlier than the Abbott Prism and HCV 3.0, respectively. Although MEFA 7.1 is cleaved by NS3/4a the degraded MEFA 7.1 remains immunoreactive. To overcome this proteolysis all the NS3/4a cleavage sites were removed from MEFA 7.1 and the MEFA 7.2 antigen was generated. MEFA 7.2 has similar expression level as MEFA 7.1 and can be purified using the same procedure. We demonstrate that MEFA 7.2 has very little degradation in the presence of NS3/4a and preserves all epitopes contained in MEFA 7.1.

Conclusion: The availability of new recombinant antigens may assist in the development of more sensitive blood screening tests for HCV.

A 4.14

Is it favourable or advisable to perform the nucleic acid testing (NAT) for multiple viruses?

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Background: NAT for HCV and HIV, reducing the 'window period', has improved transfusion safety, but also other viruses (as HBV and PV-B19) may cause post-transfusion infections. Moreover HBsAg screening cannot identify 'low-level' carriers, HBsAg-antibodies or HBV-HBV complexes or HBV mutants. On the other hand, PV-B19 is resistance to viral inactivation procedures (both heat and solvent-detergent) and, thanks to its dimensions, may cross the filters used for haemofiltration. From March to June 2003, HBV- and PV-DNA research was performing by PCR in 3000 periodic blood donors (58.8% males and 41.2 females), aged 19–53. Eight donors resulted HBsAg positive, but only 3 of them were HBV-DNA positive too; while, in our population with high incidence of IgG anti-PV Abs, five subjects resulted PV-DNA positive. Our results demonstrate that HBV-DNA PCR, with actual limits of sensitivity, did not add anything to HBsAg screening. About
PV-B19, the PCR seems the most effective method to avoid post-transfusion infections; in fact 75% of all donors resulted IgG positive, without any correlation with DNA positivity. Moreover a frequency of PV-B19 positivity equal to 1:600 requires an accurate evaluation. In fact, the possibility of performing the NAT at the same time for numerous viruses, correlated to transfusion transmission, would not involve an increment of the staff, neither of needing time for biological validation of haemocomponents nor of costs, increasing, on the contrary, the transfusion safety.

A 4.15
HBsAG initial positive samples: further serologic results and PCR investigations
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In 2003, HBsAG initial positive samples were collected and further investigated with respect to their serological status and HBV viremia. 102 plasma samples from blood donors, which were initially positive with the Abbott PrismaMax HBsAg test, were collected. Confirmatory testing (Abbott HBsAg confirmatory assay) was performed on the repeatedly HBsAg positive samples, whereas anti-Hbc testing (hepatitis B virus core antigen CORZYME® kit) as well as ALT testing (ALAT/GPT-kit) was performed on all of them. Subsequently, the QIAPena UltraSens Virus Kit extraction procedure (500 µl plasma) and two different PCRs targeting two different regions of the virus (the X-protein gene region and the core region) were performed. The PCR was repeated for HBsAg positive, 16.7% turned out to be negative after repetition in duplicate. Of the remaining 83.3% of samples, 27 (31.8%) were negative or indeterminate with the confirmatory assay. None of 102 samples showed elevated ALT, whereas 88.9% of the confirmed samples (34 of 39) and one of the nonconfirmed samples were anti-Hbc positive. HBV DNA was detected in all the confirmed samples but in none of the other samples; thus the occurrence of core mutants could be excluded. Only 26.5% of HBsAG initial positive samples could be confirmed. Further investigations might reveal the nature of these cross-reactions, which were seen more often in women than in men.

A 4.16
The TaqScreen™ MPX screening assay based on generic nucleic acid extraction and real-time TaqMan® PCR
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Background: TaqScreen™ MPX assay is being developed as a highly sensitive and reliable PCR/NAT blood screening assay. This 5-parameter multiplex assay for detection of HIV-1 (M), HIV-1 (O), HIV-2, HCV and HBV, as well as target-specific discriminatory assays will be fully automated on integrated TaqScreen MPX system, suitable for laboratories of all sizes. System is intended to support single/pooled donation screening, with configurable batch sizes that include armoured RNA/protected DNA full process controls.

Materials and Methods: Test procedure features generic nucleic acid extraction technology on precursor sample preparation instrument, and real-time multiplex PCR based on COBAS TaqMan® technology. Optimised signal threshold algorithm used for a screening classification.

Results: Preliminary analytical sensitivity performance is approximately 15 cp/ml (26 IU/ml) [HIV-1 (M)], 18 cp/ml (6 IU/ml) [HCV], 12 cp/ml (2 IU/ml) [HBV] and <50 cp/ml [HIV-1 (O) and HIV-2]. Tissue origin studies demonstrated comprehensive subtype recognition. Assay showed high specificity and very low chemical interference with plasma substances.

Conclusions: Preliminary performance data reflects significant improvements over existing NAT assays. Additional experiments suggest expansion to a 6-parameter multiplex assay appears feasible. This highly sensitive assay, in combination with the TaqScreen MPX system, may open up a promising new path towards identification of low viral-load window period donations.

A 4.17
A comparative study of 17 third-generation anti-hepatitis C virus ELISAs as WHO recommendation
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Introduction: Third-generation anti-hepatitis C virus (HCV) enzyme-linked immunosorbent assay (ELISA) is used for blood screening, so the sensitivity of the assay is very important. The sensitivity of the assays for the detection of antibody to HCV is evaluated by the serocoversion panels like as Boston Biomedica, Inc. (BBI) panels.

Materials and Methods: 17 anti-HCV ELISAs-3 evaluated by BBI serocoversion panels. PHI 205, PHI 905, PHI 906, PHI 908, and PHI 914 hepatitis C seroconversion BBI panels were used.

Results: The results of the assays were compared by Ortho HCV 3.0 Enhanced SAVe as WHO method that recommended in Report 1, January 2001. Relative serocoversion sensitivity index for each assay was calculated and evaluated to +20. Discussion: The positive score for an assay showed less sensitivity than Ortho HCV 3.0 as reference assay in serocoversion panels. In this study only one assay was sensitive as Ortho HCV 3.0 and other assays were less sensitive than the reference assay with relative serocoversion sensitivity index +1 to +20.

A 4.18
Experience with the Roche COBAS AmpliScreen HIV-1 v1.5 test applied to source plasma donor screening
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Screening for HIV-1 in source plasma donations by NAT or p24 antigen testing is required in the US. Studies were performed to support a plasma indication for the Roche COBAS AmpliScreen HIV-1 Test v1.5 in 96-donation minipools (previously licensed for whole blood donations in pools of 24). The following studies were performed: serocoversion study (10 serocoversion panels), yield study (20 Ag-positive, Ab-negative yield samples), positive pool deconstruction (ten 96-sample minipools, 1-10 positives each), and a clinical specificity study (>100 000 plasma donations). For all serocoversion panels, HIV-1 RNA was detected at 1:96 concurrent with or prior to HIV-1 p24 Ag and prior to HIV-1 Ab. All 20 yield samples were positive when diluted 1:96. Fifty of 51 positives in the deconstruction study were correctly identified. The undetected, spiked sample was derived from a fable clinical specimen containing significant precipitate, which may have limited the ability to extract sufficient RNA for detection. The clinical study comprised 104 448 donations representing 35 905 applicant and qualified donors. Three PCR positive donations from three unique donors were identified. Two were Ag/Ab- and one was p24 Ag-positive only. HIV-1 positive status was confirmed by licensed HIV-1 assays. The COBAS AmpliScreen HIV-1 test was highly sensitive and specific to HIV-1 in 96-sample minipools, while meeting the throughput and performance needs for HIV-1 screening of source plasma donors.

A 4.19
The first Italian experience in NAT testing for HBV, HCV and HIV
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In Italy only NAT testing for HCV-RNA was mandatory. The aim of this study was to report the authors’ experience, the first Italian experience, in NAT testing for HCV-RNA, HIV-RNA and HBV-DNA. In our transfusional Centre we adopted the AmpliScreen methods supplied by Roche. NAT testing for HCV-RNA was adopted in March 2001, 81 200 blood units (BU) were tested; for HIV in June 2001, 74 400 BU were tested; for HBV-DNA in March 2003, 26 300 BU were tested. Each mini-pool (MP) was prepared with 24 samples. For HCV-RNA we tested 81 200 BU in 3458 MP tested in 742 analytical sessions (AS). We observed 7 (0.94%) invalid AS, 161 (4.65%) invalid MP, 2 initially reactive MP, only 1 MP presented a repeat reactivity due to an anti-HCV positive sample. For HIV-RNA we tested 74 400 BU in 3091 MP tested in 654 AS. We observed 7 (1.07%) invalid AS, 111 (3.59%) invalid MP, 1 initially reactive MP, only 1 MP presented a repeat reactivity due to an anti-HIV-1 positive sample. For HBV-DNA we tested 26 300 BU in 1097 MP tested in 217 AS. We observed 2 (0.92%) invalid AS, 11 (1.02%) invalid MP, 3 initially reactive MP, 2 MP presented a repeat reactivity. In the first case the sample was HBsAg positive, in the second case the sample was HBsAg negative. This is a single centre experience without any of statistical relevance. The aim of this report was only to underlineate the feasibility of an implementation of NAT testing for HBV, HCV and HIV in a medium size Italian transfusional service.

A 4.20
Effective analysis of indeterminate results using TMA assay compared to other confirmatory tests
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Objective: Since March 2003 the Second Regional Blood Centre of Athens has implemented NAT testing. The scope of this study is to analyse the correlation of
Procleix HIV/HSV assay test with MEIA results and to compare these with the confirmatory INNOlIA test.

Materials and Method: All samples were tested for HIV antibodies with MEIA (Abbott AxSYM) and for HSV DNA using the Procleix HSV-1/2 HSV assay. Positive samples and samples with grey zone results with MEIA (S/CO: 0.3–0.6) were also tested with the confirmatory test INNOlIA (BioMerieux).

Results: From March 2003 to January 2004, totally 16 068 blood donation samples were collected. 100 of them (0.62%) needed to be confirmed for HIV infection. 13 blood donors (0.08%) were found positive for the presence of anti-HIV antibodies after confirmation of MEIA results with INNOlIA. HIV RNA was detected in the plasma of nine donors (0.06%). From 88 samples (0.56%) that were originally either in the grey zone or characterised as marginally positive (S/CO < 6.00), 83 and 16% were negative and indeterminate, respectively, and only one sample was found positive with INNOlIA. No HIV RNA was detected in these samples.

Conclusion: NAT testing is very important in reducing the window period in HIV infection. Moreover, it can be used as an alternative means of confirming grey zone or indeterminate results faster and more accurately than other confirmatory results.

A 4.21 Excellence sensitivity and specificity with a new Ag–Ab combination assay: Enzygnost® HIV Integral II

Bussfeld D, Petri E, Duttmann U, Krupka H, Korn K, Graziani M, Guertler L, Korn K, Young J, Cheng L, Pai A, Gallarda J

Background: Enzygnost® HIV Integral II is a new ELISA for the detection of HIV p24 Ag and Ab to HIV-1 group M, HIV-2 and HIV-1 subtype O in human plasma and serum to shorten the diagnostic window.

Materials and Methods: The sensitivity of HIV Integral II was assessed by 38 seroconversion panels, by 1461 HIV-1 or HIV-2 Ab positive specimens of various subtypes, by the French Ag SFTS Panel as well as by 64 viral lysates and cell culture supernatants contain different (sub)types. To determine specificity 15 950 specimens from random blood donors were tested with HIV Integral II. 443 HIV-negative samples contain different anticoagulants were investigated.

Results: 10/38 sc panels were tested with HIV Integral II vs. competitors’ HIV assays. On average HIV Integral II detected the first pos. specimen 2.4 and 2.3 days earlier than the two comp. HIV Ag/Ab assays. The comp. HIV Ab assay showed an average delayed detection of 4.7 days. The other 28 panels revealed excellent sensitivity of the new assay. HIV Integral II clearly detected all 1461 HIV Ab-pos. specimens. In Ag detection limit was between 19.4 and 22.9 ng/ml HIV Ag. All viral lysates and supernatants showed 100% sensitivity. Testing 15 950 HIV neg. blood donor specimens with HIV Integral II the initial specificity was 99.92%, specificity after retest was 99.93%. Anticoagulants (443/443 specimens) did not impact specificity.

Conclusion: Enzygnost® HIV Integral II showed excellent HIV Ag- and Ab-sensitivity and an outstanding specificity.

A 4.22 Quantification of West Nile Virus RNA in donated blood by TaqMan PCR

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Background: Although blood centres testing for viral targets typically require a qualitative ‘positive/negative’ answer from laboratory tests, added value may be obtained by simultaneously providing quantitative information using a blood screening assay capable of quantifying the amount of West Nile Virus (WNV) RNA. During DNA synthesis, TaqMan chemistry will result in detectable fluorescence in a dose-dependent fashion, and can thus be used to determine the titre of viral RNA in a sample.

Materials and Methods: A calibration curve is generated by TaqMan PCR from a panel consisting of WNV RNA at known input copy numbers. The amplification cycle at which probe degradation products are first detected (the Ct value) is plotted against the log input copy number for each panel member and a linear calibration curve is obtained. The Ct value from an unknown sample is then used to determine the amount of viral RNA present. An internal quantitation standard is included in each reaction.

Results: A quantitative assay with a dynamic range of 30–10 000 copies of WNV RNA has been developed which can be used to determine the concentration of virus in donated blood samples.

Conclusions: The ability to quantify the amount of virus present in blood samples provides: (1) data which allows inherent quality control capabilities relevant to the testing lab; (2) information significant to donor counselling; and (3) epidemiological data such as typical virus titre in infected samples.

A 4.23 HCV NAT screening in northern Poland and HCV RNA and HBV DNA results in donors with elevated ALT

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Background: In Poland all blood donations are tested for anti-HCV, anti-HIV, HBsAg and for ALT activity. Since 2002 NAT screening for HIV RNA is also obligatory. Aim: To analyse the frequency of (1) HCV RNA in donors with negative results of obligatory serological tests and (2) HBV DNA and HCV RNA in seronegative donors with elevated ALT activity.

Materials and Methods: [1] 160 588 seronegative donations were tested for HIV RNA; (2) 701 donations with high ALT level were tested for HBV DNA and HCV RNA. HCV RNA and HBV DNA detection was performed in pools of 29–48 donations prepared by Tecan Genesis RSP150. HCV RNA was screened by Cohas AmpliScreen HCV (Roche Diag), HBV DNA by homemade, nested PCR according to Shiriaki et al. Results: In 160 588 seronegative samples with normal ALT level, one HCV RNA positive donation was detected. In 701 samples with high ALT, no HCV RNA positive donations were detected but one HBV DNA positive sample was determined. Further examination revealed it was anti-HBe positive.

Conclusions: (1) Introduction of HCV RNA NAT allowed to identify one HCV-infected donor with no serological markers and reduce the risk of transfusion-transmitted HCV infection; (2) In 701 donors disqualified for elevated ALT one infected HBV DNA positive donor was found; if routine HBV NAT were introduced in addition to NAT HCV, we should consider to discontinue ALT testing.

A 4.24 TaqScreen™ West Nile Virus system: development of an automated platform for NAT screening of blood

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Objective: In 2002, a West Nile Virus (WNV) epidemic of over 4100 cases in the US resulted in 277 deaths. Manufacturers were requested by federal authorities to develop systems to screen the blood supply for WNV in 2003. This report summarises features and preliminary nonclinical performance for the automated TaqScreen™ West Nile Virus system.

Materials and Methods: The system consists of a Hamilton Microlab AT/Plus 2 pipettor, a COBAS AmpliPrep for automated sample preparation, a COBAS TaqMan Analyzer for PCR amplification/detection, and a computer system with Pooling and Data Management software for results compilation and reporting. Preliminary non-clinical performance studies included analysis of the system’s specificity, sensitivity for WNV, and assessment of the assay’s ability to detect other members of the Japanese encephalitis virus serocomplex.

Results: Nonclinical specificity of the TaqScreen™ WNV System was 100%; the assay showed no cross-reactivity for 125 non-WNV microorganisms. The sensitivity of the assay is estimated at 15 cp/ml for WNV. The assay detects the Kunjin variant of WNV, as well as isolates for the Japanese, Saint Louis and Murray Valley encephalitis viruses. Conclusions: In the first 2 weeks of testing blood donations, the first reported human case of WNV infection was identified using the TaqScreen™ WNV system. Conservative throughput estimates are that 700 000 samples (in pools of six) per year can be screened using two systems with two operators.

A 4.25 HBV NAT screening of mini-pools in Poland

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Background: In Poland the obligatory tests for the release of blood components are HBsAg, HBVAb and HCV RNA.

Aim: To investigate (1) the frequency of HBV DNA positive donors with negative results of the required tests and (2) serological characteristics of HBV infection and HBV DNA viral load in these donors.

Materials and Methods: From May to October 2003, 60 000 blood units were collected. They were tested for HBV DNA by the COBAS AmpliScreen HBV test (Roche Diag) in mini-pools of 24 (Tecan, Switzerland). The HBV DNA positive samples were further tested for HBsAg, HBsAb, HBcAb total/igm, HBsAg, HBcAb by IMX Abbott and for quantitative HBV DNA (COBAS Monitor HBV, Roche Diag). Results: HBV DNA was detected in two donors (0.003%). The viremia was 1.82 x 10^4 and 1.14 x 10^4 copies/ml. In both donors anti-HBe total were positive. All other serological tests were negative in one donor, and in the second one HBsAg was detected. Both of them were
repeat donors. In one donor 4/5 previous donations were HBV DNA pos; in the second donor the previous donation was HBV DNA neg.

Conclusions: (1) The frequency of HBV DNA pos/HbsAg neg donors identified by COBAS AmpliScreen was 0.003%. (2) The HBV viral load in infected donors was low. (3) HBV DNA pos/HbsAg neg donors were anti-Hbc pos and one was HBcAg pos. Since anti-Hbc or HBcAg are not routinely used as screening tests in Poland, both HBV DNA positive donations represent HBV window cases that would have been missed during routine blood bank screening.

A 4.26
Single donor testing using the Roche TaqScreen™ West Nile Virus assay
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Background: The current method of testing for West Nile Virus at this institution is performing mini-pools of six samples. Due to issues of sensitivity, a workflow study was performed to determine the feasibility of performing individual sample NAT testing.

Study Design: Over a period of 7 days, approximately 300 samples/day that had been previously tested in pools of six were relabeled and tested again as individual samples. The amount of time required to process was monitored and also compared to the results of the original pool.

Results: Over the 7-day period, 2399 samples were tested. It required 11 h a day to process the 300 samples as opposed to the 5 h it would require to test in mini-pools. All 2399 samples were reactive in both the pool and the individual runs.

Conclusions: At our institution, we average 1050 samples/day for West Nile testing. Using the current mini-pool system it takes 9 h to complete the testing using three TaqScreen™ systems. If these samples were tested as individuals, it would take almost 28 h to complete. Until there is a way to increase the throughput of the TaqScreen™ system, the only feasible way to test all samples at our institution is through the use of mini-pool testing.

A 4.27
HTLV-I/II seroindeterminate blood donors: epidemiological features in 20 cases of seroconversion
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HTLV-I/II is endemic in Brazil, with prevalences ranging from 0.04 to 1% in blood donors and screening (EIA, confirmed by WB) is mandatory since 1993. WB indeterminate results are frequent and need better understanding, due to donor counselling and deferment policies. As part of the ongoing HTLV cohort study (GIPH) at Fundação Hemominas, blood donors with abnormal serological results are re-tested every 2 years. The band patterns and epidemiological features of 20/230 with initial HTLV-I/II WB indeterminate results who later presented seroconversion were analysed. All 20 individuals converted to HTLV-I; none had HTLV-II bands. WB band patterns varied among indeterminate subjects, but p19 and p24 were detected respectively in 12/20 (60%) and 8/20 (40%), previous to seroconversion, not different from the nonseroconverters. Bands of low intensity (‘weak bands’) of precursors of gp21 were found in 12/20 (60%) (P < 0.001, when compared with nonseroconverters). The risk factors identified in the 20 individuals were: positive family member (30%), blood transfusion (25%), illegal drug use (15%), risky sexual behaviour (30%). In four cases (20%) there were no detectable risk factors for HTLV infection. Although most seroindeterminate donors are probably not infected with HTLV, delayed seroconversion makes counselling difficult in this population. The seroconversion rate must be considered and long-term studies are necessary to determine the meaning of HTLV seroindeterminate.

A5 Bacteria

A 5.1
Evaluation of particle gel immunosassay (ID-PAGIA) as screening test for syphilis in blood donors
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Although lack of transfusion-transmitted syphilis in past decade, transmission of T. pallidum via blood products is possible since organisms can live longer than 5 days in refrigerated blood. So serologic testing for syphilis continue to be obligatory. Tests are divided into nonproenomal and treponemal. Newer techniques such as enzyme immunoassays have shown excellent results.

Aim- Methods: Aim of this study is to evaluate ID-PAGIA Syphilis, Diamed AG, that uses three recombinant T. pallidum antigens (TpN15, TpN17 and TpP47) for potential use in screening blood donors. Results obtained were compared to our routine rapid plasma reagin (RPR) screening test, and positive results were confirmed with the fluorescent treponemal antibody absorption (FTA) test in a reference laboratory.

Results: During last year eight of 8966 blood donations were found RPR positive. Seven out eight RPR+ donations were found PaGIA+, while the eighth RPR+ was found PaGIA-. Five RPR+/PaGIA+ sera were confirmed as FTA+ (two RPR+/PaGIA+ blood donors could not be found so we have not been able to re-evaluate and confirmed their sera), while the RPR+/PaGIA- serum was FTA-. Concurrently 300 consecutive blood donors were tested in parallel with our routine RPR test and with PaGIA test and were found negative in both tests.

Conclusion: Our results indicate that PaGIA seems high sensitive and specific and offers advantages of rapidity and simplicity, which make this test an attractive choice for testing antibody against T. pallidum.

A 5.2
Bacterial test on apheresis platelet concentrates (APLT) incidence rate/ delay time for inoculation
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Background: Our standard procedure for APLT bacterial test inoculation (Bact/ALERT) allows 2–4 h between collection and inoculation (delay time). This period was defined, considering that the sooner the test is started, the earlier results are achieved, diminishing the possibility of temporary blood unit shortage. On the other hand, since May 2001 only 0.026% (1/3827 APLT units) positive test was found (Staphylococcus aureus). This fact led us to think about some issues: Is the bacterial contamination rate really low in our service or are we facing false-negative results due to the small delay time for inoculation? To try to answer these questions we evaluated the real storage time for transfused units.

Methods: The storage period for APLT transfused since March 2002 were evaluated using Medinfo-Hematos BigSystem, which provided date and time of collection/ transfusion for each unit.

Results: 2401 APLT were transfused with a mean ± SD of 3.5 ± 1.2 storage days and only 3.6% (86 units) were transfused before 36 h of storage.

Conclusion: Based in publications that show better sensitivity when the inoculation is performed later, we could ensure that a longer delay time for inoculation (from 2–4 to 12 h) will not ensure any expected shortage problem. After changing this procedure, we will compare the future contamination rate, in order to test if low rates observed so far are truly low incidence rate or due to a low sensitivity of our test due to the short delay time.

A 5.3
Bacterial screening test on red blood cell (RBC) incidence rate and delay time for inoculation
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Background: Our standard procedure for RBC bacterial test inoculation (Bact/ALERT) allows 4–6 h between collection and inoculation (delay time). This period was defined, considering that the sooner the test is started, the earlier results are achieved, diminishing the possibility of temporary blood unit shortage. On the other hand, since May 2001 only 0.006% (1/17 096 RBC) positive test was found (Staphylococcus epidermidis). This fact led us to think about some issues: Is the bacterial contamination rate really low in our service or are we facing false-negative results due to the small delay time for inoculation? To try to answer these questions we evaluated the real storage time for transfused units.

Methods: The storage period for RBCs transfused since March 2002 were evaluated using Medinfo-Hematos BigSystem, which provided date and time of collection/ transfusion for each unit.

Results: 9853 RBCs were transfused with a mean ± SD of 12.2 ± 8.4 storage days and only 0.94% (93 units) were transfused before 48 h of storage.

Conclusion: Based in publications that show a better sensitivity when the inoculation is performed later, we could ensure that a longer delay time for inoculation (from 4–6 to 24 h) will not ensure any expected shortage problem. After changing this procedure, we will compare the future contamination rate, in order to test if low rates observed so far are truly low incidence rate or due to a low sensitivity of our test due to the short delay time.
A 5.4 Broad-range rRNA PCR for the bacterial safety of donor blood and in the clinical microbiology
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Blood Center of the Russian Ministry of Health

We are at attempt of creating special protocol for use of the universal PCR on 16S rRNA or its genes for rapid estimation of bacterial safety of the donors’ blood and for rapid detection of Gram-specificity of bacteria in clinical material because the conventional blood culture techniques are low-sensitive, time- and labour-consuming. We have compared the sensitivity of broad-range PCR on 16S rRNA (detectable genome-equivalents counting) with the culture method (CFU counting) and have received comparable results. We have also convinced of opportunity to establish gram-specificity of bacteria by PCR with gram-specific primers.

| Dilution of infected blood | Escherichia coli | Pseudomonas aeruginosa | Staphylococcus aureus | Entercococcus faecalis |
|---------------------------|-----------------|-----------------------|---------------------|----------------------|
|                           | CFU  | PCR     | CFU  | PCR     | CFU  | PCR     | CFU  | PCR     |
| Undiluted                 |      | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   |
| 10^6                      | >100 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   |
| 10^5                      | >100 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   |
| 10^4                      | >100 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   | >10^3 | >10^3   |
| 10^3                      | >20  | >10^3   | >10^3 | >10^3   | >20   | >10^3   | >20   | >10^3   |
| 10^2                      | 0    | 0       | 0     | 0       | 0     | 0       | 0     | 0       |
| 10^1                      | 0    | 0       | 0     | 0       | 0     | 0       | 0     | 0       |

Contamination by bacterial DNA was overcome by irreversible photochemical inactivation: the mix of PCR reagents in a test tube containing psoralene before adding of an extract of nucleic acids from a sample exposed to ultraviolet.

A 5.5 Evaluation of a recombinant enzyme immunoassay for syphilis screening in a blood bank routine
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Background: Serodiagnosis plays an important role in the detection of syphilis infection. In Brazil it is mandatory to perform screening test for syphilis in blood donors routine. At our Institute, the first screening is done using a nontreponemal test, the VDRL assay. Since these tests lack sensitivity and specificity and cannot be automated, they were progressively replaced by more specific treponemal tests, like enzyme immunoassay (EIA). The purpose of our study was to evaluate the use of the Treponostika TP recombinant for syphilis screening in the blood bank routine.

Methods: A total of 7747 serum samples from voluntary blood donors collected in November 2003 were tested in parallel in VDRL (BioMerieux) and Treponostika TP recombinant (Organon Teknika). The reactive samples were submitted to TPHA (Bio-Merieux).

Results: Of the total tested, 356 samples (4.6%) were EIA reactive, 74 (0.9%) were VDRL reactive (all except one, were EIA reactive too) and 78.4% of the EIA reactive samples were positive in TPHA.

Conclusion: Although the syphilis EIA is very well suited for screening large number of samples, the test may give positive results for individuals with active, treated or inactive disease, increasing the discard of blood bags by syphilis positivity from 0.9% using VDRL to 4.6%, leading to deferral high number of repeat blood donors, that had syphilis infections many years ago, and would not represent an actual risk for blood inventory.

A 5.6 Bacterial contamination in leucodepleted and nonleucodepleted platelet concentrates
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Background: To compare the bacterial contamination rate of leucodepleted (LD) and nonleucodepleted (NLD) platelet concentrates (PC).

Material and Methods: From 1999 to 2003, 5–6% of the annual production of LD PRP and NLD Buffy-Coat single units (BC) were screened by BactALERT. LD Apheresis PC were evaluated since 2001. Samples were inoculated into paediatric bottles and incubated for 5 days. A result was considered positive if the same bacterium was detected in the bottle and PC or/and satellite components.

Results: 11 565 PC were evaluated, corresponding to 5206 BC, 6054 PRP and 305 apheresis. The age of the PC was similar for PRP and BC. 67% were screened until the second day, 27% from the third to the fifth days and 6% on the sixth and seventh days.

The apheresis PC were tested in the first day. 48 units were initially positive, but only 18 were confirmed. No bacterial growth was detected in apheresis nor in outdated PC. Bacteria identification and numbers [n] are the following:

| D1–D2        | D3–D6        | Total       |
|--------------|--------------|-------------|
| BC Coag neg  | Coag neg     | 10 [0.19%]  |
| Staphylococcus (1) | Staphylococcus (8) |         |
| Brucella species (1) |            |             |
| PRP Coag neg | Staphylococcus (4) | Listeria (1) | Staphylococcus (1) |
|                |              |            | Gram pos Bacillus (1) |

Conclusions: There was a slight but not significant increase in the number of contaminated PC obtained by BC. Half of the cases belong to older PC, a less numbered group. It seemed that age represented a greater risk rather than nonleucodepletion.

A 5.7 Evaluation of Pall bacteria detection system (PALL eBDS)
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Purpose: Pall Medical has recently made improvements to its bacteria detection system to increase sensitivity and improve ease of use. The purpose of this study was to investigate the new system, Pall eBDS, using bacteria spiked buoyant platelet concentrate stored in plasma.

Method: Buoyant platelet units were inoculated with different bacteria species at a target level of 2–10 cfu/ml. Immediately after inoculation and also after 24 h storage, samples were taken into eBDS pouches and stored for 24 or 30 h at 35 °C in a shaking incubator. The percentage oxygen in the sample pouches was then measured. Three replicates were performed for the following: Escherichia coli, Staphylococcus aureus, Salmonella choleraesuis. Two replicates were performed for: Streptococcus agalactiae, Bacillus cereus, Salmonella paratyphi, Pseudomonas aeruginosa, Staphylococcus epidermidis.

Results: In all cases, the Pall eBDS detected the presence of bacteria when samples were taken immediately post-inoculation and incubated for 24 h. The eBDS also detected bacteria in the majority of inoculated units that were further stored for 24 h. Two Salmonella and one Pseudomonas did not test positive but these were associated with platelet units that had ‘self-sterilised’ during the 24-h period.

Conclusions: Further tests are ongoing but data thus far suggest that the Pall eBDS is an easy system to use with excellent sensitivity.

A 5.8 Transfusion microbiology – an interesting case-2
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The female donor born in 1955 is a health care worker and has 15 donations to her credit.

Summary of results:

| Sample       | Screen HBsAg | Reference HBsAg | Anti-HBc | Anti-HBs |
|--------------|--------------|-----------------|----------|---------|
| 8th Feb 00   | neg          | Reactive assay A | A–not neutralised | Neg assay Z |
| 10th Oct 00  |              | Reactive assay A | Unreactive assay A | Low level reactive assay Y |
| 5th Jun 01   |              | Reactive assay A | A–Reactive assay A | Low level reactive assay Y |
| 25th Apr 02  |              | Reactive assay A | B–not neutralised | Low level reactive assay Y |
| 14th Aug 03  |              | Reactive assay A | A – not neutralised | Low level reactive assay Y |

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The apparent anti-HBc seroconversion between October 2000 and June 2001 made us consider the possibility of an HBV infection. We believe that the donor: • is falsely positive in some HBsAg assays – no reactivity has ever neutralised, • is falsely positive for anti-HBc and • the anti-HBs response is due to vaccination. (A speedier conclusion was limited by the volume of material available for testing, i.e. we would have liked to test the sample 10 October 2000 for anti-HBs and for anti-HBc using assay Z.)

A 5.9
Transfusion microbiology – an interesting case-I
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Our system has already been described whereby donors who fulfill certain criteria have computer flags set to allow their donations, false-positive in certain assays, to be released after testing in alternative assays from the ‘approved list’. This particular donor is a male born in 1938 whose record details 54 donations. His first transfusion microbiology reactivity was in July 2000 in the screen assay for HBsAg. His next sample was also reactive in the screen tests for HBsAg so we would have considered setting his record for release by alternative assay. Unfortunately this ‘second’ sample was now also reactive in the syphilis screen assay. The situation then became even more complicated by false reactivity in the HBs screen assay! The table gives a summary of the screening results – all reference results have been negative.

| Donation date       | HBsAg | Syphilis | HIV | HCV  |
|---------------------|-------|----------|-----|------|
| 10 June 2002        | POS   | POS      | POS | Neg  |
| 10 September 2001   | NEG   | NEG      | POS | POS  |
| 26 February 2001    | POS   | NEG      | NEG | NEG  |
| 17 July 2000        | POS   | NEG      | NEG | NEG  |
| 17 December 1999    | Neg   | Neg      | Neg | Neg  |

Our alternative assay system allows alternative assay release for multiple markers. However, perhaps not surprisingly, the donor was withdrawn on 29 April 2003 because of rheumatoid factor.

A6 Malaria

A 6.1
Screening of blood donors for malarial parasites – analysis of situation in the Sultanate of Oman
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A number of factors are leading to an increased incidence of malaria in areas declared free of infection such as Oman. Since 1998 no indigenous cases recorded. However malaria continues to be an important health problem due to the importation of cases from endemic areas. In Oman risk of transfusion malaria is maintained at low levels by a policy of careful questioning about donor travel history. Aim is to assess the need and usefulness of donor screening for malaria as an adjunct to travel history taking into account the success of Malaria Eradication Programme in containing malaria. Over a period from November 01 till July 03 a total of 24 276 blood donors at the Central Blood Bank, Muscat, were tested for malarial parasites using the rapid immunochromatographic test AMRAD’s ICT malaria P.F/P.v. Over the study period only four donors were found ICT positive. Donor details were sent to the DEH&ME, Muscat, where donors were called upon and fresh sample taken, tested on thick and thin smears at regular time intervals. All four reported negative and the DEH&ME, Muscat, where donors were called upon and fresh sample taken, tested negative and the DEH&ME, Muscat, where donors were called upon and fresh sample taken, tested four negative and the DEH&ME, Muscat, where donors were called upon and fresh sample taken, tested four negative.

A8 Red cell processing and pathogen reduction

A 8.1
Novel high-performance method and devices for electrostatic blood separation
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The blood separation method with use of centrifugal forces is most widespread. Specificity of this method is based only on very small differences of the density of blood components. Therefore efficiency of method is limited. For different blood components the ratio of their electrical charge to mass is another parameter that can be used with the purpose of blood separation. However workable process of direct electrostatic blood fractionating is difficulty achievable, as blood is highly conducting fluid and depth of electrostatic field penetration in blood is small. Such electrostatic separation can occur in very thin layers only close to electrodes. The effects of electrochemical decomposition of blood and electrode polarisation also complicate the use of electrical fields. The application of electrical fields for the blood separation requires to form strong electrical fields with deep uniform penetration in separated blood. We have developed alternative method and devices for electrostatic blood separation. During separation the blood slowly circulates in powerful constant magnetic field, travelling or rotating with high speed. So each blood component is exposed to action of Lorentz force. Discussed process of blood separation is extremely high-performance, as the powerful uniform electrical field arises in all depth of separated blood simultaneously, at the same time the blood can move slowly arbitrarily and there are no electrode effects in the absence of electrodes.

A 8.2
Phase 0 evaluation of the Baxter ALYX: collection of double dose red cells from apheresis donors
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1Welsh Blood Service and 2Baxter R&D Europe

Increasingly stringent donor selection criteria and testing algorithms threaten to reduce the donor base in the UK. Automated collection of multiple components offers the potential to increase production despite a shrinking donor pool. We therefore evaluated the ability of the Baxter ALYX to collect a double dose of red cells from selected donors. 16 plateletpheresis donors who fulfilled Council of Europe criteria for double red cell donation were recruited. The aim was to collect 360 ml of RBCs. SAG-M was added in the ratio 1:2.1. The final product was filtered through an Asahi filter and split into bags A and B. Both bags were samples on days 1 and 42. Bag A was additionally sampled at days 7, 14, 21, 28 and 35. 15 procedures were completed in a mean (±SD) time 24 ± 1.8 min. One was aborted due to haematoma formation. The volume of RBCs collected was 361 ± 1.1 ml with Hb content in Bag A vs. Bag B of 54.0 ± 0.9 vs. 52.9 ± 1.1 g per unit. Hct was 56.6 vs. 56.1%. All units were leukodepleted to <10^6 WBC per unit and bacterial screening was negative. K+, Na+, pH, pO2, pCO2, glucose and lactate were all acceptable during storage. Haemolysis at day 42 was 0.22 vs. 0.23%. The procedure was well tolerated and staff found the machine easy to operate. All donors expressed the wish to donate on the ALYX again. ALYX can effectively and safely collect double dose RBCs from selected donors and should be considered as part of the strategy to augment supply in the face of donor attrition.

A 8.3
Gamma Irradiation: effect on biochemical parameters of erythrocytes
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Introduction: Today it is proven beyond doubt that when blood is mixed with an anticoagulant solution and stored at 4 °C the red cells change in shape, become more rigid, shed lipid, exhibit various biochemical changes. Recent clinical studies suggesting a lack of efficacy of stored blood transfusions have led to a renewed interest in storage-related changes and their functional implications.
Aims and Objectives: To study the biochemical changes during conventional preservation of irradiated and non-irradiated blood.

Material and Methods: Ten units (350 ml each) of whole blood was taken from healthy donors and divided into two parts. One part from each unit of blood was subjected to gamma irradiation of 35 Gy and then stored at 4°C. Estimation of free plasma haemoglobin, potassium and lactate dehydrogenase was done from these irradiated blood bags (cases) and 10 non-irradiated blood bags (controls) on day 0, 7, 14 and 21.

Results: There is a statistically significant increase in cases than controls for all parameters.

Conclusion: These experiments indicate that irradiation of blood results in increased storage lesions. This might not have any clinical significance in most patients. Although there are reports of hyperkalaemia in foetal intravascular transfusion, neonatal transfusions and patients receiving massive transfusion with irradiated stored blood. There is a need for further in vitro studies to study the consequences in these groups of patients.

A 8.4
Preparation of leuko-reduced red blood cell concentrates (RBCC) on Haemonetics MCS+®

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

Material and Methods: Collection of 13 double RBCC using the MCS+ SDR protocol and LN948PF disposable sets integrating RC2H filter (Pall). All subjects were male donors meeting the French requirements. Two study groups defined. Group 1: 17 products gravity-filtered at RT between 2 and 10 h after collection. Group 2: 16 products refrigerated and filtered at +4°C within 24 h after collection. In vitro quality parameters of the leukoreduced RBCs evaluated before and after filtration and on day 14, 28 and 42 of storage.

Results: All the results complied with the French regulatory characteristics for apheresis leuko-reduced RBCC: minimal volume (without SAGM) of 125 ml, haemoglobin content ≥40 g, haematocrit between 50 and 70% and leukocyte level ≤1 x 109 per unit. The only significant difference found between the groups concerned the leukocyte log reduction performance (P < 0.001): 4.1 ± 0.5 for group 1 (RT), 5.2 ± 0.6 for group 2 (cold). Haemolysis was minimal in both groups, 0.26 ± 0.08 and 0.26 ± 0.06%, respectively, at day 42.

Conclusions: The RBCC produced by apheresis using the SDR protocol on MCS+ and disposable set LN948PF, suspended in SAGM and filtered at RT or at 4°C meet the French requirements for such product. The RBCC obtained from apheresis on MCS+ and filtered either at RT or +4°C are equivalent. This will simplify the preparation process of these RBCC.

A 8.5
Assessment of the clinimed packaging system for the transportation of red cell components

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UKBTS guidelines require transportation of red cell components at a surface temperature <10°C. A validation was performed on Clinimed UBP110 (capacity, six red cell units) and UBP130 (16 units) packaging systems. Transportation boxes were filled with either red cells or Medicool pack inserts at 4°C and stored at 4, 22 or 32°C for 12 h. Internal box temperature was determined using four temperature loggers placed at different specified locations. The maximum acceptable transportation time at an ambient temperature <22°C was defined by the shortest time for the red cell pack insert surface temperature to reach 10°C, when stored at 22°C, on any of the four temperature logger positions in each box type. With red cells, the UBP 110 and the UBP 130 maintained the internal box temperature <10°C for 6 h 30 min when stored at 22°C. At 32°C a surface temperature of 10°C was exceeded at approximately 2 h for both box types. Clinimed UB110 and UB130 systems are suitable for transportation of red cell components for a max. of 6 h 30 min at temperatures up to 22°C. Storage data at 32°C provided an evidence base in the event of transportation at unexpectedly high ambient temperatures. Further work suggests that the correct positioning of Medicool packs improve performance of both boxes. This may allow the upper ambient temperature limit and transportation time to be raised. This work will allow a nationally standardised NBS procedure for transporting blood components.

A 8.6
Performance of Haemonetics ACP 215 automated blood glycerolisation/deglycerolisation system

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The system (Haemonetics Model 215) used in this study is an automated, functionally closed system for the glycerolisation and deglycerolisation of human RBCs. This technology automates the red cells deglycerolisation process and does not expose the blood to air or bacterial contaminants, thereby enabling the shelf life of thawed red cells to be extended to 14 days as opposed to 24 h using previous technology. The performance of this system was evaluated in terms of the automation of the process as well as the quality and yield of the thawed red cell concentrates upon storage. Performance of the equipment was reflected by the quality of the red cell concentrates tested. This process was carried out against a required set of protocols specified by the manufacturer. The mean supernatant haemoglobin level, mean osmolality level and post-wash Haematocrit levels were tested and all met with AABB Standards requirements. Bacterial sterility was also performed and all tested negative. In conclusion, the Model 215 indeed provides a closed, automated system for RBC deglycerolisation with a storage period of 14 days for deglycerolised red cell concentrates. Red cell yield was acceptable and haemolysis minimal. This extended shelf life facilitates inventory management of frozen blood supplies.

A 8.7
Quality control of red cells – comparable study in Czech Republic

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Objective: Blood donations vary in their composition due to considerable variation in donor cell number and collected volume. General rules and guidelines of quality assurance (QA) and good manufacturing practice (GMP) were adapted for use in all blood centres in the Czech Republic. We were focused on QC of red cells in additive solution, buffy coat removed (AS-BCR) originated from 16 different blood centres.

Material and Methods: Results of each unit were related to current QC standard for AS-BCR (Htc 0.50–0.70, Hb ≥ 43 g/unit and WBC < 1 x 106/μl) within 24 h after collection. EDTA additive solution was used for all tested units.

Results: Out of 114 of AS-BCR 34 units were processed manually (30%) and 80 units by plasma extraction system (70%). 56 units were collected in three blood centres, each with production more than 10 000 collections a year. 34 units obtained from eight blood centres with production between 5000 to 10 000 collections a year and 24 units are from five small blood centres (<5000 collections a year).

Conclusions: The QC results of tested units showed that the quality consolidation of blood components was effected. The quality of transfusion products in Czech Republic corresponds with the national standards as well as European one.

A 8.8
Use of ACP 215 apparatus – our own investigation

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Two methods are used for freezing red blood cell concentrates (RBCs): low or high concentration of glycerol as cryoprotectant. Thawed RBCs prepared according to the above procedures cannot be stored for longer than 24 h. With ACP 215 apparatus used for automatic freezing and thawing it is possible to prolong the storage time of thawed RBCs. In our study we evaluated the quality of RBCs frozen with the use of ACP and stored at ~80°C. These observations will be presented in a separate work. The usefulness of ACP for washing RBCs was also tested. We studied the quality of RBCs washed with manual centrifugation method and with automatic method with ACP. In both cases closed system was used. Additive solution (ADSD) was added after washing. During 1 week of storage red blood cell count, haemoglobin, haematocrit and percent of haemolysis were tested. No statistically significant differences have been
observed. After 1 week of storage the following results for RBC haemolysis were determined: 0.46 ± 0.13% no preparation; 0.36 ± 0.14% for manually washed and 0.62 ± 0.21% for ACP. No microorganisms contamination has been observed. All data will be displayed on the poster. ACP 215 is a simple, friendly and easy to operate piece of equipment for obtaining washed RBCs within a closed system with additive solution (ASI) added. Storage of RBCs can be prolonged to at least 7 days after completion of washing procedure. Additionally, the products obtained are of constant parameters.

A 8.9 Evaluation of Pall RC2D leucocyte depleting filter for red cells
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Background: The aim of the study was to evaluate the Pall Leukotrap RCPL system containing an improved red cell filter RC2D (instead of RC1M) and the ATS-LPL for plasma and platelets.

Methods and Materials: After collection of whole blood (WB) donations into current Pall RCPL systems the units were placed onto cooling plates for transportation to the processing centre and held prior to processing. Two WB hold conditions were trailed (8°C (n = 12) and 17–22°C (n = 12). The WB units were then sterile connected onto Pall RCPL systems containing RC2D Filter and processed as per routine centrifugation and processing conditions. Filtration was performed as per manufacturers instructions for use.

Results: Mean leucocyte residuals of the red cell units were <0.007 ± 0.007 × 10⁶ (unit) and 0.023 ± 0.011 × 10⁶ (unit) for whole blood held for 8°C and 17–22°C on cooling plates, respectively. The mean filtration time for all red cell units through RC2D was 30 min (range 14–36). Council of Europe Guidelines were met for parameters: volume (ml), haemoglobin (g/unit) and haematocrit (%) for all leucocyte depleted red cell units.

Conclusion: The Pall RCPL filter system containing RC2D filter effectively fitted into current blood processing practices at BCT. The CE guidelines for residual leucocyte level and other red cell parameters were met in all SAG-M red cell units produced.

A 8.10 The effect of two leukocyte depletion in line filters on the whole blood filtration
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Aim: The aim of this study was to compare the difference of the leucocyte reduction efficiency and quality of fresh-frozen plasma (FFP) from filtered whole blood produced by two types of in-line filters (only filter material were surface modified by two methods A and B).

Methods: Whole blood was filtered within 6 h of collection at an ambient temperature (10–12°C). Samples were taken pre- and post-filtration for analysis of WBC numbers, coagulation factors and complement activation (n = 8 for each type of filter).

Results: All filtered units (232−267 ml) contained <2.5 × 10⁶ residual leucocytes. No significant difference between group A and B. But group B seems take longer time to filtration than group A (5.3±4 vs. 4.1±2). Neither group A nor group B shows statistically significant losses of total protein, album, IgG, IgM, fibrin, factors VIII, IX, VWF and C3 (P > 0.05). Factor V, XI and AT-III decreased significantly in two group filters. Group B showed more significant losses IgA content and factor V activity (6.0%, 35.1%) than group A (1.7%, 12.6%). It appeared to be related to the lower wet ability and positively charged surface of group B filters.

Conclusions: Two types of filters can remove leucocytes effectively, and no significant changes were observed in the quality of fresh-frozen plasma (FFP) from filtered whole blood.

A 8.11 Washing of red cells: product and process quality versus wash-solution temperature
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Introduction: Washing red cell (RC) units can prevent undesirable complications. Washing and additive solutions are normally used at room temperature and therefore RCC core temperature post-wash exceeds 10°C. We studied the potential cell-damage by interrupting the cooling chain. Method: 10 double dose RC units were split into two groups (10 units each). Both groups were washed in an automated closed system (ACP 215, Haemonetics, USA) 1 week after collection. Group 1 was washed and resuspended using solutions stored for 24 h at 4°C, group 2 with solutions stored at room temperature. RC units were immediately stored after the washing process at 4°C, samples were taken after washing and on day 14. Wash-efficacy (protein depletion), potassium, haemolysis (cell damage) and ATP (cell viability) were analysed.

Results: (1) Group 2 RC units were warmed up (°C) to 26°C ± 0.5 (surface) whereas group 1 only to 20.0 ± 0.5. Protein depletion after washing was not significantly different between the groups. Potassium (mmol/l) after 2 weeks of post-wash storage was 20.2 ± 2.0 vs. 19.0 ± 1.2, cold vs. warm, respectively; P = n.s.). Haemolysis in percent was lower and ATP higher in group 2, but differences did not reach significance.

Conclusion: Our data demonstrate that wash solutions at 20°C in contrast to 4°C solutions result in higher post-wash temperatures of RC units, but do not cause increased cell damage, reduced wash-efficacy or cell-viability.

A 8.11.2 Introduction of Optilink24 and Optipress software reduced production losses in WBS
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The WBS has been using Optipress II’s for 6 years. To ensure a fully GMP compliant record for the processing of donations the Optipress data management system was introduced in April 2001. The Optilink system is connected to each Optipress and records operator, donation number, processing protocol, time taken and gross weights of the prepared components. This system also allows database interrogation, which assists in managing the process and identifying training needs. Problems experienced with Optipresses include kinks in the tubing of the red cell transfer pack line leading to red cells running over into the plasma pack (run over). Kinks in the plasma line would lead to plasma running into the red cell transfer pack (run dry). Introducing the Optilink system reduced some losses due to these faults (Table 1) which may be explained by the process requiring operator identification, requiring slightly more time. A major benefit is that operators can be identified and retraining provided as appropriate. The introduction of updated software for all Optipresses in May 2002 also reduced the number of losses.

Table 1. Production loss figures for run overs and run drys

| Financial year | Run-overs | Run-drys |
|---------------|-----------|----------|
| 1997–1998     | 117       | Not recorded |
| 1998–1999     | 71        | 65       |
| 1999–2000     | 28        | 261      |
| 2000–2001     | 23        | 179      |
| 2001–2002     | 35        | 68       |
| 2002–2003     | 4         | 5        |
| April–December 2003 | 1   | 0        |

A 8.13 Automated separation of whole blood – comparison of Compomat G4 and MacoPress/Sepamatic (prototype)
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Introduction of Optilink

Background: Reduction of separation time as well as recording of electronic data required for GMP are the reasons to replace manual processing by automated separation. Preparation of blood components should be reproducible and easy to handle. In our studies we compared the prototype MacoPress/Sepamatic with the Compomat G4.

Material and Methods: We compared 538 separations with MacoPress (Macopharma) vs. 768 separations with Compomat G4 (Biotest) using a top and bottom blood bag system (Macopharma). After hard spin centrifugation we separated whole blood into plasma, RBC anduffy-coat. For the platelet concentrates (PC) we pooled four buffy coats with one unit plasma. We compared quality data, reliability and separation time. We compared 538 separations with MacoPress (Macopharma) vs. 768 separations with Compomat G4 (Biotest) using a top and bottom blood bag system (Macopharma). After hard spin centrifugation we separated whole blood into plasma, RBC and Buffy-coat. For the platelet concentrates (PC) we pooled four buffy coats with one unit plasma. We compared quality data, reliability and separation time. Cell counts were measured on a Sysmex SE-9500 analyser.

Results: There was no significant difference in quality data as cell contamination in plasma, RBC and PC. Platelet concentrates processed on Macopress contained slightly more platelets compared to products processed on Compomat G4. The rate of technical failure because of not correct position of one sealing head was higher on MacoPress and positively charged surface of group B filters.

Introduction: Washing red cell (RC) units can prevent undesirable complications. Washing and additive solutions are normally used at room temperature and therefore RCC core temperature post-wash exceeds 10°C. We studied the potential cell-damage by interrupting the cooling chain. Method: 10 double dose RC units were split into two groups (10 units each). Both groups were washed in an automated closed system (ACP 215, Haemonetics, USA) 1 week after collection. Group 1 was washed and resuspended using solutions stored for 24 h at 4°C, group 2 with solutions stored at room temperature. RC units were immediately stored after the washing process at 4°C, samples were taken after washing and on day 14. Wash-efficacy (protein depletion), potassium, haemolysis (cell damage) and ATP (cell viability) were analysed.

Results: Group 2 RC units were warmed up (°C) to 26°C ± 0.5 (surface) whereas group 1 only to 20.0 ± 0.5. Protein depletion after washing was not significantly different between the groups. Potassium (mmol/l) after 2 weeks of post-wash storage was 20.2 ± 2.0 vs. 19.0 ± 1.2, cold vs. warm, respectively; P = n.s.). Haemolysis in percent was lower and ATP higher in group 2, but differences did not reach significance.

Conclusion: Our data demonstrate that wash solutions at 20°C in contrast to 4°C solutions result in higher post-wash temperatures of RC units, but do not cause increased cell damage, reduced wash-efficacy or cell-viability.
Conclusion: The prototype of MacoPress is an alternative to the Compomat G4. But there are still some technical corrections necessary to make the separator as reliable as the Compomat G4.

A 8.14
Performance qualification of the Fresenius HemoCare bloodpack: T3988 ‘Wide boring’
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Background: Whole blood can be separated into: plasma, buffy coat and red-cell conc. (RCC) by differential centrifugation and separation on a separation device. Because of the high haematocrit of the RCC, 60% of the process time is needed for expression of the RCC. By increasing the internal diameter of the tubing at the bottom of a T&B system by 0.7 mm, a decrease of the process time is expected.

Methods: 220 units of whole blood were collected with the new T 3988 ‘wide boring’ blood pack and separated on a routine base. Quality control parameters were checked and the whole process time was monitored. Free haemoglobin was measured up to 42 days.

Results: Process time of a ‘wide boring’ bag is significant shorter compared to a standard blood bag. Average decrease: 86 s. Slight increase in free haemoglobin is measured probably due to the increased express rate of the red cells.

Materials and Methods: Aims: The double plateletpheresis produces more platelets compared to the single unit procedure. The purpose of this study was to evaluate the performance of Gambro’s Orbisac, an automated system for production of buffy coat platelet concentrates requiring and a difficult to optimize process.

Background: Manual buffy coat PC production is a time-consuming, physically demanding and a difficult to optimize process. A total of 22 pooled PC from four buffy coats were produced by the manual procedure. The aim of the study was to compare platelet concentrates (PLTs) produced using the Orbisac centrifuge (Gambro BCT) to PLTs produced using the standard manual method OptiPure® (PLT Baxter) regarding the procedure, the quality and the ergonomics.

Materials and Methods: On the first 27 PLTs the content of platelets, RBCs and leucocytes were measured in the primary pools; the final products and the residuals. The weight and the haematocrit were likewise determined. Recovery of platelets from the pools was calculated. Evaluation of swirling at days 5 and 7 and pH measurement at day 7 was performed. On the next 140 PLTs weight and platelet content were measured. The results were compared to the contents of the manually produced PLTs. Ergonomics were evaluated by scoring and commenting any physical strains related to the procedure.

Results: The mean platelet content of all the PLTs was 309 ± 10^3 /ml (range 165–443). This was 20% more than the platelet content of standard PLTs. 4.8% of the PLTs did not meet the requirements of the Council of Europe. The mean recovery of platelets was 80% (range 66–87). The mean leucocyte concentration was 0.13 ± 10^9 l. At day 7 swirling was graded 3 (highest score), the mean pH was 6.93 (range 6.78–7.08) and the mean platelet content was 93% of the initial content. Compared to the manual processing the ergonomics were far better.

Conclusion: The Orbisac centrifuge offers an opportunity of producing high-quality PLTs with a high yield.

A 9.2
Evaluating production of platelet concentrates using the Orbisac centrifuge
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Objective: The aim of the study was to compare platelet concentrates (PLTs) produced using the Orbisac centrifuge (Gambro BCT) to PLTs produced using the standard manual method OptiPure® (PLT Baxter) regarding the procedure, the quality and the ergonomics.

Materials and Methods: A total of 22 pooled PC from four buffy coats were produced by Orbisac and the quality controls compared with those from 10 PC also from four buffy coats produced by our conventional manual method.

Results: 28 persons donated platelets, and their median body weight and height was 70 kg and 174 cm, respectively. Preapheresis platelet count was 280 000/µl. The total processing volume and processing time was 3988 ml and 97 min. Collection efficiency was 54.4%. Product volume was 501 ml. Platelet yield was 6.4 × 10^11 platelets per collection. Donor platelet count was reduced 39% and the time needed for more than 95% donors to recover platelet count more than 95% of preapheresis procedure was 14 days.

Conclusions: Double platelepheresis is safe and useful method for Korean donors to collect platelets. For Korean donors, preapheresis platelet count should be more than 200 000/µl and blood donation deferral period after double platelepheresis should be more than 14 days.

A 9.3
Evaluation of Orbisac: an automated system for production of buffy coat platelet concentrates
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Background: Manual buffy coat PC production is a time-consuming, physically demanding and a difficult to optimize process.

Aim: The purpose of this study was to evaluate the performance of Gambro’s Orbisac, an automated system for production of buffy coat PC and to compare it to our routine manual procedure.

Materials and Methods: A total of 22 pooled PC from four buffy coats were produced by Orbisac and the quality controls compared with those from 10 PC also from four buffy coats produced by our conventional manual method.

Results: The mean platelet content of all the PLTs was 309 ± 10^3 /ml (range 165–443). This was 20% more than the platelet content of standard PLTs. 4.8% of the PLTs did not meet the requirements of the Council of Europe. The mean recovery of platelets was 80% (range 66–87). The mean leucocyte concentration was 0.13 ± 10^9 l. At day 7 swirling was graded 3 (highest score), the mean pH was 6.93 (range 6.78–7.08) and the mean platelet content was 93% of the initial content. Compared to the manual processing the ergonomics were far better.

Conclusion: The Orbisac centrifuge offers an opportunity of producing high-quality PLTs with a high yield.
Results: The average platelet count per unit was $3.07 \times 10^{11}$ with the Orbisac system and $2.6 \times 10^{11}$ with our routine procedure. The recovery was highly improved from 60 to 93 %. Swirling was present in all the PC from both Orbisac and manually produced PC.

Conclusion: Orbisac provides good ergonomics and it is a rapid and user-friendly way for producing optimal quality buffy coat platelet concentrates with a high consistency. The Orbisac system allows increased productivity and better resource optimisation.

A 9.4

The washing and resuspension of a platelet concentrate in Macopharma SSP solution

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Introduction: The regulation dated 28th May 2003, stipulates that a platelet concentrate must have a pH between 6.5 and 7.4. However, the solutions in current use no longer meet this criterion. In order to find the most suitable solution, three products were tested: Macopharma SSP solution (1), Macopharma SQA solution (2) and BRAUN solution (3).

Method: A manual technique and an automatic technique were employed. Tests were carried out on the initial apheresis platelet concentrate (APC), plasma depleted APC at 0 h, the supernatant, plasma depleted APC at 6 h and plasma depleted APC at 24 h. The following parameters were recorded: platelet count, average platelet volume, swirling, aggregates, potassium, pH and proteins.

Results: Swirling was observed and the average platelet volume was constant for solution (1), as opposed to the other two solutions. 40% of the potassium was retained in the cell-free solution (1), as opposed to 20% in the other two solutions. The pH remained within range in solution (1) and was out of range in the other two solutions. Given these results, the Macopharma SSP solution appears to be the most satisfactory for preserving the functional quality of platelets, within 6 h (legal expiry period) and longer (up to 24 h).

A 9.5

Responsiveness of pooled platelets produced by the OrbiSac BC System and stored in different media

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1Sanquin Blood bank Region Southeast, Maastricht, The Netherlands, and 2Gamбро/BCT, Blood bank Technology, Zaventem, Belgium

Introduction: The Gambro OrbiSac BC system automatically produces platelet concentrates (PCs) from buffy coats (BCs). We were interested in the influence on platelet activation of three different storage media in PCs thus prepared: autologous plasma, Composol (Fresenius Hemocare) and T-Sol (Baxter). Materials and methods: Five BCs were pooled with additive solution into a ring-shaped container. Subsequently, this ring was centrifuged and the PC is transferred into a new bag. We measured the percentage CD62-positive platelets by flowcytometry on day 1, 4 and 7 during storage. We also determined responsiveness of the platelets (expressed as %CD62-positive after activation with a thrombin analogue).

Results: Platelets in T-sol have a significantly higher percentage of CD62-positive platelets on day 1 before activation. After activation, on day 1 and during storage, platelets in T-sol remain the least responsive. Also PCs in Composol are significantly less responsive than PCs stored in autologous plasma (P < 0.01).

Table 1. Mean (SD) of %CD62-positive cells

| Day | n | Before activation | After activation | Before activation | After activation |
|-----|---|--------------------|------------------|------------------|------------------|
|     |   | activation         |                  | activation       |                  |
| Plasma | 14 | 16.1 (4.2) 62.2 (8.0) | 22.1 (3.4) 62.2 (8.0) | 19.8 (6.5) 53.3 (10.1) | 22.7 (3.7) 53.3 (10.1) |
| Composol | 19 | 19.8 (6.5) 53.3 (10.1) | 22.7 (3.7) 53.3 (10.1) | 19.8 (6.5) 53.3 (10.1) | 22.7 (3.7) 53.3 (10.1) |
| T-sol | 7 | 34.4 (5.0) 37.1 (11.0) | 44.2 (3.4) 37.1 (11.0) | 34.4 (5.0) 37.1 (11.0) | 44.2 (3.4) 37.1 (11.0) |

Discussion: In contrast to PCs stored in plasma or Composol, PCs stored in T-sol show less responsive than PCs stored in autologous plasma (P < 0.01). Platelets in T-sol remain the least responsive. Also PCs in Composol are significantly less responsive than PCs stored in autologous plasma (P < 0.01). On day 1, T-sol PCs have a significantly higher percentage of CD62-positive platelets than PCs stored in plasma or Composol. On day 4 and 7, the difference between the three storage media is less pronounced.

A 9.6

Evaluation of Trima®Accel® (TA) productivity and inter Satellite center (SC) variability

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Background: CT Galicia has an important automated blood collection (ABC) activity, our ABC strategy combines high productivity with short procedure times. In October 2002 we upgraded our Trima’s (V 4.2) towards TA. We evaluate during 1 year the productivity, procedure times (Times) and inter-satellite centre (SC) variability for TA.

Materials and methods: A total of 4339 ABC were performed in seven SC. Collection focus was to collect platelet (PLT), plasma (PLM) and red blood cells (RBC) in maximum number of ABC, target average Time was 60 min. We compared productivity results for all seven SC with results of the individual SC to evaluate inter-SC variability. Transfusion dose (TD) is defined as: PLT = $3.5 \times 10^{11}$, PLM = 210 ml, RBC = 225 ml – 80% HCT.

Results: Constant high productivity was shown for all SC, inter-SC variability is good with exception for number of ABC by TA/day. The SC with highest productivity performs 97% more ABC by TA/day than the SC with lowest productivity which results in a difference of 90% in TD by TA/day between both.

A 9.7

Collection of plasma-reduced platelet concentrates (PPC) and plasma on Trima® Accel® (TA)

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Background: Transfusion of PPC has shown clinical benefits for patients (less allergic reactions and better ABO compatibility). Moreover PPC-collection helps in collecting much more plasma by apheresis procedure (AP). The goal of this study was to evaluate routine feasibility of PPC-collection with TA and its impact on plasma collection/revenue by AP.

Materials and methods: PPC and plasma AP were performed on 55 routine donors. PPC were collected at a concentration of $3500 \times 10^3 /l$ in plasma. AP was performed at the blood bank using a single PRP column. The aim was to collect 452 ml of plasma resulted in an extra revenue of 41.2 per AP compared with AP with platelets in plasma. No donor adverse event was reported.

Table 2. Mean (SD) of %CD62-positive cells

| Day | n | Before activation | After activation |
|-----|---|--------------------|------------------|
| Plasma | 14 | 16.1 (4.2) 62.2 (8.0) | 22.1 (3.4) 62.2 (8.0) |
| Composol | 19 | 19.8 (6.5) 53.3 (10.1) | 22.7 (3.7) 53.3 (10.1) |
| T-sol | 7 | 34.4 (5.0) 37.1 (11.0) | 44.2 (3.4) 37.1 (11.0) |

Discussion: In contrast to PCs stored in plasma or Composol, PCs stored in T-sol show a higher percentage of CD62 expression. Both PCs stored in Composol and T-sol have a lower responsiveness, as of day 1.

A 9.8

Evaluation of a filter for leukocyte reduction of pooled platelet concentrates

J Leal

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Background: The minimum leukocyte reduction (LR) level is more difficult to achieve in platelet concentrates (PC), particularly when they are obtained by the platelet-rich plasma (PRP) method. The flow influences the filter performance. The aim was to evaluate the effectiveness of a filter for the leukocyte reduction of pooled PC obtained from PRP.
Material and methods: Twenty-one pooled PC obtained from PRP were filtered using the system BioP 10 Plus BBSS 8P [Fresenius HemoCare, Germany] with a manually adjustable flow control. The pools were tested before and after filtration. The evaluated parameters were the volume, volume loss, leukocyte count, leukocyte retention, platelet count and plate recovery. The LR capability was evaluated having the Council of Europe (CE) recommendations as standard.

Results: See table [mean ± SD, n = 21 pools].

| Parameter               | Before-filtration | After-filtration |
|-------------------------|-------------------|------------------|
| Pool vol. (ml)          | 352±1 ± 45.2      | 327.8 ± 42.2     |
| Vol. loss (ml)          | ---               | 24.35 ± 5.5494   |
| Leukocyte count         | 51.7 ± 38.97      | 0.0323 ± 0.0377  |
| (<10^7 single unit equiv.) | ---             | 99.9444 ± 0.0578 |
| Leukocyte retention (%) | ---               | 99.9444 ± 0.0578 |
| Log 10                  | ---               | 3.5 ± 0.44       |
| Platelet recovery (%)   | ---               | 95.78 ± 4.36     |

The mean flow rate was 57 ml/min.

Discussion and conclusion: The obtained LR levels comply with the CE standards. However, in accordance to other studies and to the manufacturer specifications lower flow rates (30 ml/min) could increase the LR capability up to 4 log. The global analysis of the results seems to demonstrate the effectiveness of the evaluated filter for the tested parameters.

A 9.9

Effect of lack of agitation of platelets stored in PAS-III M as assessed by in vitro assays

HS Hormey,1 S MacDonald1, O Drummond1, K McColl1, HA Leaver1, IR MacGregor1 and CV Prowse2

1Scottish National Blood Transfusion Service, National Science Laboratory, and
2South-East Scotland Blood Transfusion Service, Edinburgh, UK

The aim of this study was to investigate the effects of storing platelets in PAS-III M without agitation. Two concentrations of platelets were used. Four identical platelet pools were eventually performed. The platelets and plasma were collected using the ‘LRS procedure time was 52 min (range 36–80, median 53). Average platelet yield was approximately 1350 x 10^6 /single unit equiv.)

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A 9.10

Evaluation of Gambro TRIMA automated BCT system version 4

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Objective: To evaluate the performance of the Gambro TRIMA Automated BCT System V4 in blood bank setting.

Method: The evaluation involved 20 voluntary, regular Apheresis donors. Written consent was obtained for the procedure. A total of 20 single-donor-platelet procedures were eventually performed. The platelets and plasma were collected using the ‘LRS Platelet and Plasma’ sets. Platelet yield of 4.0 x 10^11/bag was targeted for every procedure.

Result: The first three donors experienced prolonged procedure time due to technical problems in that the RBC detector was out of range. After adjustment this problem did not recur. The other 17 donors were successful in their donation and their average procedure time was 52 min (range 36–80, median 53). Average platelet yield was 4.1 ± 10^11 (range 3.4–4.7, median 4.0). Average volume of plasma collected was 326 ml (range 300–473, median 300). Average PCs volume was 243 ml (range 185–250, median 250). 100% had <1 x 10^6 residual leucocytes.

Conclusion: Evaluation showed that the 52 min procedure time is relatively short compared with Haemonetic MCS + system (95 min) and Baxter Amicus system (90 min). Moreover platelet yield is sufficient for a transfusion dose and comparable with the other systems. Therefore the Gambro TRIMA Automated BCT System could be considered for use in Singapore Blood Bank setting.

A 9.11

Recovery and flow cytometry of cryopreserved platelets: controlled vs. uncontrolled – rate freezing

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Aim: To investigate of correlation intergroup variations of the platelet count, morphological, functional recovery and surface antigen expression.

Material and methods: Platelet concentrates were examined before and after uncontrolled-rate and our own controlled-rate cryopreservation (using 6% DMSO). The platelet count was determined by Technicon H-3. Morphological score of platelets (MSP) was examined using phasecontrast microscope. Cell functions were estimated using hypotonic shock response and aggregation with ADP. Surface antigens were investigated by monoclonal antibodies anti-CD41 (Serotec), anti-CD42b, anti-CD62p, anti-CD63 (CLB), and Annexin V-FITC (Roche), using Epics XL.

Results: Platelet count (51.1 ± 5 vs. 86.2 ± 6), the recovery of discs (57.9 ± 3 vs. 51.6 ± 4) were higher and MSP (294 ± 7 vs. 270 ± 10) were superior (P = 0.01) in controlled-rate setting. Also, better HS-answer and aggregation were obtained in controlled-rate group. Differences between cryopreserved vs. control group for GPIb/CD42b, CD62p, CD63 and PS was significant (P < 0.05), regardless the freezing procedure used. Contrary, CD62p expression was higher (P = 0.05) in controlled-rate vs. uncontrolled-rate setting.

Conclusion: Controlled-rate vs uncontrolled-rate cryopreservation resulted in higher percent of viable platelets and less decrease of the GPIb expression – with better expected postthaw platelet in vivo recovery.

A 9.12

Quality analysis of blood components obtained by automatic collection system

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Blood components can be obtained by conventional manual method from whole blood as well as by apheresis methods. The aim of our study was to estimate the quality of three components (platelet concentrates PCs, red blood cells RBCs and plasma FFP) collected within apheresis procedure which were intended to collect all this components during one apheresis procedure. Products were processed with blood collection separator Trima (Gambro). Platelets count, leukocytes count, hypotonic shock, expression of CD42b and CD62p antigens were studied in platelet concentrates. Erythrocytcs count, platelets count, leukocytes count, hematocrit, hemoglobin and percent of hemolysis were studied in red blood cells. Platelets, erythrocytes and leukocytes counts were studied in plasma. We obtained: PCs with yield 3–7 x 10^11 platelets/U; RBCs units with 284 ± 5 ml volume and 64 ± 2% hematocrit and plasma units with 200 ml volume. We didn't observe significant change in pH of RBCs and PCs during storage. Percentage of hemolysis in RBCs didn't extend 0.4% in last day of storage. We also did not observe significant differentiation in expression of antigens during storage in PCs. All products were leukodepleted e.g. they had <1 x 10^6 leukocytes/U. Blood collection separator Trima is a simple, easy to use apparatus. We can obtain any combination of three products from one donor using Trima. There products have good quality and parameters according to programmed values.

A 9.13

Processing platelets from pooled buffy coats for the Intercept®system

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Platelets in additive solution for a photochemical treatment (PCT) using the Baxter Intercept System must meet specifications defined for platelet concentration, red cell content and percentage of plasma in the final product.

Material and methods: Whole blood is collected into Macopharma, Leucoflex LCR5 and Intercept System must meet specifications defined for platelet concentration, red cell content and percentage of plasma in the final product.
A 9.14

The impact of Trima Accel on a blood transfusion center apheresis unit
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Background: To evaluate the impact of Trima Accel (Tr5) on donors, collection and
platelet (plt) products, taking as comparison Trima version 4(Tr4).

Material and methods: A total of 70 donors, 64 males and six females, underwent
single-dose (SDP) and double-dose platelethpheresis (DDP) using both versions. The
donors were monitored for the amount of citrate infused and adverse reactions. For
each collection, the time (T), efficiency (CE) and rate collection (CR) were evaluated.
For plt products, the evaluation was the difference between obtained and programmed
yields, split capability and leucocyte content. A failure was defined as plt yield <2 x10^{11}. The content of the LRS chamber was evaluated.

Results: Tr5 had a 17.5% reduction in the infusion of citrate and a drop of adverse
reactions from 11.8 to 2.9%. For SDP the decrease in TC was more evident in females
than in males (14.5% vs. 5.4%) and the DDP TC was 6.7% faster with Tr5. CE and CR
were improved with Tr5, 17.5 and 30%, respectively. The obtained yields were higher
than the programmed in both devices (mean differences 0.5 x10^{11} and 0.2 x10^{11} for
Tr5 and Tr4, respectively). There were four failures with Tr4, three of them charac-
terized by a LRS chamber fulfilled with plt aggregates, but none with Tr5. The new
device allowed the diversion of seven donors from SDP to DDP. All products collected
by both devices met the criteria for leucodepletion.

Conclusions: Tr5 provided a better approach in terms of donor safety, process effi-
ciency and productivity.

A 9.15

The evaluation of a new sterile docking device, Denco TCD® B40
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Objective: To evaluate a new sterile docking device TCD® B40, Denco Inc., focusing on
the reliability, weld quality, training, usability, safety, service and maintenance.

Methods: Four TCD® B40 devices were tested. In the laboratory phase, two different
plastic tubes, with different dimensions and wall thickness, containing various blood
components were welded together. The integrity and strength of 100 wet-to-wet
connections was tested using an over-pressure-tester. In the production phase, more
than 20 blood bank technologists made 6000 welds in pooled platelet production. All
welds were inspected visually. Over-pressure tests were performed weekly.

Results: During the laboratory phase, six over-pressure tests were failed. During the
production phase, none of the 160 tests were failed, instead about 70 welds were found
to leak during production. Majority of these was due to a user error. Operators typically
had a learning curve to reduce the number of faulty welds from up to six per day at the
beginning to down to 0–1 per day after 3 days of operating. The smoke emitted during
welding, difficulty of setting the tubes to the tube holders and the overall time required
for welding caused complaints. Service or maintenance were not needed.

Conclusion: The TCD® B40 was found reliable and overall weld quality was found
good. The usability and safety, however, should be improved to ease the training, to
require less experience and care from the user, and to better trap the emitted gases.

A 9.16

MCS®+platelets show good function preservation stored to 7 days as assessed by thromboelastography
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Background: Preservation of platelet functionality with extended storage is important
in order to ensure the efficacy of the platelet product. With the thrombelastogram,
(TEG), the angle (slope) measures the rate of fibrin – platelet clot formation and
the maximum amplitude (MA, mm) is a function of the participation of both platelets and fibrinogen in clot formation and strength.

Methods: Apheresis platelets were manufactured using the MCS®+device (Hemo-
netics, Braintree, MA, USA) using either the SPDP-D (n = 4) or SPDP-E (n = 5) protocol.
A sample was removed from each product for testing on day 1, day 5 and day 7. The
platelet rich plasma (PRP) was diluted with platelet poor plasma (PPP) to a count of
100 x 10^{11} / l. Both the PRP and PPP samples were studied. Either 110 ml of PRP or PPP
were placed in the TEG cups and 20 ml CaCl2 (0.25 mol) was added. Ten millilitre of a diluted
Russel Viper Venom (RVV) solution was then added to initiate coagulation. No exo-
genous phospholipid was added. TEG tracings were obtained up to 1 h per sample. For
MA assessment, the MA in the PPP was subtracted from the PRP-MA, in order to
remove the fibrinogen contribution to the MA. Data were analysed using ANOVA.

Results: Results are presented in the table. Data are the means ± 1 SD.

|                | Day 1 | Day 5 | Day 7 |
|----------------|-------|-------|-------|
| Angle (%)      | 78.2 ± 2.4 | 75.6 ± 6.3 | 78 ± 6.4 |
| Angle (°)      | 4.01 ± 7.6 | 4.30 ± 8.2 | 4.65 ± 8.3 |
| P              | 0.76   | 0.18   |       |

Conclusions: Platelet functionality as assessed using these measures was well pre-
served to 7 days.

A10 Fresh frozen plasma

A 10.1

Donation of autologous plasma in patients undergoing hepatic surgery
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Purpose: The significance of an autologous blood donation program for patients
planning hepatic surgery was compared between the two periods, i.e. before-and-after
the initiation of this programme.

Methods: First, whole blood ([O.1 × BV × H]/(1-H)) was drawn. Autologous plasma
([O.1 × BV × H]) was separated from it by gentle centrifugation and red blood cells
were reinused to the patient. Second, whole blood ([O.1 × BV] was drawn again,
centrifuged, and autologous plasma ([O.1 × BV × H]) was obtained. Concentrated
red cells ([O.1 × BV × H]) were stored in a refrigerator at 4° and plasma ([O.1 × BV] was stored at –40°. To prevent further anemia, patients were administered
EPO (24000iu/W). Results: For seven patients (4 males, 3 females) whose mean age was
69 years (range 57–80) and whose body weight was 50kg (45–59), the donation period
was 11 days (6–15), the volume of red blood cells donated was 814ml (300–1350), and
autologous plasma was 761ml (210–1380). EPO effectively prevented anemia (pre-
donation Hb 12.3, pre-operation Hb 11.8 g/dl) and the average fall in serum protein
concentration was small (6.3, 6.1g/dl). Perioperative liver function, and coagulation
parameters were also the same for the two periods. Four of the seven patients avoided
homologous blood.

Conclusion: Collection of autologous plasma at 10% per blood volume in addition to
red blood cells is feasible. However, the significance of the procedure seems obscure,
because the number of patients studied was small.

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A 10.2 Methylene blue treated plasma: pharmacokinetic and toxicological profile of MB and photoproducts
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3Blood of the German Red Cross Chapters of NSTOB, 4MacoPharma Tourcoing/France and Langer/Germany
Aims: The MacoPharma Therafflex® System uses MB and visible light for virus inactivation of plasma for transfusion. Addition of MB in a concentration of 1 µM. After illumination MB and its photoproducts (Azure B, Azure A/C, Thionine) are removed by an integrated depletion filter (final dye concentration 0.1 µM). To investigate the toxicological profile of MB and photoproducts, a series of toxicological investigations were conducted.
Methods: Genotoxicity studies were performed with MB and photoproducts in vitro and in vivo. The investigations in vitro were performed in rats by i.v. infusion with doses of ≥20 mg/kg body weight. Pharmacokinetics, reproductive and single toxicity were also investigated. Light-treated MB plasma was also administered intravenously to beagles, and haematological and biochemical parameters were examined.
Results/conclusion: In vitro genotoxic effects of MB and Azure B were observed in prokaryotes and mammalian cells, but not in various rat organs following i.v. infusion of MB and its photoproducts. Infused 14C-MB and Azure B are rapidly cleared from the circulation and from organs: After 24 h infusion >1% MB was detected in clinically relevant organs during the observation period of 48 h. No signs of intolerance were observed in beagles after infusion of light-treated MB plasma. General toxicology studies showed no toxicologically relevant effects of MB at the dose level administered during routine clinical use of MB plasma.

A 10.3 The influence of methylene blue-light treatment on fibrinogen activity and fibrin polymerization
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Background: Methylene blue-light treatment (MBLT) is efficient in inactivating viruses in plasma. However, it may cause alterations in fibrinogen (Fg). We investigated the effects of MBLT and subsequent use of MB removal filter (MBRF) on Fg activity and fibrin polymerization.
Methods: Apheresis plasma (n = 10) were processed as follows: (i) WBC filtration; (ii) the filtered plasma was treated, using macopharma system; (iii) removal of MB and its metabolites by filtration. Plasma samples from each step were tested for alterations in Fg activity, Fg antigen (Ag), thrombin time (TT), reptilase time (RT) and fibrin polymerization curves.
Results: Fg activity decreased after MBLT (mean 3.12, 2.20, and 2.11 g/l, for samples from steps A, B, and C, respectively) whereas Fg-Ag remained stable (mean 2.70, 2.75, and 2.79 g/l). TT was significantly prolonged (mean 6–7 s) after MBLT with no further changes after MBRF. A similar trend was observed using RT. Fibrin polymerization triggered by both thrombin and reptilase were delayed and the slopes of the polymerization curves were decreased slightly, with concomitant changes in fibrin opacity, in groups B and C as compared with A. Conclusion: MBLT resulted in only a slightly decrease in Fg activity and fibrin polymerization. The slope of the polymerization curves were decreased slightly, with concomitant changes in fibrin opacity, in groups B and C as compared with A.

A 10.4 Octaplas and Uniplas efficacy and safety in obstetric and gynaecological emergencies
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The efficacy and tolerance of solvent-detergent plasma (SDP) was evaluated in all transfused women (n = 38) in two large maternity hospitals from April 2002 through October 2003. One patient’s record was not traced and was excluded.
Results: Thirty-eight women (mean age 34.7 ± 12.9 years) received 57 transfusions (Octaplas – 36; Uniplas – 2), mean dose 958 ± 492.4 ml per patient (15.3 ± 7.7 ml/kg). 36/38 patients had severe haemorrhage (mean blood loss 3345.8 ± 2738.1 ml). One woman with factor V deficiency received Octaplas before LSCS and one for HELLP syndrome. 22/38 (58%) had abnormal coagulation screens before plasma transfusion. In these patients mean blood loss and plasma volume transfused were higher than in those without coagulopathy. Pre- and post-transfusion APTT was measured in 41 transfusions, PT and fibrinogen in 42. Mean APTT improved from 50.1 ± 18.4 to 32.7 ± 6.9 (t = 6.40; P ≤ 0.001); PT from 21.0 ± 5.2 to 15.6 ± 1.9 (t = 7.71; P ≤ 0.001); fibrinogen from 15.5 ± 0.7% to 2.74 ± 0.86 (t = 9.15; P ≤ 0.001).
Conclusion: Octaplas and Uniplas in therapeutic doses have very good overall clinical tolerance in patients with obstetric and gynaecological emergencies and are associated with correction of coagulopathy and favourable outcome.

A 10.5 Plasmapheresis with the PCS2: version G.1 software
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Objective and design: In 2001, Haemonetics® introduced the ‘high separation’ core bowl (HS bowl) and software version G to collect virtually cell-free apheresis plasma with the PCS2. Recently, a revised software version G.1 was presented. In this study we validated the G.1 software with the 622HS and 623HS disposables.
Methods: Procedures: Performing (A) 622HS (n = 75); (B) 623HS (n = 79). In all procedures, the plasma was concentrated 10x for in vitro analysis. Residual WBCs (flow cytometer), RBCs (Burker) and platelets (automated impedance counter) were measured.
Results:

| Procedures | A (n = 75) | B (n = 79) | Requirements |
|------------|-----------|-----------|--------------|
| WBCs (10³/µl) | 0.1 ± 0.08 0.03–0.4 | 0.1 ± 0.09 0.02–0.5 | <3 |
| Mean (±SD) | Range |
| RBCs (10⁹/l) | 0.6 ± 0.6 0.6–99 | 0.6 ± 0.6 0.6–420 | ≤6000 |
| Median (±SD) | Range |
| Platelets (10³/l) | 1.4 ± 0.9 2.4–4.6 | 1.4 ± 0.8 1.0–3.1 | ≤50 |
| Mean (±SD) | Range |

*In 151/154 (98.1%) of the procedures, a range of <0.6–3.9 × 10³ RBCs/l was found. In three procedures a range between 99 and 420 ± 10³ RBCs/l was found. No irregularities were observed during the 154 plasmapheresis procedures.

Conclusions: In 100% of the cases, the plasma quality was according to the (European) requirements. In 2% of the procedures outliers from the median RBC cell count were found, but still within the specifications.

A 10.6 The effect of storage at +4°C for 5 days on coagulation factors in FFP
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Introduction: Over the past 5 years FFP usage in the UK has increased by 20%. Although there has been improvement with regards to the correct usage of FFP, there is still a considerable amount of FFP wastage at our hospital (245 U in 8 months) due to incorrect ordering procedures and the short ‘time-to-transfuse’ window (currently 4 h). To see if FFP wastage could be decreased further by increasing the ‘window’ to 24 h or longer, the levels of factors II, V, VII, VIII and IX and in thawed FFP stored for 4°C at 5 days were measured.
Materials and methods: Coagulation factor levels were measured using an ACL 9000 analyser. INR, APTT and fibrinogen were measured using the ACL Advance Fuga. FFP was obtained from NBS and Octapharma and thawed using either a waterbath, a dry heat oven or a microwave oven.
Results: Factor VIII activity declined by 35.2 and 17.5%, in NBS and Octapharma FFP, respectively, after 24 h storage at 4°C, and 56% in both products after 5 days storage at 4°C. INR, APTT, fibrinogen level, and factors II, V, VII and IX activities did not show any significant changes, even after 5 days storage at 4°C.
Discussion: This study supports the new UK FFP guidelines which state: ‘FFP can be stored at ≤4°C in an approved blood storage refrigerator, before administration to the patient so long as the infusion is completed within 24 h of thawing’. From our results it would appear that the ‘time-to-transfusion’ window could be increased even further.

A 10.7 Cia and Csa in plasma from different membrane and centrifugal apheresis procedures
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Background: Complement activation may occur during plasma collection. We have compared Cia and Csa in plasma from two automatic membrane apheresis (Baxter AutoC- and Haemonetics Filter Core [FC]) and from three centrifugal apheresis (Haemo- mechanics PCS3 Rev F, Rev G, and High Separation Core [HSC] procedures).
Materials and methods: Five groups of 30 donors donated with each five procedures and another group of 10 again with Auto-C. Plasma was assessed after various periods of storage. Results: Mean C3a was 4724 ng/ml (range: 2400–7360; 18 months storage) and >4149 ng/ml (2408–>4640; 3 months) and mean C5a 32.1 ng/ml (10.6–57.2; 18 months) in Auto-C groups. Mean C3a in FC was 1151 (526–2991), 1092 (349–3498) and 507 (307–815) ng/ml and mean C5a was 26.6 (4.9–74), 18.9 (9.5–42.6) and 30.9 (10.7–62.3) ng/ml at days 0 and 6, and 12 months, respectively. Mean C3a, at 18 months, was 903 (422–1707), 439 (156–1271), and 482 (4–1964) ng/ml in Rev F, Rev G, and HSC, and mean C5a was 9.8 (9.2–39), 20.2 (6–35), and 12.5 (0.8–27) ng/ml, respectively. Conclusion: Significantly less (P < 0.0001) C3a in plasma from the three PCS2 centrifugal and the FC membrane apheresis procedures than from Auto-C membrane apheresis. C5a similar in Auto-C and FC, and more than in Revs F&G, and HSC. Differences in biocompatibility of apheresis procedures may influence complement activation. Impact on tolerance of transfusion plasma and quality of plasma for pathogen reduction treatment or fractionation should be considered.

A11 Plasma-derived coagulation proteins

A 11.1 Self-sufficiency in anti-D immunoglobulin in Brazil

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Background: The worldwide supply of anti-D immunoglobulin is dependent on anti-D plasma collection in the USA. We studied the prevalence and the titration of anti-D in Brazilian donors, in order to estimate if this source could cover the country demand. Methods: During a 41-month period, 339 221 blood donors were submitted to antibody screening; if the test was positive, an antibody identification was performed. The donors with an anti-D were then called for an anti-D titration. For calculating the need of anti-D we estimated a 300 ml dose per woman, 3 million deliveries per year, and at least 32 would be eligible for entering the plasmapheresis programme, and we estimated that each eligible donor would donate 4 l of plasma per year.

Results: 366 donors (0.1%) presented an anti-D. 315 out of 80 678 female blood donors presented an anti-D (6.39%). Among the Rh-negative female donors, the anti-D prevalence was 3.98%. The mean anti-D titration was 16, and 20% of donors had a titration greater than 32. There would be 3000 female donors with an anti-D. At least 366 donors with an anti-D were then called for an anti-D titration. For calculating the need of anti-D we estimated a 300 ml dose per woman, 3 million deliveries per year, and at least 32 would be eligible for entering the plasmapheresis donation programme, and we estimated that each eligible donor would donate 4 l of plasma per year.

Conclusion: Theoretically, Brazil would be self-sufficient in anti-D, if it started a plasma fractionation programme.

A 11.2 Risk of Parvovirus B19 transmission through plasma derivatives obtained from Brazilian plasma

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Background: Parvovirus B19 is a non-enveloped virus that resists to viral inactivation techniques. The infection can be life-threatening in immunodeficient recipients. We present the results of nucleic acid test (NAT) for Parvovirus B19 in Brazilian plasma sent for fractionation.

Methods: From November 2002 through November 2003, 169 547 plasma bags were tested for Parvovirus B19; these bags were collected on the South-East part of Brazil. The NAT test was done on 100 samples pool, by in house PCR. Results: Eight positive pools were found, representing a 4.7/100.000 rate of Parvovirus infection. One infection for each 21 201 donations would be expected. As each blood donation is transfused, on average, to 1.6 persons, in Brazil, the risk for Parvovirus transmission by blood transfusion is 1/13 250. However, near 85% of Brazilian adult population have protective antibodies against B19; therefore the risk for acquiring the virus through blood transfusion is quite small. Nevertheless, considering that the plasma batch size in fractionation industry is at least 10 000 bags, one out of two batches of plasma derivatives produced from Brazilian plasma would be contaminated by B19.

Conclusion: The Parvovirus prevalence in Brazilian population shows that testing manufacturing plasma for Parvovirus B19 in mini-pool is crucial, in order to minimize the risk of virus transmission through plasma derivatives administration.

A 11.3 Passive HTLV-I/-II antibody in pregnant patients receiving IVIG therapy for NAIT

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Intravenous immunoglobulin (IVIG) is a concentrated immunoglobulin purified from large pools of donor plasma. Depending on the protocols of the manufacturers, some do not include screening tests for the human T-lymphocyte viruses (HTLV-I/-II) on plasmapheresis donors. IVIG blocks the reticuloendothelial system and it has been used to treat many immune disorders including neonatal allomunence thrombocytopenia (NAIT). Recently we have experienced with two healthy pregnant women, with a previous history of an infant with NAIT, who required regular, prophylactic IVIG therapy during the second and third trimesters of their pregnancy. Prior to elective delivery, maternal platelets were collected. In both women, the results of the pre-donation ELISA screening test for anti-HTLV-I/-II were positive. The confirmatory western blot was found to be negative in one and indeterminate in the other. According to Health Canada regulations, both women were ineligible for directed blood donation to their newborns. However, subsequent repeat testing for anti-HTLV-I/-II by ELISA > 3 months after each delivery were negative. Thus, it is likely that the anti-HTLV-I/-II antibodies (Ab) detected were passively acquired from the IVIG infusion. Passive transfer of Ab against syphilis, hepatitis A and B, cytomegalovirus, and HIV (before 1989) following the IVIG therapy has previously been reported. These two cases represent the first report of the passive acquisition of anti-HTLV-I/-II Abs through IVIG infusion.

A 11.4 Efficacy of a ‘home-made’ fibrin glue in reducing bleeding in liver resections

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Introduction: Liver resection is related with haemorrhagic risk, due to the difficulties to obtain an effective haemostasis on liver with the standard techniques. Several reports show the efficacy of FG in reducing bleeding. We compared the efficacy of a commercial fibrin glue with a ‘home-made’ product obtained through an automated device. Methods: 30 patients undergoing liver resection for cancer have been evaluated. Ten patients received ‘home-made’ FG, while the others received Tissucol Baxters. Age, sex, weight, height and peripheral blood volume (PBV) were similar in the two groups. In all the patients the same amount of FS was used on the liver cut surface. ‘Home-made’ FG was made with Cryosel Thermogenosis DISITECO system. Blood loss occurring from presurgery to day 5 was evaluated in terms of RBCs loss according to the formula: Blood loss = PBV × (Basal Hct - day 5 Hct) + RBC transfused.

Results:

| Parameters | ‘Home-made’ FG | Commercial FG | Student t-test |
|------------|---------------|---------------|---------------|
| Basal Hct% | 41.09 ± 4.02  | 39.97 ± 5.22  | NS            |
| Cut surface cm^2 | 138.33 ± 130.7 | 94.45 ± 66.4 | NS            |
| Patient transfused [%] | 2 (20%) | 5 (25%) | NS            |
| Vol. RBC transfused/patient (ml) | 100 ± 216 | 130 ± 285 | NS            |
| Hct day 9% | 36.3 ± 4.3   | 33.2 ± 3.6   | P < 0.05      |
| RBC loss (ml) | 30 847 ± 26 706 | 44 607 ± 33 818 | NS            |

Conclusions: ‘Home-made’ FG seems to be at least as much effective as commercial FC in reducing bleeding in liver surgery.

A 11.5 Random quality control: Is it always the right approach?

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Objective: The difference in coagulation factors levels in cryoprecipitate units, in relation to ABO blood group has been well documented. This study refers to the
practical aspects of this biological phenomenon and its impact on quality control performance.

**Materials and methods:** Factor VIII and fibrinogen were determined in 365 units of cryoprecipitate prepared from whole blood stored for 4-18 h at 20-24 °C using, 1,4-butanediol cooling trays. Measurements were performed by coagulation analyzers: Factor VIII-ACT 300 (Instrumentation Laboratory), Fibrinogen-CA-1500 (Symyxex).

**Results:**

| Factor | Parameter | Group O (n = 82) | Group A (n = 130) | Group B (n = 117) | Group AB (n = 36) |
|--------|-----------|------------------|------------------|------------------|------------------|
| Factor VIII | Mean ± SD, IU/unit | 994 ± 40 | 1404 ± 52 | 1454 ± 45 | 1404 ± 46 |
| % Conforming units | 79 | 96 | 98 | 100 |
| Fibrinogen | Mean ± SD, mg/unit | 286 ± 87 | 290 ± 95 | 281 ± 79 | 298 ± 92 |
| % Conforming units | 100 | 99 | 99 | 100 |

Percentage of conforming units according to Factor VIII limit ≥ 80 IU/unit and Fibrinogen ≥ 150 mg/unit. Conformation with the AABB standards (100% units > 80 IU Factor VIII/unit) was achieved in units from A, B, and AB groups. Group O cryoprecipitate contained 1.4 times less FVIII (P < 0.05) with only 78% of the units meeting the required criteria. No significant difference (P > 0.2) in fibrinogen content was found.

**Conclusion:** Official requirements for cryoprecipitate quality should consider the ABOgroups distribution in the donor population. Cryoprecipitate should be tested in pools, built so they will represent the percent of the different blood groups in a given population.

**A 11.6**

Use of one-stage clotting method for determination of factor VIII activity during manufacture

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The FVIII activity during manufacture of purified, twice virus inactivated preparation of the FVIII from cryoprecipitate was supervised by one-stage clotting method and with a chromogenic substrate. The FVIII activity was measured in keep with advisable NIBSC technique, using for construction of calibrating curve SHP calibrated against WHO-standard. Reagents Dafttin (Baxter), Pathromtin (Dade), Platelein L (Organon), Alexin HS and APTT Reagent (Sigma), STA APTT Kaolin (Stago), Erylgy-kaolin and APTT-ellagic acid (local production) were used as APTT-reagents. The FVIII activity determined by various APTT-reagents changes from 8.0 up to 12.0 IU/mL. Thus the FVIII activity determined in the same sample by chromatographic substrate and the same SHP made 12.5 ± 1 IU/mL. Closer to this magnitude was the activity, received by APTT-Reagent, Erylgy-kaolin and APTT-ellagic acid. However, at use as the standard the 6th International Standard FVIII-C, concentrated, the value of activity determined by a method of chromogenic substrates, consists 11.6 ± 1.0 IU/mL. Values close to this value of FVIII activity have been received by Pathromtin, Dafttin and Platelein L.

The values of activity received with STA APTT Kaolin and Alexin HS, were much lower and made 7.8-8.5 IU/mL. The resulted data testify to an opportunity to use one-stage clotting method for definition of FVIII activity in cryoprecipitate. Thus it is necessary to compare preliminary the values of activity, received by two various methods.

**A 11.7**

A quantitative PCR method for HCV removal validation in plasma-derived products

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Viral validation is a prerequisite for evaluation of plasma-derived products. This study aimed to develop the quantitative PCR method for removal validation of HCV from plasma-derived products. The standard for quantification was established from high titer of HCV RNA positive plasma, assigned an 5.9 × 10⁶ IU/ml of HCV RNA. The nested RT-PCR was performed after extraction viral RNA by QIAamp viral RNA isolation kit. The first round of PCR was done by GeneAmp PCR System 9700™ and for the amplifications were amplified by LightCycler™ to quantify HCV RNA. Several quantitative PCR conditions such as annealing temperature, MgCl₂ concentration, and primer concentrations were optimized. The linearity was conserved with the diluted series of the quantitative standard ranging from 5.9 × 10⁵ to 5.9 × 10⁴ IU/ml. The coefficients of variation values of the inter- and intra-assay reproducibility were 6.61 and 0.79%, respectively. Coagulation factor spiked with HCV RNA positive plasma was filtered with viresolve NFP filter and the reduction factor was 4.63. With this system, it was concluded that this system could provide a high level of confidence and cost saving step as a tool of HCV removal validation in the plasma-derived products.

**A12 Immunoglobulin therapies**

**A 12.1**

Stability level of polymers and aggregates in the rabies immunoglobulin

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Rabies immunoglobulin is a liquid- or freeze-dried preparation containing human immunoglobulins, mainly immunoglobulin G (IgG). On the basis of the quality control procedures, Distribution of molecular size test item was chosen for the stability test being sensitive enough to indicate alterations of the preparation during the shelf-life period. Samples stored at 2-8 °C for up to expired date. The lots tested were formulated with the container-closure system for marketing. The basic principle of size exclusion chromatography (SEC) is that molecules are partitioned between solvent and a stationary phase of defined porosity. The larger molecules are excluded from the stationary phase and therefore elute first the column. The acceptable criterion for the sum areas of polymer and aggregates in this preparation is not more than 5% of the total area of the chromatogram. So, high level of Polymers and aggregates in bio-molecules which are used clinically can raise the incidence of allergic reaction including sever anaphylactic shock in the patients. In this study, the level of polymers and aggregates in 20 batches of local and imported rabies immunoglobulin were measured by using SEC with HPLC system. The results shows that the percent of the total area related to polymer and aggregates are significantly variable during the shelf-life period. There is no difference between local and imported products.

**A 12.2**

Mannan-binding protein and α2 macrogluglobulin as innate immunity factors in Serbian blood donors

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Mannan-binding protein (MBP) is an acute phase protein, which activates complement through the so-called lectin pathway. Lectin pathway of activation is initiated by binding of MBP to repeating carbohydrate moieties. It is a C-type lectin which preferably interacts with mannose and N-acetylgalcosamine residues. MBP is physically associated with two serine proteases, MASp-1 and -2, their activity being regulated primarily by the α2 macrogluglobulin (α2M). Several lines of evidence indicated that the presence of specific immunoglobulins has a significant enhancing effect on haemolysis via the lectin pathway. In this study, mean concentration values for MBP and M were determined in blood donors. Concentrations were determined using laser nephelometric assay and radial immunodiffusion. Preliminary results show that MBP concentrations in our population are higher compared with data available in literature, although it does not refer to α2M. Likewise, statistically significant differences were demonstrated in the distribution of MBP concentration among various blood groups: A vs. 0, 0.007; B vs. 0, 0.08; A, B, AB vs. 0, 0.04. Noted differences might indicate varying immunological competence in different blood types. The preliminary results of this study point to the need of further investigation.

**A 12.3**

Comparison of toxoplasma antibodies with IF and ELISA techniques

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Toxoplasmosis is caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*. Transmission may occur by eating uncooked meat, contaminated vegetables,
blood transfusion, organ transplantation, and across the placenta from the mother to the fetus. Antibodies to T. gondii may persist in the serum at high titer for years. Sera from 50 patients suspected to toxoplasmosis (32 females and 18 males) were collected for detecting toxoplasma IgG and IgM antibodies by ELISA technique and toxoplasma antibodies by IF (immunofluorescence) with total anti-human immunoglobulin. Sera from 50 patients’s sera tested for toxoplasma IgG antibody (by ELISA) and IF method, 35 (70%) sera were positive. Frequency of toxoplasma IgG positivity in female (78.1%) is higher than male (55.6%), but this difference is not significant \(P = 0.089\). Eighteen patients (36%) were positive for toxoplasma IgM antibody, which is significantly different from controls \(P < 0.05\). There is good correlation between ELISA results for IgG antibodies with IF results. Our result showed there is a good relationship between IF and ELISA results for detecting IgG antibody. Unfortunately we did not detect IgM antibody by IF technique. It is suggested for detection of IgM antibody the ELISA method is preferred because of its greater sensitivity.

A 12.5 Prophylactic use of the cytomegalovirus immunoglobulin in the kidney transplantation
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Background/Aims: The risk of cytomegalovirus (CMV)-induced morbidity and/or mortality in immunosuppressed patients underwent kidney transplantation is significant. The goals of this study were to introduce a simple screening methods for detection and preparation of the protective CMV antibodies (CMVIG) and to present the effects of CMV-disease prevention in kidney recipients.

Methods/Results: Serum testing to CMV antibodies was performed using ELISA tests (Abbott and Behring) and by semi-quantitative test (System IMX-CMVigG Abbott). Selected and collected plasma (267 ± 79 ml), was collected from 800 blood donors (age: 18–23 years) and frozen at –30 °C until fractioning. The results of Paul-Ehrlich Institute research significantly proved the quality of this preparation. In clinical setting, our own CMVIG was applied prior and after transplantation. Therapeutic protocol of the CMVIG i.m. usage was: 0.3 ml/kg on 0 day of the transplantation (6 h before procedure), and 0.3 ml/kg once a week (during next 5 weeks). CMVIG was well sustained, without undesirable side effects and post-transplantational serum control of the recipients was negative.

Conclusion: The application of CMVIG in combination with antiviral drugs was proved positive in prevention of the CMV-disease. The successful therapeutic application of i.m. CMVIG was stimulating for its further application, together with the efforts to start i.v. CMVIG production.

A 13.1 Prevention of cytostatic injuries of bone marrow by temporary inhibition of the blood forming
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Cytostatic/radiation injuries of blood-forming organs are main reason to stop successful cytostatic or X-ray therapy in cancer patients. The interrupted active proliferation of bone marrow cell precursors considerably increases the probability of irreversible cytostatic injuries, including dangerous mutations. Method of temporary inhibition of proliferation during system cytostatic or X-ray therapy in cancer patients is discussed. Such preventive temporary inhibition of proliferation in bone marrow allows practically to exclude irreversible cytostatic or X-ray injuries of blood-forming organs. Several sessions of autologous transfusion with consecutive plasma reinfusions plus stepwise cryoconservations of the viable blood cells should be carried out 1–2 weeks before cytostatic therapy. It is necessary to carry out complete final reinfusion only after the sufficient accumulation of red + white blood cells, and platelets, and the day before of short-term session of high-dose cytostatic therapy. As a rule, the negative feedback in blood-forming system supports the concentration of blood cells extremely high-precisely. The results final reinfusion of superfluous amount of the viable cells causes substantial cell concentrating of blood and stops temporarily the proliferation of bone marrow cell precursors. Offered approach is preventive and allows considerably to reduce irreversible injuries in granulocyte branch of the blood-forming, and protection of red blood branch and platelet system.

A 13.2 Collection of granulocyte concentrates from donors primed with G-CSF
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Background: The greater number of cells can be collected by leukapheresis from donors primed with G-CSF and higher cell doses administered. The aim of the study is presentation of granulocyte collection results from G-CSF stimulated donors.

Materials and methods: Family members \(n = 10\) were stimulated with the G-CSF \(5\) mg/kg of body weight s.c. in single dose, \(24\) h before collection. The unstimulated group was community donors \(n = 10\) who received dexamethason \(8\) mg i.v. Leukapheresis were performed on CS-3000 plus. The Dextran 70 000 and the Na citrate were used in collection procedure. Cells were counted on CELL-DYN 3200 (Abbotti). Results were statistically compared using Student’s t-test.

Results: Stimulated donors: PMN \(30.7 ± 8.1 \times 10^9/l\); Yield \(40.23 ± 6.42 \times 10^9/l\). Unstimulated donors: PMN \(3.4 ± 0.5 \times 10^9/l\); Yield \(16.02 ± 1.3 \times 10^9/l\). The differences between groups were statistically significant \(P < 0.01\). Mean cell dosis was \(2.48 ± 10^10/m^2\) for the patients that received granulocyte concentrates from stimulated donors and \(0.98 ± 10^10/m^2\) for patients that received granulocyte concentrates from unstimulated donors.

Conclusion: Granulocyte concentrates obtained from donors primed with G-CSF contain a larger number of collected granulocytes, which are clinically efficacious and well tolerated by recipients.

A 13.3 Erythrocyte ghosts as drug-delivery system
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The treatment of leukaemia with chemotherapeutic agents is coupled with severe side effects. Therefore, it is of great importance to deliver the drugs directly to the target cells. The aim of our project is to investigate human erythrocyte ghosts as a drug-delivery system for cyclophosphamide (CP). The permeability of molecules and the kinetics of phagocytosis of ghosts was investigated to analyse the efficiency of the drug release in dependence on the preparation. Human erythrocyte ghosts were obtained by
A15 Immunotherapy

A15.1 Phenotype and functionality of mature dendritics cells produced from PBMC of healthy donors
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Aim: The aim of the study was to define the phenotype and evaluate the functionality of mature human Dendritic cells (DC).
Materials and methods: Immature DC (iDC) were produced by stimulating adherent peripheral blood leukocytes (PBMC) from 30 healthy donors with GM-CSF and IL4 for 7 days. The yield of iDC production was similar for all donors and ranged from 15 to 100 x 10⁶ cells. Maturation of these iDC was induced by overnight stimulation with CD40-L.
Results: iDC expressed high levels of CD86, HLA-I but low or undetectable levels of HLA-II, CD40 and CD80. In contrast, mDC further up-regulated HLA-I and CD86 and expressed high amounts of HLA-II, CD40 and CD80. mDC were tested for their capacity to stimulate an allogeneic T-cell response. mDC strongly stimulated proliferation of allogeneic T-cells in a dose-dependent manner. T-cell proliferation was observed even at a T/DC ratio of 1/100. Moreover, mDC-activated naive antigen-specific T-cells. In fact, mDC from HLA-A0201 donors loaded with the HLA-A0201 bound MELAN/A peptide (ELA/GIGLTV) induced specific cytotoxic T lymphocytes after two rounds of in vitro stimulation as assessed by tetramer staining, intracellular IFN-γ staining and cytotoxic assay.
Conclusion: The mDC may be used in the induction of specific cytotoxic T-lymphocytes with different cancer peptides and could proceed, in this way, in anticancer therapy.

A15.2 Extracorporeal photochemotherapy in relapsing/remitting multiple sclerosis
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Ospedale San Gerardo, Monza-Apheresis Unit, IRCCS Don Gnocchi, Milano-Bicocca, Neurology Institute, and NovusPharma, Milano, Italy
Background: ECP has been proposed for several T-cell-mediated diseases (Systemic Sclerosis, Pemphigous Bullosus, GvHD). We performed a pilot study on RR-MS patients with 3 relapses in the last year, entered the study. Treatment schedule: two ECP/month with 8-MOP added to the yield and finally, UV-A irradiation (2 J/cm²) was performed.
Results: Patients were given 5, 6-11 and 1 x 10⁹ irradiated cells/ECP (median dose). PB WBC count did not change all over the study period, as well as CD4/CD8 ratio. Three patients had no relapses, and two had three relapses each, responsive to low-dose PDN. In two patients PDN total dose tapering was achieved and three did not require PDN all along the study period. EDSS did not worsen in four of the five patients. MS activity evaluated by means of MRI was reduced compared to the pre-ECP period.
Conclusion: We observed a decrease in the number of relapses, EDSS stabilized in 80% of the patients. Similar findings were observed as to the occurrence of new cerebral lesions in MRI after gadolinium enhancement. A prospective multicenter study is now required to assess the possible usefulness of ECP in the treatment of RR-MS.

A17 Red cell immunohaematology

A17.1 Comparison of tube technique by PEG-IAT and GLISS for antibody identification
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Background: There are several methods for antibody identification. We developed a new potentiator – GLISS, a hypotonic buffered low ionic strength solution with glycine (Sigma, USA) prepared in house for antibody identification. The purpose of this study was to compare the tube technique by PEG-IAT and GLISS for antibody identification.
Methods: We performed antibody identification using tube test technique by PEG-IAT and GLISS on 77 frozen samples of our inventory with known antibodies previously identified by association of techniques: gel test, LISS-IAT, PEG-IAT and enzyme treated red blood cells panels (Gamma Biologicals Inc., USA). PEG-IAT by tube test technique was performed as described elsewhere. Fifty microliters of 3% red blood cells suspension panel (ASEM, Brazil) was dispensed in each tube with 100 µl of serum and 100 µl of GLISS followed by reading after 15 minutes incubation at 37 °C. The tubes were washed three times with saline and 100 µl of anti-IgG (ASEM, Brazil) were added. Following centrifugation, the tubes were observed for agglutination and hemolysis.
A 17.2
Alloimmunization to red cell and platelet antigens in stem cell transplanted patients
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Background: Clinical trials have suggested that leukoreduction of blood components reduces post-transfusion alloimmunization. Stem cell transplanted patients, although immunosuppressed, are at risk for the development of red cell (RBC) and platelet (PLT) antibodies. This study attempts to determine the incidence of RBC and PLT pre and post-transplant alloimmunization.

Methods: We conducted a retrospective analysis of all patients undergoing stem cell transplants, autologous and allogeneic, between 2002 and 2003. We transfused only leukoreduced blood components and routinely screened these patients for alloimmunization on the day of the transplant (day 0). Indirect antiglobulin tests (IAT) using the microcolumn method and platelet antibody screening (solid phase tests – immune-complex formation involving drugs and detection of antibodies to cisplatin). We studied three patients, in different oncologic contexts (cancer of cavum, testis and pancreas), under different chemotherapy protocols all including cisplatin in unequal doses, with serious hemolytic episodes occurring after re-exposure to cisplatin, regardless of the clinical oncologic context and the intensity of the dose used. This drug-dependent hemolysis is a rare but potentially serious complication of cisplatin therapy, which must be taken into account.

Results: The screening for alloimmunization on day 0 facilitates the surveillance of these patients. Alloimmunization is a rare phenomenon in this group of patients despite the fact that they are one of the most heavily transfused.

Conclusion: Based on this study GLISS can be used for antibody identification. GLISS is more sensitive (P ≤ 0.01) than PEG-IAT for identifying clinically significant antibodies.

A 17.3
Drug-induced immune hemolytic anemia due to cisplatin
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Background: The first description of cisplatin therapy-associated hemolytic anemia was in 1980 [Getaz et al.; more studies have followed which include other drugs of the platinum family. Several mechanisms are responsible for the hemolysis associated to platinum derivatives. We studied three patients, in different oncologic contexts (carcinoma of cavum, tests and pancreas), under different chemotherapy protocols all including cisplatin in unequal doses, with serious hemolytic episodes occurring after re-exposure to the drug and hemoglobin drops of up to 5 g.

Methods: The patients’ sera and eluates were tested against RBCs in the presence of cisplatin and against cisplatin-treated RBCs according to the methods for demonstration of immune-complex formation involving drugs and detection of antibodies to cisplatin.

Results: The patients’ RBCs were all DAT positive (IgG). The patients’ sera reacted strongly with cisplatin-treated RBCs using the immune-complex method as well as with the drug sensitizing method (the latter used on only two of the three patients).

Conclusions: We demonstrated an antibody to cisplatin. These clinical reports have in common an episode of intravascular hemolytic anemia, which occurs following re-exposure to cisplatin, regardless of the clinical oncologic context and the intensity of the dose used. This drug-dependent hemolysis is a rare but potentially serious complication of cisplatin therapy, which must be taken into account.
cells were considered: species I (normal disks), species II (polygonal) and species III (crenated).

Results: Four groups of curves (corresponding to A, B, AB and O groups) were ob-
tained showing significant differences between them. Also the rates of alteration (K1, and K2) for each group were calculated following the Dienes’ theory.

Conclusions: It was evident that the polysaccharide terminal of each ABO antigen has an important role in the effects of low-power laser radiance at high level of applied
energy. The less altered was the B group, followed by AB, A and O groups in this order.

A 17.7 Investigation of effect of routine antenatal A/D prophylaxis on fetomaternal haemorrhage estimation
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Routine antenatal A/D prophylaxis (RAA/D) was introduced to further reduce the incidence of Rh(D) immunization from the current rate of 1.5%. This study aims to
determine the effect, if any, of RAA/D on FMH estimation and recommend the best
method of testing. Thirty-two clinical samples were tested by Kleihauer test (KT) and by
flow cytometry (FC) using direct and indirect anti-D and anti-Hf methods. Forty-eight
spiked samples representing different levels of FMH with and without addition of an
equivalent prophylactic dose of anti-D were tested by all three FC methods. With clinical
samples anti-Hf method overestimated FMH volume in the majority of samples
compared to all other methods. Direct and indirect anti-D methods were comparable.
With spiked samples, anti-Hf method significantly underestimated FMH volume
compared to the total volume. Both anti-D methods gave results close to target value
and addition of anti-D Ig made no significant difference. As expected, KT gave a wide
variation in volume compared to FC methods. There are several explanations for the
contradictory results between clinical and spiked samples for anti-Hf method including
fetal cell HbF content at birth. Results of the spiked samples show the difference between
methods is unlikely to be due to prophylactic A/D. Anti-Hf is complex and less robust,
making it more difficult to achieve accurate results. Both direct and indirect A/D methods
are suitable for use in FMH estimation even when RAA/D has been administered.

A 17.8 A new technology for antibody screening adapted to a fully automated system
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We have developed a new automated technology for the detection of irregular
antibodies suitable for automation and high throughput. The method does not require
centrifugation steps thanks to the use of magnetic red blood cells (MRC). This
methodology requires washing steps under magnetic field and is based on the fixation
of sensitised RBC on the surface of a well coated with monoclonal anti-human
globulins. In a first step, serum and ready-to-use RBC were distributed on a microplate
and incubated 10 min at 40 °C, and excess of unbound immunoglobulins is removed
by washing. In a second step, RBC were transferred in the antiglobulins-coated plate
and placed 4 min on a magnet plate. Wells in which antigen-antibody interactions
have occurred display a confluent layer of RBC (positive reaction). The negative
reaction appeared as a pellet in the middle of the well. The test can be read by eye or
by an automatic reader. The patterns in the well are stable for at least 24 h at room
temperature. Antibody-positive plasma samples were obtained from routine screening
and negative controls from random blood donors and patients. They were prior
characterised using gel tests. The first results are obtained in about 25 min, and the
detection limit is close to 10 ng/ml with anti-RH. Comparative studies showed that
our new technology, without any centrifugation steps, is reliable and sufficiently
sensitive and specific enough to detect irregular antibodies linked to RBC.

A 17.9 A new technology for blood grouping and phenotyping adapted to a fully automated system
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Current immunohaematology testing methods have limitations including throughput,
safety and adaptability to automation. DIAGAST have developed a new technology for
grouping and phenotyping suitable for high throughput automation. This method is
based on magnetic haemagglutination assays. In order to avoid all centrifugation steps,
magnetic fields are simply applied on microplates. In a comparative study, 307 samples
from healthy regular blood donors and from hospital patients were collected. The samples were tested for ABO, Rh (D, C, c, E, e) and K, and reverse grouping, in parallel,
by the new microplate agglutination method and a commercially available blood
testing system (reference method). These preliminary experiments show 97% of
concordance of the results compared to the reference method. No false-negative results
were detected using this new technology. False-positive reactions were found only in
reverse grouping tests and represent 3% of the tested cases. Moreover the use of magnetic
fields instead of centrifugation enables the detection of weak antigens (e, K, Ax) on RBC. The
global sensitivity of the test is similar to the reference technique and the
tests are performed in less than 30 min. Then, this new technique shows the
possibility to get a fully and reliable automation without centrifugation. It will ensure
transfusion safety and will be a relevant contribution to immunohaematology.

A 17.10 Prevalence and incidence of non-RhD pregnancy immunization in the Netherlands
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Background: From 1998 Dutch pregnant women (200 000/year) are screened for
irregular erythrocyte antibodies (IEA) early in pregnancy. Evidence for benefits, risks
and costs of screening is unequivocal. The OPZI study (Detection and Prevention
Pregnancy Immunization) evaluates the program in a nationwide study. The aim is to
describe the epidemic of non-RhD IEA with clinical relevance: IgG IEA directed against
an antigen with fetal expression.

Methods: Screening is performed in 86 labs all over the country. IEA-specificity is
usually determined centrally. Data are collected from electronic databases, lab records
and obstetric caregivers. Period: September 1, 2002 to June 1, 2003.

Results: We observed 58% relevant IEA in 506 pregnancies: prevalence 506/150 000
(0.33%). Following anamnestic 30% of IEA where known before pregnancy, hence
incidence is 0.23%. Specific (prevalence): anti-K 139(58%) (23.6%), -c 84 (14.4%), -C to
-e 20 (3.4%), -Cw 53 (9.1%), -E 165 (28.2%), -Fya 44 (7.5%), -Kk 24 (4.1%), -S 33
(2.3%) and others 24 (4.1%). The father was antigen-negative in 46%, ranging from
6% (anti-c) to 83% (anti-K).

Conclusion: Prevalence: Non-RhD pregnancy immunization in the Netherlands is
0.33%, incidence 0.23%, especially anti-c, -K and -C. The father is antigen-negative
in 46%. The correlation of each IEA with hemolytic disease of the newborn is determined
in an ongoing study. The now presented data indicate that, compared to other prenatal
programs, prevalence and incidence of IEA seem to justify nationwide screening.

A 17.11 Changes in techniques of RBC antibody detection and incidence of delayed transfusion reactions
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New sensitive techniques for RBC antibody detection might result in an increase of
incidence of delayed hemolytic transfusion reactions (DHTR) and delayed serologic
transfusion reactions (DSTR). Because of bloodgrouping automatisation at Clinical
Hospital Centre, Ortho column agglutination test (CAT) replaced the tube test (TT) in
1998. In 2000, DiaMed test (GT) replaced the TT in crossmatching, too. DHTR and
DSTR data from 1998 to 1999 and data from 2000 to 2002 were reviewed. These data
were compared with our historical data (1990-1997) when TT was used. With
introduction of micromethods in pretransfusion testing, we observed evident increase
of total incidence of both DHTR and DSTRs, from 1:3437 to 1:2300 (P < 0.001). The
incidence of DHTR was almost the same, but the incidence of DSTR increased from
1:8333 to 1:2500 (P < 0.00001 units transfused. The introduction of GT in
crossmatching resulted in another increase of total incidence of DHTR/DSTRs, from
1:4167 to 1:1389 (P < 0.001). The incidence of IEA seem to justify nationwide screening.

A 17.12 Multicentric quality assessment of immunohaematology testing in Croatia
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Introduction: Immunohaematology tests provide the safety basis of transfusion treat-
ment. In 1993, the Croatian Institute of Transfusion Medicine introduced a program of
quality control for immunohaematology tests in transfusion institutions of Croatia, in
order to get an insight into the quality of pretransfusion immunohaematology testing.

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Quality assessment is performed once a year. Participation in the program is voluntary and results are anonymous. Evaluation of the data collected allows a more objective comparison of results and provides better insight into quality of work of the transfusion service in Croatia.

Results: Cumulative results of 11 years (1993–2003) multicentric quality assessment of immunohematology pretransfusion testing in the transfusion service of Croatia are as follows: number of responding transfusion laboratories were from 92 to 100%; irregular antibody testing accurate results were from 81 to 100% and cross matching accurate results were from 79 to 100%.

Conclusion: Mean result of accurate results for irregular antibody testing is M = 91.3% and mean result for accurate cross matching is M = 98%. The data obtained straight point to the need of reorganization of transfusion service in Croatia, in order to improve the quality work in pretransfusion immunohematology testing.

A 17.13

New ABO-identity test for pretransfusion bedside testing

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Background: Most fatal complications of blood transfusion are due to errors, which occur at the patient bedside. Therefore, ABO-Identity testing at the bedside is mandatory in several countries.

Aim: To develop a bedside test which is particularly suited for the requirements of a non-laboratory environment.

Material: A plastic chip has been developed composed of a donor side and a recipient side with two reaction chambers each (A–B). The chip contains at each side one injection port that fits in luer syringes and from which blood is equally distributed to the two reaction chambers via microfluidic meanders. The reaction chambers contain predefined antibody reagents.

Method: A luer syringe filled with patient whole blood or with segment blood is connected to the respective injection port of the chip. Injection is stopped as soon as a blood droplet is visible in the reaction chambers. The chip is then rocked gently back and forth. Results (haemagglutination) can be read after 1 min.

Results: From 100 EDTA-blood samples tested 14% of each blood group A, B, AB, 0, included in ABO, A1B, A2B, 24% of ABO were identified correctly.

Discussion: The ABO identity test presented here is a closed system with one single pipetting step for recipient and segment blood each. This, together with the use of syringes without needle, may help to increase the safety for the nurse and reduce the risk of bedside testing errors. Performance evaluation studies are currently under way.

A 17.14

Detection of antibodies in tube and column agglutination technique

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Background: The direct antiglobulin test (DAT) can show negative results in a proportion of patients with clinical evidence of AIHA. This may be due to autoantibody levels below the lower detection limit of the traditional DAT. The developed column agglutination test (CAT-Ortho BioVie) seems to show increased sensitivity.

Methods: We performed the DAT with polyclonal and IgG anti-human globulin sera using the standard tube and the CAT methods on 3% RBC suspensions collected from 97 patients of the: surgical patients (47), non-hemolytic anemia (11), hemolytic anemia (11), thalassemia (10), leukaemia (10), other conditions (8). The eluates (warming technique) were prepared for all samples with a positive result in one or more methods.

Results: Of 97 samples 44 showed negative DAT with both methods. In 26 patients we detected positive results only by CAT-DAT: positive eluates and warm autoantibodies were found in 15 subjects (three patients with non-hemolytic anemia, two with hemolytic anemia, three with leukemia, six with thalassemia and with other conditions. Conclusions: CAT-DAT shows increased sensitivity versus tube-DAT. Further study is needed to determine its specificity.

A 17.15

The Kell blood group system: four unusual antibody cases in South African patients

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Background: The antibodies to the 24 blood group antigens in the Kell system are considered to be clinically significant. Four unusual antibody cases were referred to the South African National Blood Service Reference Laboratory.

Material and methods: The samples were tested using standard serological techniques. Selected panel cells including various rare antigen types, were used for antibody identifications.

Results: Case 1: An avid type IgM antibody (titre 64) was identified as anti-Kp. The patient required blood following surgery. No recent immunisation was known. Case 2: High titre anti-Jk4 was demonstrable in an auto-immune haemolytic anaemia (AIHA) case. The auto antibodies and anti-Jk4 antibodies could be differentiated by titration. Case 3: Anti-K was identified in a 7-month-old infant. The anti-K was detected 11 days after an unbilical cord blood transplant. Case 4: Anti-K was identified in a pregnant woman. The previous infant had been unaffected and the husband had typed K-. Intraperitoneal death occurred at 22 weeks. Subsequent typing of the patient’s second husband showed that his cells typed K+, K-.

Discussion: The four unusual Kell system cases highlight various important aspects of antibody identification. Typically type IgM antibodies, e.g. Anti-Kp may occur as type IgM saline reacting antibodies. Clinically significant antibodies may be masked by alloantibodies in AIHA and a patient’s history may have a significant impact in antenatal cases.

A 17.16

An example of anti-Co3 in gipsy woman with Co(a−b−) phenotype associated with mild HDN

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Background: In the Colton blood group system three specificities are defined: CoA (Co1), CoB (Co2), a Co ab (Co3). Co(a−b−) individuals are extremely rare and only few examples have been reported in the literature. Anti-Co3 has caused mild and severe HDN requiring neonatal transfusion with maternal blood. This report describes anti-Co3 in 25-year pregnant Gipsy woman with three previous pregnancies.

Methods: Serologic methods were used to identify patient’s phenotype, specificity and titer of antibody at 5th month of gestation and at the delivery. She was typed Co(a−b−) and the antibody was an anti-Co3, class IgG, subclass IgG1, titer of 4000 (LESS-IAT 37 °C).

Results: Anti-Co3 could cause severe HDN requiring neonatal transfusion with maternal blood. Sister of the patient was typed Co(a−b−) too. But both of them were HCV positive and they had been taking intravenous heroin. Transfusion Department and Blood Bank of Brno Faculty Hospital contacted Centrum Hema, Quebec, Canada for sending Co(a−b−) erythrocytes. We obtained 4 Units of cryopreserved erythrocytes 0 RhD positive Co(a−b−). The patient delivered in 37 weeks without any problems. Baby boy did not require neonatal transfusion, only phototherapy by the blue light was performed.

Conclusion: This case represents high titer anti-Co3 associated with only very mild HDN.

A 17.17

Implementation of an immunohematology quality control program in a large country, Brazil

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Background: External quality control in immunohematology is a critical tool for blood transfusion testing improvement. Brazil is the fifth largest country in the world. It is a socially, economically, and geographically heterogeneous country, with different education background levels. Objective: The purpose of this study was to create an strategy for implementation of an immunohematology external quality control program in Brazil.

Material and methods: In 2002, the Brazilian Agency of Sanitary Vigilance (ANVISA) qualified eight public blood centers from five different regions to coordinate the program. Letters of invitation and a profile survey with 30 questions were sent to all public Brazilian blood transfusion services and centers. External quality control panels composed by four blood samples to perform ABO/Rh typing, direct antiglobulin test, unexpected antibody screening and identification were specially designed, produced, and distributed twice a year by each one of the coordinator center.

Results: Of total blood transfusion services (n = 749), 530 (70.8%) of all 27 Brazilian States answered the survey and participated in the first panel with an average of 96.2% of correct answers. With new agreements, a total of 615 (82.1%) blood services participated in the second panel with an average of 96.2% of correct answers.

Conclusion: This study demonstrated that this was a successful strategy and provided excellent cover, despite of regional differences.
A 17.18

Duffy genotyping in a Saudi population
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Background: The Duffy blood group system is one of the most clinically important blood group systems involved in blood transfusion risk and occasionally in neonatal haemolytic disease. The molecular basis of the Fy(a-b+) polymorphism has been elucidated. We report here the first molecular investigation of the Duffy blood group in a native Saudi population.

Materials and methods: DNA was extracted from buffy coat samples from 154 randomly selected, healthy Saudi blood donors. Initially Fy(a-b) and Fy(a+) alleles were investigated for all samples. Specific primers for the GATA mutation and Fy(a) alleles were also used to type Fy (a-b-), Fy (a-b+), and Fy (a+b-) individuals.

Results: Initial genotyping for Fy(a-) and Fy(a+) alleles, showed that 44 samples typed as Fy(a+) Fy(a+). 45 samples were found to be Fy(a-) Fy(a-). 28 were Fy(a+) Fy(a+). From 37 samples serologically typed as Fy(a-b-), we found that 16 samples genotyped as Fy(a-b-); however one sample typed as Fy(a+) Fy(a+). Sequencing results of a representative Fy(a-) individual revealed a C at position -33. For the individual phenotyped as Fy(a-b-), but genotyped as Fy(a+) Fy(a+), a Tnt was found at position 265 confirmed the presence of Fy(a+) allele.

Conclusions: The high frequency of the Fy(a+) allele and the identification of the Fy(a+) allele within our Saudi cohort highlight the need to perform Fy(a-) genotyping in different ethnic groups.

A 17.19

External quality control in immunohematology 8 years of experience
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Background: In 1996, an External Quality Control Program was set up covering the southern region of Portugal, Autonomous Regions (islands) and later on African countries with Portuguese idiom.

Material and methods: Three times a year human red cells and serum samples are sent to participants in order to perform ABO and Rh(D) typing, Rh phenotyping, crossmatch, antibody detection and identification. The tests cover common problems from the routine activity of a laboratory. The errors are divided into technical and non-technical.

Results: From 1996 to 2003 the number of participants rose to 45 representing within Portugal 95% of the southern public/private health institutions. A total of 1005 exercises were sent to participants. Non-technical errors, probably related to transcription mistakes, accounted for 47% (n = 1180) of the errors. In the last 4 years, 10 laboratories were consistently classed as good performers and four as bad.

1996–2003 Tests [n] Errors [n] Rate [%]

| Test Type       | n   | Errors | Rate |
|-----------------|-----|--------|------|
| ABO             | 2286| 85     | 3.8  |
| Rh (D)          | 2286| 65     | 2.8  |
| Rh phenotyping  | 2106| 57     | 2.7  |
| Antibody screening | 873 | 10    | 1.1  |
| Antibody identification | 649 | 49    | 7.5  |
| Crossmatch      | 1692| 22     | 1.3  |
| Total           | 9892| 288    | 2.9  |

Conclusion: The higher rate of errors occurred in antibody identification, when more than one antibody was present, followed by ABO typing. The high incidence of non-technical errors reflects the importance of the clerical aspects of the laboratory work.

A 17.20

Silent Kidds, an alternative approach
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Kidd antibodies can be undetectable a few months after being produced, but are responsible for about 30% of all cases of DIHT. Incompatible transfusion to patients with Kidd antibodies often results in rapid cell clearance and a negative DAT. We have shown that when conventional antibody techniques fail to resolve such cases, red cell phenotyping is a useful additional approach. We report on a 57-year-old male with no recollection of transfusion, but previously had undergone open-heart surgery. Five days post-transfusion of 4 Units of apparently compatible blood, he had signs of a DIHT, with black serum, dark urine and serum bilirubin level of 85 µmol/l [normal range: 0–21 µmol/l]. Routine pre-transfusion tests, DAT, enzyme IAT, two stage IAT and a manual polybrene test on pre- and post-transfusion samples all proved negative. Phenotyping pre-transfusion samples showed the patient to be N-, s-, P1a-, Jk(b-); post-transfusion samples showed mixed populations using anti-N-, s- and P1a, but no circulating Jk(b+) red cells. One of the transfused units was confirmed as Jk(b+). Subsequent serum samples demonstrated a weak anti-Jk(b-) reactions [with homozgyous cells only, using DiaMed enzyme IAT]. A month on the antibody was undetectable. Transfusion of Jk(b-) blood gave no reaction. Following the successful use of phenotyping in two similar cases, it is now included as part of the routine protocol when detection of antibodies prove difficult, especially where there is clear evidence of a DIHT.

A 17.21

Another example of auto-anti-Kp
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Introduction: Autoantibodies found in patients with warm auto-immune haemolytic anaemia (AIHA) often show a red cell specificity that is apparently related to the Rh system. Antibodies with specificity for high-frequency antigens in other blood group systems are rare. We report an unusual case of auto-anti-Kp.

Case study and result: A 65-year-old Caucasian male with pre-existing myelodyplasia and colitis presented with acute AIHA (Hb 5.6 g/dl). Anti-K was detected in the plasma but no autoantibody was present. The DAT was positive (IgG 3+). A red cell eluate did not react with one Kp(b+) cell present on the red cell panel in use; the anti-Kp specificity was confirmed using additional Kp(b+) cells. The patient’s cells were group O D- K- Kp(b+). One month after recovery, the eluate no longer showed anti-Kp specificity.

Discussion: Auto anti-Kp has been reported in AIHA, but in these cases free auto anti-Kp was detected. This case is unusual in that although the red cell eluate showed auto anti-Kp specificity, no free autoantibody was detected in the plasma. The assignment of a specificity was fortuitous, because the cell panel in current use included an example of the unusual Kp(b+) phenotype. Disease-associated transient suppression of Kell antibodies has been reported and it has been suggested that this may explain the transient nature of autoantibodies to Kell system antigens, but in this case no suppression of the Kp(b+) antigen was noted.

A 17.22

Immunogalileo automat for blood donors immunohematologic tests: throughputs and work organization
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Background: Since April 2003, we have been routinely using two Galileo automats to test 800–1200 donations per day (2:00 000 per year). We report our experience in terms of automat throughputs and organizational aspects.

Methods: Galileo is a full automat with random access [different combinations of tests for each sample] and continuous access [loading of new samples while tests are being performed]; it uses hemagglutination in microplates. We perform: AB forward and reverse grouping and RH typing on the first two donations; short AB on each donation; short AB on a bag segment for first-time donors; antibody screening for first-time donors and when medically requested. Manually, we perform weak D testing, antibody identification and titration, DAT, detection of hemolysins.

Results: We began with a bi-directional link automat-LIS, using the random access feature, but we had to abandon this method because of the very low Galileo throughput in these conditions. We then adopted manual sorting of the samples and sequential testing on Galileo (same test for all loaded samples). We also had to add, over 900 donations, another technician for manual tests.

Conclusions: Two Galileo with two technicians, and 0.2–0.3 technicians for manual tests allow ensuring our routine. With our workload, the random access is not usable, unless there is a third automat. Over 1000 donations, including 120 or more first-time donations, technician overtime is often needed.

A 17.23

Comparison between two automatic methods for the detection of red cell alloantibodies
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Background: The aim of this study was to compare the sensibility and specificity of two column agglutination test (CAT) systems, employing a 3% screen panel and a 0.8% one, by a fully automated procedure.

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Materials and methods: 54 frozen samples, containing 60 irregular antibodies, were tested with an automatic system (AutoVue Ortho CD), both with the 3% and the 0.8% screen panels (Surgiscreen 0.8% and 3%, Ortho CD). The titre of each sample was determined with specific cells of the two panels. 2572 samples were screened with AutoVue, 1521 using the 3% screen panel and 1021 with the 0.8% one. On positive samples, antibody identification and titration were performed.

Results: All the 54 samples reacted with the specific cells of the two panels. The reac-
tions scores were higher with the 0.8% system in 23 cases (43%), with the 3% system in six cases (11%) and in 25 samples (46%) it was the same. Fifty antibodies (83%) showed higher titres with the 0.8% cells and 10 (17%) with the 3% ones. Thirty-three out of 1551 random samples (2.1%) reacted with the 3% panel and 22 out of 1021 (2.1%) with the 0.8% panel. Thirteen out of 33 (0.8%) and 12 out of 22 (1.2%) were not confirmed.

Conclusion: The known antibodies were confirmed in all the samples with both methods. In 83% of the cases the titre was higher with the 0.8% cells. Specificity resulted not significantly different with the two panels.

A 17.24
Gel Card sensitivity and accuracy in resolving ABO blood grouping discrepancies
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Background and study design/methods: Current protocol requires that samples resulting in No Type Determined for ABO blood type (NTD) by the Olympus PK 7200a method be resolved by Ortho Microtyping System Gel Card testing. From January 2003 to October 2003, 3123 samples required Gel Card resolution. Thirteen percent of these samples resulted in NTD determinations and were submitted to the Reference/Consultation for final resolution by tube method.

Results: Gel Card NTD samples were divided into seven categories: weak A1 cells, weak B cells, positive DAT, forward/reverse discrepancies, weak D, weak anti-A and weak anti-B. Notably, weak reverse typing accounted for 51% of these NTD with weak B cell results comprising 38% of that total. The consultation/reference final deter-
mination correlated 100% with the forward typing in these instances.

Conclusion: The data suggests that the Gel Card procedure may not allow for detection of weak reverse groups, possibly due to final red cell concentration and effect of increased diluent concentration and storage time on the B antigen. Tube typing uses the standard 4–5% final red cell concentration and allows for increase in the serum/cell ratio as well as increased room temperature incubation time.

A 17.25
ABO and RhD genotyping by PCR-SSP
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Background: The molecular basis of many blood group antigens has enabled develop-
ment of molecular genotyping techniques to identify blood group alleles. We evalu-
ated the ability of PCR-SSP to determine ABO and RhD blood groups using buffy
coat cells from routine EDTA samples from an extended phenotype panel as a source of DNA.

Materials and methods: Samples (n = 28) were phenotyped for ABO and RhD and DNA extracted using a QIAamp Kit. Primers specific for the common ABO alleles (A1, B, O1 and O2) and eight exons of RHD were synthesised according to published genomic DNA sequences. Primers, MgCl2, DNA concentrations and cycle numbers were all optimised before achieving standardised blood group specific PCR-SSP assays.

Results: In ABO typing, multiple non-specific product bands occurred with all primers in both antigen-negative and -positive samples. Similarly, amplification of RHD exons 5 and 6 produced false positive bands in all RhD negative samples tested (n = 5/28). Both protocols required extensive modifications of published primer and MgCl2 concen-
trations and amount of template DNA, in order to achieve expected results. Using the optimised assays resulted in complete concordance between DNA typing and serological phenotypes.

Conclusions: It is apparent that published blood genotyping methods may not be automatically applicable for routine practice even in another experienced laboratory.

A 17.26
Severe HDN due to anti-Cw
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Introduction: The red cell surface antigen Cw (RH8) occurs in about 2% of Caucasians. Although Cw alloimmunisation is not uncommon (estimated at 1 in 1100 pregnancies;

Bowman and Pollock [1993], reports of Cw HDN are extremely rare and are usually of mild to moderate severity. We report two cases of Cw alloimmunisation causing severe HDN requiring exchange transfusion at delivery. Anti-Cw was eluted from the affected infants' red cells and apart from Cw HDN, no other underlying pathology was noted.

Case 1: SD delivered at term with an anti-Cw titre of eight. Infant WD was DAT positive ([lgG 4+] and Cw positive, cord Hb13.1 g/dl and serum bilirubin 305 mmol/l]. An exchange transfusion was required.

Case 2: DW delivered at term with an anti-Cw titre of 32. Infant IW was DAT positive ([lgG 4+] and Cw positive, cord Hb 11.7 g/dl]. Two exchange transfusions were required. Both babies were referred back to hospital approximately 6 weeks post delivery with anaemia (Hb 6.7 and 7.2 g/dl, respectively). One infant required two top-up transfu-
sions.

Conclusion: In these two cases if anti-Cw had not been identified in routine antenatal testing a delay in the diagnoses and treatment of the Cw HDN may have ensued with the risk of severe hyperbilirubinemia and kernicterus. Using Cw-positive screening cells for antenatal testing alerted the hospitals to the risk of this rare cause of HDN.

Reference: Bowman JM and Pollock J. Maternal Cw Alloimmunization, Vox Sang 1993; 64: 226–230.

A 17.27
Post-transfusion hyperhaemolysis in a patient with sickle cell disease: case report
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Introduction: Delayed haemolytic transfusion reactions are seen more frequently in patients with sickle cell disease (SCD), and are characterized by a positive direct antiglobulin test and the appearance of previously undetected red blood cell (RBC) alloantibodies. It has been described a syndrome of post-transfusion hyperhaemolysis in patients with hereditary hemolytic anemia, characterised by destruction of both autologous and transfused RBCs with negative serological findings. Case report: A 45-year-old male with SCD multiple transfusions and known alloan-
oantiglobulins was admitted for painful vaso-occlusive crisis. Isoimmune exchange transfu-
sion was performed using 4 Units of ABO compatible c negative, E negative, Jkb negative packed red cells. After 48 h, he developed joint pains, pyrexia and his Hb from 8.5 dropped to 4.3 g/dl. Direct antiglobulin test was negative and antibody screening revealed the presence of the known alloantibodies anti-c, anti-E and anti-
Jkb. He received 2 Units of compatible blood without response. Transfusions were discontinued. He was treated with IvIg (sandoglobulin) and corticosteroids. His Hb raised to 8.6 g/dl and the patient made an uneventful recovery. No serological cause for the observed haemolysis could be found on repeated samples.

Conclusion: The syndrome of post-transfusion hyperhaemolysis in SCD is successfully managed with intravenous immunoglobulin, steroid treatment and avoidance of further transfusion of RBCs.

A 17.28
Study of 188 DAT-positive patients, part II: implications for transfusion treatment
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Materials and methods: Described in Part I.

Results: 140 DAT-positive patients (DATs) at first admission were proved as not transfused/grafted but 38% requested transfusion of which 76% urgently. Positive Ab screening in 85% DATs with urgent request revealed 15 polyspecific, five specific (three c, one C + e, one c) autoAbs and 34 alloAbs (nine with autoAbs). Anti-E, K, C and c were found in 21, 11, 7 and 4% urgent DATs, respectively. 71% DATs requested blood during 2 years: 26% in one, 51% in two to nine and 23% in more than nine occasions. Transfusion was at least once cancelled in 10% and postponed for >4 h in 35% DATs (in 17% >24 h), in 77% and 92% at first admission. 55% postponements >24 h were due to auto, 30% to allo and 15% to auto + alloAbs. Monoclonal patient typing could not be done due to prior transfusion in 17% and polyvalent without laborious procedures in another 54% DATs. In D-positive DATs prophylactic Rh-K-Jk-matched transfusions (PAM) would be often neg for E-K-Jk (22%), E-K-Jka (18%), E-c-K-Jkb or E-K (15%). For each blood unit 6.7, 8.3, 25.0 and 1.9 donors should be screened, respectively. In D-negative DATs units would be neg for C-E-K-Jka (39%) and C-E-K-Jkb (28%), with 5.3 and 3.8 donors screened, respectively.

Conclusions: Due to many urgent transfusions, positive Ab screening and cancelled/postponed requests, rational approach at least in our case seems to be: autoAbs absorption + identification = complete typing at first admission, and Rh-K-Jk PAM + serum antibody identification at blood transfusion.
A 17.29
Study of 188 DAT-positive patients, part I: serologic features
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Materials and methods: 188 DAT-positive patients (DATs) >6 months (hemolytic/serologic reactions excluded) analyzed by DAT Screening I, DiaPanels and DiaCidel (DiaMed).

Results: DATs were 52.5 (0.5–95) years, 57% female, 59% found at blood grouping/cross reactions, only 15% at AIHA investigation, coming from internal medicine (58%), hematology (28%), hemodialysis (11%) and “unusual dpts” (18%), with often diagnosis: primary AIHA (16%), lymphoproliferations (13%), renal insufficiency (9%), solid tumors (8%) and cytosis (5%). In 153 IgG DATs, 77 warm autoAbs and 76 negative eluates were found. In 28 IgG + C3f DATs 17 were warm, three warm/cold AIHA and eight to drug or immune complexes [IC]. In seven C3d DATs, four were cold AIHA and three due to drugs/IC. Antibody screening was positive in 109 (58%) DATs: 28% polyspecific, 10% specific autoAbs (anti-e, e + c, E, E + c, c and N, and 39% polyspecific auto + allo and 11% alloAbs were found. Anti-E was found in 22, K in 15, c in eight, C in seven, D and HLA in five, Jka in four, S and Lu(a) in two, Jkb, Fya, Fyb, Cw, M, Kn1 and Ck1 in one patient. 14 patients had ≥1 alloAbs. Three eluted anti-E, one E + c and one K alloAb pointed to five possible HTRs. In 1, 1+ or 2 DATs elution was more often negative (67%) than in 3+/4+ DATs (11%).

Conclusions: DATs are common over 50, but few patient groups may be prone to routine DAT. DATs and alloimmunisation seem connected: either autoimmune conditions make patients prone to alloimmunisation or immunomodulation caused by alloimmunisation leads to autoimmune events.

A 17.30
Multiple RBC alloantibodies, including anti-Di, after ABO compatible PBSC transplantation
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2Blood Transfusion Centre, Gdańsk, Poland

Background: Data on RBC alloantibodies appearance after PBSC transplantation from ABO compatible donor are limited. We present a case with three types of RBC alloantibodies.

Case report: A 32-year-old man with CML, never transfused, conditioned with fludarabine and carboplatin and transplanted with non-myeloablative regimen and 9 months of immune suppression with prednisolone, tacrolimus and mycophenolate mofetil. His conditioning regimen was associated with a high incidence of venous thromboembolism (VTE), which required dabigatran treatment. His alloantibodies (alloAbs) profile before and after transplantation included anti- E + c, anti-Di and K. The presence of anti-Jk a + E + Di in the donor, but not in the patient, was noted. The syndrome was observed despite intensive post-transplant immunosuppression likely due to RBC transfusion after transplantation and/or activation of donor cells by G-CSF. This is the first description of anti-Di after allogeneic PBSC.

A 17.31
Transfer of cholesterol from red blood cells to plasma in vitro
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Exchange between erythrocyte cholesterol and the free cholesterol in serum has long been known [Hagerman and Gould, Proc. Soc. Exper. Biol. Med. 1951;78:129]. Our aim was to measure the transfer to plasma in our system and to determine if adenosine-5’-triphosphate (ATP) was needed. Fresh (ATP approximately 4.08 µmol/gHb) and aged (ATP ~0.3 µmol/gHb) red blood cells (RBCs) were labeled with [4-14C]-cholesterol by incubation for 6 h at 37°C with constant agitation (n = 4). Two millilitre of 10% suspensions of the labeled RBCs were centrifuged, the supernatant removed and the RBC button resuspended in saved autologous plasma. The samples were incubated for 32 h at 37°C with constant agitation. The radioactivity in the supernatants was quantified using liquid scintillation spectrometry. The transfer of labeled cholesterol to plasma was 38.97 and 34.44% for the fresh and aged cells, respectively. The difference was not statistically different (P = 0.36).

Conclusion: Labeled free cholesterol diffuses freely from the RBC pool to the plasma and is not ATP-dependent.

A 17.32
External quality assessment program in red cell serology: a 4-year experience in Thailand
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Objective: To evaluate the efficiency and show the improvement of laboratories performing routine blood bank tests over 4 years.

Methods: There were 36, 58, 73 and 78 blood banks participating from 2000 to 2003. Only 17 laboratories participated for 4 consecutive years and could be evaluated for their improvement. They were requested to perform ABO grouping, Rh (D) typing, antibody screening, antibody identification and direct antiglobulin test on eight blood samples. The correct results and the summary of all participant results were sent back using code numbers for each laboratory.

Results: The return rate was 100%. Regarding ABO grouping, an error rate ranged from 0 to 11.8%; due to clerical errors. The error rate in Rh typing ranged from 5.9 to 35.3%, mostly due to interpretation of weak D phenotype. The error rates in DAT were found to be 0–17.6%. Errors in antibody screening were decreased from 29.4% in the first year to 5.9% in the following years. Antibody identification was not routinely performed in all laboratories but the number was increasing each year from 82.4 to 94.1%.

Conclusion: We conclude that an EQA program in red cell serology should be maintained to compare results from different laboratories and to identify which laboratories need improvement on test procedures. Moreover, training programs, especially capacity building for laboratory personnel, need to be considered. Thus, the patients will receive appropriate and safe transfusions.

A 17.33
ABO and Rh phenotype frequencies of four major ethnic groups in Rivers State, Nigeria
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Most reports on the frequencies of blood group antigens center on the racial differences with little or no report on the ethnic variations of these antigens. Information relating to the frequencies of the ABO and Rh phenotypes in blacks has been determined by testing African Americans or small groups of Africans with little or no regard for ethnic variations. Data are not available on the frequency of ABO and Rh antigens among ethnic groups in Port Harcourt. Two millilitre of venous blood was collected into an EDTA tube from each of 400 persons of mixed ethnic groups recruited for the study. The study population comprised 167 Ijaw (41.8%), 141Ekereves (35.2%), 50 Ekpeyes (12.5%) and 42 Ogonis (10.5%). RBCs were phenotyped for ABO and Rh antigens according to standard serologic methods. The following frequencies for the eight antigens of the ABO and Rh were obtained A (23.8%), B (15.5%), AB (1.0%), O (59.8%), D (95.0%), C (17.7%), E (20.5%), c (99.8%) and e (98.7%). The most frequently occurring Rh antigen being c. The antigens occurred independently of the ethnic groups (P > 0.05) except the antithetical Ee, which was found to be statistically significant when subjected to Pearson Chi-square test (χ² = 9.890, P < 0.02). One of the frequency of the E antigen was found to be c- while 20 (5.0%) were D-. This study illustrates the great variability of blood group phenotypes within the ethnic groups of Rivers State and Africa at large.
A18 Molecular diagnostics in immunohaematology

A 18.1 Cases of heteropaternal superfecundation in Southern Africa

D McNlinden

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Introduction: Over a 6-year period, 2774 disputed paternity cases have been investigated in our parentage-testing laboratory. Thirty-nine cases involved the testing of twins. Two cases (5.12%) demonstrated that each twin was fathered by a different man, heteropaternal superfecundation.

Materials and methods: DNA profiling was performed using the ABI Prism 310 Genetic analyser. DNA amplification was performed on a 2400 Thermocycler, using the Profiler Plus and Cofiler PCR amplification kits.

Results: In the first case, the alleged father tested was not excluded as the biological father of twin 1, with an identity index of 42 000:1.He was conclusively excluded as being the biological father of twin 2 with a total of eight exclusions on DNA testing. In the second case, the alleged father was excluded at 10 different DNA loci as being the biological father of twin 1, and not excluded as being the father of twin 2, with a identity index of over 356 000 000 000:1.

Conclusion: In accordance with international DNA paternity testing standards, a man who is excluded at more than three different DNA loci may be conclusively excluded as the biological father of that particular child. By contrast, if the calculated paternity index of an alleged father who is not excluded is greater than 399.1, it is considered ‘practically proven’ that he is the father of that child. Using these standards, we have demonstrated twice that it is possible for a set of non-identical twins to have different fathers.

A 18.2 The two juvenile haemochromatosis genes as modifiers of the p.C282Y homozygous-related phenotype

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Background: The most common form of haemochromatosis is an adult-onset condition, which has mainly been associated with the HFE1 p.C282Y/p.C282Y genotype. The phenotypic expression of this genotype is very heterogeneous and depends on a complex interplay of genetic and non-genetic factors. Aim of the present study was to determine if mutations in one of the two juvenile haemochromatosis genes, i.e. HFE2A and HFE2B (JAMP), were associated with more severe iron overload phenotypes in p.C282Y homozygous patients.

Materials and methods: We studied, by DHPLC and subsequent sequencing analysis, the HFE2A and HFE2B coding region in a cohort of 310 p.C282Y homozygous patients that had a transferring saturation level greater than or equal to 45%.

Results: We found 14 p.C282Y homozygous patients carrier of an additional mutation in either HFE2A (n = 9) or HFE2B (n = 5) and observed that their iron indices were effectively among the most elevated of the cohort.

Conclusion/Discussion: Our results revealed that mutations in either the HFE2A or HFE2B gene could explain one part of the p.C282Y/p.C282Y-related phenotype heterogeneity by accentuating the iron burden. They are in accordance with previous studies, performed in different models of knockout mice, which have evidenced that search for modifier genes could enable us to more precisely distinguish those p.C282Y homozygous patients with a higher risk to develop a severe iron overload and, consequently, clinical complications.

A 18.3 D-negative individuals carrying RHD gene sequences in Spanish population

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RHD-positive haplotypes have recently been reported in D-negative Caucasian individuals. Alterations found in the RHD gene of these individuals include gene conversions and point mutations.

Objectives: To estimate the incidence of RHD-positive/D-negative individuals in our population and to analyse the molecular structure of RHD alleles detected.

Methods: 96 D-negative blood donors (13 tr, 53 rt, 27 rt′, 2 rDt′ and 1 rD′rD) were studied. DNA samples were screened using a rhesus box analysis method. Samples carrying RHD-positive haplotypes were further studied by RHD exon scanning and RHD-specific PCR. RHD typing was performed on a panel of 21 anti-D monoclonal antibodies and absorbtion/elution studies were performed in samples carrying RHD-positive haplotypes.

Results: Of the total samples studied, 11 have RHD-positive haplotypes (10 rt′; 1 rD′). In four samples (rD′) we detected RHD/CE recombination: three RHD-CE(4–7)–D with lower C expression) and one RHD-CE(9)–D. Dα phenotype was demonstrated in five of the seven samples with normal RHD exon scanning results. Sequence analysis is undergoing. Any of the samples carried the RHDα allele.

Conclusions: We have confirmed the presence of RHD-positive/D-negative individuals in our population (up to 10% of rD′). Grossly recombined RHD genes are found in a significant number of these cases. Almost half of the individuals carrying RHD-positive haplotypes displayed the Dα phenotype. The results of this study will allow us to improve the strategy of RHD genotyping.

A 18.4 Molecular genetic RHD characterization of 577 cases with serologic suspect for weak D

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Background: Blood samples are routinely analysed with two monoclonal sera for rhesus D antigen. To further elucidate the D-antigen status, red blood cells (RBC) are analysed with other IgM and IgG sera. In cases with weak positive results patients may be phenotypically weak D or partial D.

Methods: Here we present data on 577 cases that were RHD-genotyped by a multiplex-fluorescent assay or sequenced within D exons 1–10 and compare molecular genetic and serologic data.

Results and discussion: (A) 577 samples were analysed in total, 246 samples (43%) were genotyped weak-D; 80 (14%) were a D-category; 242 (42%) had a wild type RHD but weak serologic result in the first analysis; nine (2%) had to be analysed because of a positive DAT. We have confirmed the presence of RHD genes are found in our population (up to 20% of rD′). We have confirmed the presence of RHD.

A 18.5.3 Apparent exclusion of maternity in a twin chimera carrying only her twin brother’s blood cells

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In a case of disputed paternity 13 polymorphic short tandem repeat (STR) marker systems including the amelogenin sex test were investigated in blood samples of the mother, her child and the alleged father. In three marker systems the mother was excluded from maternity of her child. Furthermore the X- and the Y-specific fragments of the amelogenin gene were detected in her blood. As the mother had a twin brother, twin chimerism was assumed as a possible reason for this phenomenon. Therefore samples from blood, buccal cells, fingernails and eyebrows were taken from both twins. Red cell antigens were investigated by serology and by PCR-SSP. All red cell marker systems were identical in the blood of both twins. The investigation of the above-mentioned set of polymorphic STR-loci and of three more loci in blood, buccal cells, fingernails and hair of the proposita and her twin brother gave the following results: the DNA profiles obtained from her blood samples were identical with all samples (blood, buccal cells, fingernails, hair) obtained from her twin brother. In her buccal cells and her fingernails a mixture of her own and her twin brother’s alleles were found. In the hair sample, the true genotype without admixture from her brother’s alleles could be defined. These results showed that maternity of the proposita could no longer be excluded. Therefore samples taken from different tissues, especially hairs, should be tested in these cases in order to find out the true genotype.
Abstracts 111

A 18.6 Molecular genetic analysis of ABO variants found in Japanese
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Objective: The ABO system is the most important blood group system in transfusion
medicine. Since the discovery of the system, a large number of variant phenotypes
have been described. To understand the molecular genetic basis of this polymorphic
system, we have analyzed genomic DNAs obtained from Japanese individuals with
ABO variant phenotypes.

Materials and methods: The ABO variant phenotypes focused in this study were A\(\text{u}\),
A\(_4\), A\(_5\), A\(_6\), B\(_2\), B\(_3\), B\(_4\), B\(_5\), B\(_6\), A\(_{AB}\), A\(_B\), A\(_B\), A\(_B\), and A\(_B\). The DNA samples obtained from
peripheral white blood cells of the individuals were analyzed using PCR-based methods,
and nucleotide sequence analysis was performed for the exons 6 and 7 at the ABO locus.

Results: We identified 22 different alleles. Twelve of them were SNPs on A allele, four
were SNPs on B allele and six were chimera genes between A and B alleles. Sixteen of
the 22 alleles were identical to the variant alleles reported previously. The other six
alleles have not been reported, of which one was found in A\(_B\) phenotype, two in A\(_u\),
one in B\(_2\), one in B\(_3\), and one in A\(_B\).

Discussion: The results indicate that a variant phenotype could be caused by different
alleles, and that some variant alleles results different variant phenotypes, depending on
the other normal allele, O or A/B allele.

A 18.7 Comparison of two PCR assays for RHc/c genotyping in a population of highly
diverse ancestry
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Several RHc/c genotyping assays have been developed on the basis of DNA sequences
of individuals of Caucasian ancestry. These assays may not be applicable to
populations of highly diverse ancestry because of the genetic variation in RH between
different ethnic groups. We determined the genotype for RHc/c in 138 unrelated blood
donors with a highly diverse background and compared the results with those obtained
by serological testing of RBCs. DNA samples from these blood donors were tested for C
and c alleles of RHCE by two PCR assays: a multiplex PCR that includes primers
specific for C and c alleles of RHCE, and a promoter region-based PCR-RFLP system (Tanaka
et al., 2000) and a promoter region-based

Materials and methods: The ABO variant phenotypes in this study were A\(\text{u}\),
A\(_4\), A\(_5\), A\(_6\), B\(_2\), B\(_3\), B\(_4\), B\(_5\), B\(_6\), A\(_{AB}\), A\(_B\), A\(_B\), and A\(_B\). The DNA samples obtained from
peripheral white blood cells of the individuals were analyzed using PCR-based methods,
and nucleotide sequence analysis was performed for the exons 6 and 7 at the ABO locus.

Results: We identified 22 different alleles. Twelve of them were SNPs on A allele, four
were SNPs on B allele and six were chimera genes between A and B alleles. Sixteen of
the 22 alleles were identical to the variant alleles reported previously. The other six
alleles have not been reported, of which one was found in A\(_B\) phenotype, two in A\(_u\),
one in B\(_2\), one in B\(_3\), and one in A\(_B\).

Discussion: The results indicate that a variant phenotype could be caused by different
alleles, and that some variant alleles results different variant phenotypes, depending on
the other normal allele, O or A/B allele.

A 18.8 Tetragametic chimerism detected in a healthy female with mixed-field
reactions in blood grouping
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Background: We investigated the case of a healthy female with blood group AB, while
her biological father showed blood group O. Further analysis, including blood speci-
mens, buccal swabs and finger nails revealed a tetragametic chimerism.

Methods: Blood grouping was done using standard gel centrifugation test cards, ABO
genotyping by SSP and SBT, and HLA-class IUI typing by standard NIH cytototoxicity
test and SSP, STR- and VNTR-typing was performed on blood and buccal swabs. The
tetraphenoty was analysed by GTO-based chromosomes.

Results: The proposita’s RBCs were typed AB with a mixed field agglutination while
the genotype showed A0 and B0. With respect to HLA typing one paternal and two
maternal haplotypes were found. Interestingly, both paternal haplotypes were detected
in four out of 24 tested loci only, using whole blood and buccal swabs for STR- and
VNTR-typing. The karyotype was identified as 46XX. The family members appeared to
be normal in all findings.

Conclusion: Through investigation of DNA polymorphisms it was possible to deter-
mine a rare case of tetragametic chimerism as being the result of double parental
contribution of nuclei.

A 18.9 Prenatal determination of fetal RHD status from maternal plasma using
real-time PCR
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Reference Laboratory, Bristol, UK, and Gynecology Clinic Ljubljana, SLO

Background: Non-invasive fetal genotyping became feasible after it became possible
to isolate cell-free fetal DNA from maternal plasma. We tried to predict the RhD status
and gender of the fetus by using real-time PCR.

Materials and methods: Peripheral blood samples were obtained from 96 RhD-
negative pregnant Caucasian women. The DNA was extracted from the plasma and
test for the presence of intron 4, exons 7 and 10 of the RHD gene by real-time
PCR. The detection of the SRY gene confirmed that the DNA was that of a male fetus.
If the PCR results for the RHD and SRY gene were negative, we assumed that the
fetus was a RhD-negative female. The presence of fetal DNA in these cases was
confirmed by the testing of eight polymorphic bialleles in the mother’s buffy coat
and plasma. If at least one allele was negative in the mother’s buffy coat DNA and
positive in the plasma DNA, we presumed that it was the fetal DNA, which was
actually being tested.

Results: The prediction of the fetal RhD status and gender was 100% accurate. The
results were serologically confirmed after deliveries. The results of RhD and SRY
typing predicted a RhD-negative female child in 15.6% of the cases. Of these, we were
not able to confirm the presence of fetal DNA in only one case.

Conclusions: Our real-time PCR method and strategy used proved to be reliable for
the determination of the fetal RhD status from maternal plasma.

A 18.10 Association of ABO gene mutations resulting in rare B subgroups
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Background: B allele subgroups are rare (<1%) and only limited molecular genetic
analysis have been reported.

Methods: Samples from two unrelated donors were submitted to ABO serology and
molecular analysis. ABO phenotypes were determined by agglutination and absorp-
tion-elution tests using monochononal/polichonal antibodies. Exons 6 and 7 of ABO gene
were cloned and sequenced.

Results/conclusion: Red cells from both donors did not react with anti B and anti AB
reagents. However, red cells were able to absorb anti A, which could be eluted
subsequently. Both samples had strongly positive reactions with anti H. First donor
presented a weak serum antibody that agglutinated B cells (at 4 °C) and H substance
was found in saliva – subgroup serologic status was defined as Bel. Second donor
saliva was unavailable and serum lacked antibodies that agglutinated B cells –
subgroup status was not completely defined. The molecular study demonstrated the
association of three non-synonymous substitutions in both B alleles compared with
A001: 467C > T, 646T > A and 829G > A found in the A102, Ax01 and A302,
respectively. B allele also presented a weak serum antibody that agglutinated B cells (at 4 °C)
and H substance was found in saliva – subgroup serologic status was defined as Bel. Second donor
saliva was unavailable and serum lacked antibodies that agglutinated B cells –
subgroup status was not completely defined. The molecular study demonstrated the
association of three non-synonymous substitutions in both B alleles compared with
A001: 467C > T, 646T > A and 829G > A found in the A102, Ax01 and A302,
respectively. B allele also presented classic substitutions described in group B. These
results showed two B subgroup alleles that differ from those previously described.
Presumably, this association of amino acid substitutions reduced significantly the
enzymatic activity of the encoded B transferase, resulting in an important decrease of
B antigen expression.

A 18.11 Detection and molecular characterization of partial D variants in India
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Background: Partial D variants are of clinical importance as these can form anti-D if
exposed to normal D antigen. The frequency of various partial D variants differ from
population to population.

Aim: To identify partial D variants and classify them by multiplex PCR.

Methods: Study population consisting of 10 000 samples of different caste groups in
western India, were screened with partial D kit. In addition to these 32 referred cases of
partial D were also investigated by multiplex PCR and partial D kit.

Results: A total of 10 000 subjects were screened with partial D kit (given as gift from
Scottish National Blood Transfusion Service). The incidence of partial D is 0.15% in
population studied. One-third of partial D belonged to DDFR category by partial D kit.

© ISBT 2004 Blackwell Publishing Ltd. Vox Sanguinis (2004) 87 (Suppl. 3), S93–S145
A 18.12

ABO exon and intron sequencing analysis revealed a novel B-allele in an Indian family
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Background: An ABO-typing discrepancy, found during routine testing, was evaluated including serological and molecular typing strategies. Subsequently, a family study was conducted.

Material and methods: ABO blood group typing was performed on all tested samples by using a standard gel centrifugation test cards. Exons 6 and 7 of the ABO gene were analysed using PCR-SSP and DNA sequencing of genomic DNAs from members of the family containing the weak B phenotype. Additionally, absorption/elution techniques were performed.

Results: Using gel centrifugation test cards negative reactions with the propositus RBCs were obtained with anti-A, anti-B and anti-AB using monoclonal antibodies, but a weak positive reaction with anti-AB only using the polyclonal test card. Reverse typing showed 4+ reactions with A1 and A2 RBCs while no reactions with B and O RBCs. In the B-allele of the propositus and one B-allele of his mother a 27 base duplication/insertion was found leading to a duplication of the respective intron6/exon7 area. Using absorption/elution methods we were able to show that weak B antigens were present on the erythrocytes of the propositus. Conclusions: In this study a new allele with a 27 base duplication encompassing nucleotide positions 234 to 260 was identified in the weak B phenotype.

A 18.13

DO alleles in sickle cell disease patients
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Background: The Dombrock blood group system consists of two antigens (DOa and DOb) and three high incidence antigens (Gya, Gyb and Gyd). Recently, the DO gene was cloned and sequenced, the molecular background associated with Hy-negative and Jola-1 were reported and different combinations of the DO single nucleotide polymorphisms were identified (Rios et al., Transfusion 2002). Genotyping for DO in patients who had received multiple transfusions is helpful in the selection of RBC for transfusion. This study aimed to determine the DO alleles (DO A/DO B, HY and JD) in a group of multi-transfused patients with sickle cell disease (SCD).

Methods: We tested DNA from 84 SCD patients by PCR-RFLP analysis (Rios et al., Transfusion 2001, 2002), to determine the changes on nucleotides 378 C > T; 793 A > G (DO A/DO B), 323 G > T (HY), 898 C > G (HY1/HY2) and 350 C > T (JD).

Results: Six (7.1%) of the 84 SCD patients studied were typed as DO A/DO B, 40 (47.7%) as DO A/DO B and 32 (38.1%) as DO B/DO B. Two (2.4%) samples genotyped as DO B/HY1 and four (4.8%) samples genotyped as JD/DO B.

Conclusion: We describe results of PCR-RFLP assays that, for the first time, give the frequency of DO alleles in SCD patients. Dombrock genotyping in SCD patients can prevent the allologeneic transfusion and aid in the antibody identification process. It is also helpful for the selection of antigen-negative donor RBC for transfusion.

A 18.14

Fetal RHD genotyping by analysis of maternal plasma at early gestational ages
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Background: Non-invasive prenatal identification of fetal RHD status is a goal of obstetrical practice, in order to prevent maternal immunization and to help in the management of alloimmunized pregnant women, without inherent risks of the invasive procedures. We assessed the accuracy of fetal RHD genotyping by analysis of maternal plasma from RhD-negative pregnant women during the first trimester of pregnancy by using the conventional PCR assay.

Methods: We analyzed plasmas from 15 RhD-negative pregnant women between 4 and 14 weeks of gestation. Peripheral or umbilical cord bloods from respective neonates were used as control. Commercially available kits were used to extract DNA from maternal plasma (Easy-DNA Invitrogen). Conventional PCR assays were used to determine RHD genotype, using the combination of size of amplified products associated with the RHD gene in both intron 4 and exon 10/TUTK.

Results: There was complete agreement between RHD genotype results obtained with DNA from maternal plasma, and fetal RhD type determined by hemagglutination at birth.

Conclusion: Our findings indicate that fetal RHD genotyping of maternal plasma by conventional PCR assay is also a reliable method of prenatal diagnosis; which can be safely obtained at early gestational ages.

A 18.15

Molecular studies of weak D phenotypes in Brazilian blood donors
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Background: Weak D samples are typically caused by missense mutations in their RHD alleles. Our aim was to perform molecular studies of weak D phenotypes in Brazilian blood donors of heterogeneous ethnic origin.

Methods: DNA samples from 312 blood donors with weak D expression characterized by serology, were tested by a PCR system using sequence specific primers that detect the common weak D types (Wagner and Gassner, 2001), by a multiplex PCR that detects the RHD gene hybrid alleles (Maakan-van Wijk et al., 1998) and by a PCR-RFLP that detect the mutation 1025T > C (exon 7) specific for the DAR allele.

Results: Of the 312 samples studied, 13 (4.2%) were identified as partial D DAR, two (0.6%) as partial D category VI type 1, one (0.3%) as partial DML and 296 (94.9%) confirmed as weak D. The distribution of weak D types found in this population is: type 1 (31.6%); type 2 (4.1%); type 3 (24%); type 4 (21.8%); types 1,2 (38%); types 1,3 (2%); types 1,4 (16%).

Conclusion: Molecular analysis showed that 16/312 (5.1%) of the weak D phenotype samples studied carried a partial allele. Weak D types 1, 2 and 4 contributed more than 85% of all molecular weak D types. These findings show for the first time the frequency of weak D types in Brazilians.

A 18.16

Alleloanti-D immunization by weak D type 1 RBCs
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Background: The weak D phenotype is caused by many different RHD alleles encoding altered RhD. Among the common weak D types in whites, only weak D type 2 has showed to induce alloanti-D immunization (Flegel et al., 2000).

Case report: A donor phenotyped as RhD-negative at the first donation with a commercial IgM monoclonal anti-D reagent, was found to be weak D+ with an IgG alloanti-D. Among the common weak D types in whites, only weak D type 2 has showed to induce alloanti-D immunization (Flegel et al., 2000).

Conclusion: Our results indicate that weak D type 1 phenotype may cause alloanti-D immunization and it should be detected in blood donors. The sensitivity of detecting weak D depends on monoclonal anti-D reagent and on the exact conditions of the methods. Molecular genotyping could be helpful to confirm weak D expression on RBCs.

A 18.17

Assignment of RHD zygosity by amplification of the hybrid Rhesus box
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RHD is flanked by the homologous 5′ and 3′ Rhesus boxes. The RHD deletion, found in most RhD-Caucasians, was theoretically due to recombination of the 5′ and 3′ Rhesus boxes resulting in the formation of a hybrid Rhesus box. The aim of this work was to
evaluate a PCR-SSP method for RHD zygosity assignment by amplification of the hybrid Rhesus box. We studied blood samples of 95 trios (father/mother/child). The Rh phenotype was performed by hemagglutination. The RH deletion was determined by PCR with primers that selectively amplify a segment of the hybrid Rhesus box. The most probable genotype was assigned according to frequency tables. Serological and PCR inconsistencies were analysed by a PCR-RFLP method to detect the hybrid Rhesus box and genotyped for D, Cc, Ee, RhD- individuals (n = 52) and all RhD+ members whose heterozygous status was confirmed by family studies (n = 40) were PCR+ for the deleted allele. In the rest of the RhD+ samples (n = 193) the zygosity assigned by PCR agreed with the most probable genotype except for 10 cases. One homozygous and nine heterozygous samples according to serology typed PCR+ and PCR-, respectively. PCR-RFLP analysis confirmed the results of PCR-SSP. No discrepancies in RH phenotyping and genotyping were found. The discrepant results may be attributed to low frequency haplotypes that confound the zygosity determined serologically. This assay is a reliable method for RH zygosity testing and allow a better management of sensitized pregnant women.

A19 Platelet immunology

A 19.1

Intrauterine death involving fetomaternal alloimmune thrombocytopenia due to HPA 5a antibodies

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Background: Fetomaternal alloimmune thrombocytopenia (FMAIT) was first described in the 1950s by Harrington et al. Inherited platelet antigens in the fetus, lacking in the mother, cause the production of maternal platelet antibodies which cross the placenta and destroy fetal platelets resulting in life threatening fetal thrombotic disease. No records exist for the frequencies of FMAIT in the South African population. Preliminary statistics are available for the frequencies of HPA (human platelet antigens) 1-5 and -15. The case presented is of a 19-year-old Black female who presented with a miscarriage in the 32nd week of pregnancy. The clinical picture of the baby was typical of NAIT with intracranial hemorrhage and petechiae.

Materials and methods: ACD samples were taken from the mother and father and tested for HPA antibodies. Donor database is checked for availability of HLA/HPA compatible platelets. Cross-match negative (Immucor) platelets are transfused. A genotype was also performed on this sample. A serum sample was obtained from the mother and sent to Platelet Immunology Center in Cambridge for platelet immunofluorescence test and monoclonal antibody-specific immobilisation of platelet antigens assay (MAIPA).

Results: The mother typed negative for HPA 5a and the father and fetus positive for this antigen. The MAIPA test was positive for anti-5a platelet antibodies. It is extremely likely that the cause of the intrauterine death was due to the maternal platelet antibody, anti-5a.

A 19.2

Case of surprising HIT and PTP coincidence

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PTP and HIT are two immune syndromes causing thrombocytopenia with some clinical similarities but profound differences in their pathogenesis requiring different approaches for diagnosis and treatment. PTP is caused by platelet alloantibody, which extends its specificity and destroys both incompatible transfused and also patient’s thrombocytes. The patient is threatening with bleeding complications. HIT-Ig activates platelets and mediates thromboembolic complications. Differential diagnosis between them must be therefore carefully considered. Here we present one case of PTP and HIT coincidence, which is very improbable and has not been reported yet. A 70-year-old woman underwent a total hip joint endoprosthetic operation. She was treated with fraxiparin and after the operation she was transfused with 3 Units of packed red cells. On the 7th day, after the operation severe thrombocytopenia with petechie and haematruia and also deep venous thrombosis of vena femoralis appeared. LMWH was replaced by UFH, platelets and plasma was transfused without any therapeutic effect. On the 9th day, HIT diagnosis was laboratory confirmed and heparin was discharged. Nevertheless platelet count not elevated until corticoid was therapy was concluded on the 11th day. On the 13th day, platelet antibodies were detected and PTP diagnosis was established. Although PTP and HIT coincidence is statistically almost impossible, it can happen and therefore it must be considered.

A 19.3

Management of NAITP, prospective study in a random sample of Egyptian pregnant women

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NAITP is due to maternal alloantibodies against foetal platelet specific antigens (85% anti-HPA1a, 15% due to others) resulting in neonatal thrombocytopenia that may cause intracranial haemorrhage (ICH) and death. The incidence is 1/1000-1/2000 pregnancies. The aim is to study a management protocol of NAITP to reduce morbidity and mortality due to bleeding complications. A total of 5000 pregnant females are screened for HPA1a platelet phenotype. In the HPA1a negative women, samples are taken for anti-HPA1a antibodies. Alloimmune women will be followed until week 36-38, where they have delivery by CS. Fetal cord blood sample is taken immediately and platelets count is performed. Neutones with thrombocytopenia <35 000/μl are transfused with HPA1bb platelet concentrates to treat thrombocytopenia and prevent ICH.

Results:

| Type                | Total No. | HPA1bb | Prevalence |
|---------------------|-----------|--------|------------|
| Pregnant females    | 6545      | 252    | 3.75%      |
| Potential father    | 166       | 10     | 6.02%      |
| New born            | 139       | 31     | 2.2%       |
| Total No            | 252       | Follow up | 224 |
|                     |           | Alloimmunization | 22 |
|                     |           | Incidence | 9.8% |
| Total no.           | NAITP     | Incidence | followed thrombocytopenia |
| of deliveries       |           | Degree of Transfusion | ICH |
|                     | 20        | All moderate, except for one severe | 0 |
|                     | 4         | 20%     | 1 baby     |

The above results show that HPA1bb is more common in the Egyptian compared to the Caucasian population. The rate and severity of alloimmunization is comparable to the Caucasian women. Prospective identification of the problem by antenatal diagnosis and applying this simple management protocol can probably reduce the morbidity and mortality among the affected neonates.

A 19.4

Management of platelet transfusion therapy in refractory onco-haematological alloimmunized patients

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Background: The approach to refractoriness includes 1 h post-transfusion CCI computation, antibody screening, and evaluation of clinical detrimental factors. Alloimmune refractoriness is managed either with HLA-matched or cross-matched platelets. Methods: Refractory patients are recognized by 1 h post-transfusion CCI computation. Sera from patients with CCI < 7 and without clinical detrimental factors are tested for HLA/HPA compatible donors. Cross-match negative (Immucor) platelets are transfused. Platelet transfusion is restricted to patient with <10 × 10⁹/l platelets or <20 × 10⁹/l if bleeding. Platelet concentrates are all leukodepleted; irradiation is restricted to patients assigned to progenitor cell transplantation or treated with fludarabine.

Results: Eight of 147 patients had immune refractoriness; six of them had anti-HLA antibodies and two had HLA + HPA antibodies. All patients received cross-matched platelets: three of them were also provided with HLA-HPA compatible platelets. Overall they received 175 cross-matched platelet transfusions. During the study none of them had major bleeding episodes and five deceased for progressive disease.

Conclusion: Major bleeding does not occur adopting restrictive platelet transfusion therapy. Steady clinical and CCI evaluation allows quick detection of refractory state. Refractory patients equally benefit from HLA-compatible and cross-matched platelet transfusion.
A 19.5 Oxaliplatin-induced immune thrombocytopenia: a case report
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Background: In very rare cases an immune thrombocytopenia may be induced by Oxaliplatin as a side-effect. Here is reported such a case. A 59-year-old woman, suffering from a rectal carcinoma with multiple liver, lung and bone metastases, received a chemotherapy including 5 Fluorouracil and Oxaliplatin doses regularly. Eight hours after her 20th treatment, she presented gingivorrhagia followed by leg purpura and a severe thrombocytopenia with a decrease of the platelet (PLT) count to 5,109/l against 253,109/l before the 20th treatment. Two PLT units were transfused and the PLT count rose to 20,109/l a day later.

Methods: For PLT antibody (Ab) testing, an immunocapture (IC) assay and the Monoclonal Ab-specific Immobilization of PLT Antigens (MAIPA) assay were used with pooled PLT from donors together with patient’s PLT.

Results: Thirty-two hours after the gingivorrhagia, IC assays were negative on pooled or patient’s PLT and patient’s serum incubated without Oxaliplatin but positive with. Similarly, MAIPA was positive when tested on pooled and patient’s serum with Oxaliplatin and negative without. Positive results were obtained on glycoproteins (GP) IIa, IIb/IIIa and IV/ in a higher reaction on the GP IIb/IIIa.

Conclusion: In case of a sudden severe thrombocytopenia due to Oxaliplatin, an immune reaction must be suspected and PLT Ab testing performed.

A 19.6 Platelet autoantibodies by flow cytometry. Revision of 1859 studies by thrombocytopenia
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Flow Cytometry is a valuable technique for platelet antibody detection. We analysed the results obtained by this method in autoantibodies detection along near 4 years.

Methods: We performed IgG, IgM and Polyclonal platelet-associated direct anti-globulin test (DAT), ether elution and indirect antiglobulin test (ITCL) and without chloroquine treatment (IT). The results were evaluated by Facs-canto cytemeter. We analysed 1859 studies between 1999 and 2003. The 1859 results were remitted for thrombocytopenia.

Results: The DAT was positive in 3% of patients, ITCL only in 7%. The elution confirmed 60% of positive DAT, but also was positive in the 6.5% of negative DAT. In 988 studies (53%) all the tests were negatives, 343 (18%) studies were considered sure positives, 182 (10%) were probably unspecific (by negative elution). In 335 (18%) studies we could not performed elution by insufficient platelets sample (48% of this had positive DAT or ITCL).

Conclusions: In our opinion the eluate is the most specific test for autoimmune thrombocytopenia. Our results seem to be lower than in other reports, but there are a great percentage of incomplete studies due to a few sample or very few platelets count and of course not all the studies remitted had a real autoimmune thrombocytopenia, we need to analyse these data with a more clinical information to know the real value of this method.

A 20.1 Testing of neutrophil antibodies in blood transfusion centre of Slovenia in 2000–2003
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Background: The detection of neutrophil antibodies (Abs) is important for the diagnosis of autoimmune neonatal neutropenia, autoimmune neutropenias, the investigation of TRALI, sepsis, drug-induced neutropenias.

Methods: We used the granulocyte immunofluorescence and agglutination test (GIFT and GAT) for detection of neutrophil Abs and the lymphocyte immunofluorescence test (LIFT) for their discrimination from anti-HLA class I. Fresh granulocytes and lymphocytes of donors typed in HNA-1a–c, HNA-2a, HNA-3a and HNA-4a were used for testing. 38 patients (13 neutropenia/leucopenia, four FNHTR, two suspected drug-induced neutropenia, two suspected cases of TRALI, three mother of an infant with neutrophil Ab, 16 unknown diagnosis) aged 5 months to 93 years and 11 blood donors involved in suspected TRALI were tested.

Results: Neutrophil Ab were detected by GIFT and GAT in four infants (age 5, 8, 11 and 16 months, respectively). In the rest of the patients and in blood donors, Abs were not detected.

Conclusions: In four of the seven infants, neutrophil autoAb were present and a diagnosis of autoimmune neutropenia of infancy was confirmed. In cases of suspected drug induced immune neutropenia and TRALI, neutrophil Abs were not detected. For patients older than 3 years, more strict clinical indications for testing are needed.

A21 HLA

A 21.1 Automated DNA extraction by the Qiagen Biorobot MDX and import of data into a LIMS
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Background: HLA-typing for our registry of volunteer stem cell donors requires efficient DNA extraction for up to 2000 samples per campaign. Through long-term storage of gained DNA, further typing of potential donors can be performed very fast.

Methods and results: We evaluated yield and quality of DNA extracted by the Biorobot MDX by analysing concentration and ratio of 96 EDTA whole blood samples from volunteer donors (average DNA concentration: 22.2 µg/ml; SD: 6.9 µg/ml; average ratio 1.94, SD 0.05) and of 32 buffy coats (average DNA concentration: 37.7 µg/ml; SD: 28.7 µg/ml; average ratio: 1.72, SD: 0.18). Furthermore, we performed DNA extraction from 32 buffy coats that had been stored at -30 °C for >1 year. Extraction showed best results when stored buffy coats were diluted with PBS. Average DNA concentration was 23.6 µg/ml, SD was 10.9 µg/ml. Average ratio of stored samples was 1.77, SD 0.08. Number of samples with a DNA concentration less than 10 µg/ml was three out of 146 (2%). For 18 HLA-loci, results from DNA extracted by the Biorobot were compared to results in our database. No deviations were found. The Biorobot MDX was integrated into our LIMS by a unidirectional interface that transfers sample number, number of and position on the storage plate and extraction status (valid/invalid) automatically into our LIMS.

Conclusion: Automated DNA extraction with the Biorobot allows efficient DNA extraction from volunteer stem cell donors and import of storage and quality data of the DNA directly into our LIMS.

A 21.2 The study on the haplotype of MICA and MICB microsatellite locus in Guangzhou Han population of China
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Shanghai Blood Center, Shanghai, China

This study is to investigate genetic polymorphisms and haplotypes of microsatellite in the exon 5 of the MICA gene and intron 1 of the MICB gene based on 106 samples of Guangzhou Han population by PCR and fluorescent technique. The corresponding haplotype frequencies, linkage disequilibrium values and relative linkage disequilibrium values were estimated. The results show that the genotype distributions of MICA and MICB microsatellite meet Hardy-Weinberg equilibrium in this population. In total, five alleles of MICA microsatellite and 14 alleles of MICB microsatellite were observed. MICA A5 was the most common allele (0.2877), whereas A4 was the least common one (0.1121). MICB CA14 was the most common allele (0.3252), and CA19 and CA28 were the least popular ones (0.0047). Twenty-one kinds of MICA-MICB haplotypes occurred at frequencies of more than 1% (linkage disequilibria value > 3.84, P < 0.05), and they were strong linkage disequilibria. The polymorphisms and haplotypes distributions of MICA and MICB microsatellite in this population have their own genetic characteristics. The microsatellite of the exon 5 of the MICA gene and intron 1 of the MICB gene could be used as the genetic markers in the studies of anthropology, linkage analysis of genetic disease genes, individual identification and paternity test in forensic medicine.
A21.3 Study on HLA haplotypes in Jiangshu-Zhejiang-Shanghai Han population
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To investigate HLA haplotypes in Jiangshu-Zhejiang-Shanghai Han population based on 166 families by serological and molecular biological HLA typing methods, and to analysis the distribution characteristic of HLA haplotypes. The results show that allele frequencies of more than 10% for HLA antigens were A2, A11, A24, B13, B46, B66, DRB1*04, DRB1*07, DRB1*09, DRB1*11 and DRB1*15. In the analysis of HLA haplotypes, 128 kinds of A-B haplotypes and 182 kinds of B-DRB1 haplotypes were found, comprising 19.28% (128/664) and 27.41% (182/664) of total theoretical haplotypes, respectively. 18 kinds of A-B haplotypes and 23 kinds of B-DRB1 haplotypes occurred at frequencies of more than 0.5% (linkage disequilibrium value > 0). 351 kinds of A-B-DRB1 haplotypes were found, comprising 52.86% (351/664) of total theoretical haplotypes, and 8 kinds of A-B-DRB1 haplotypes occurred at frequencies of more than 0.5% (>0). The common A-B-DRB1 haplotypes were A30-B13-DRB1*07 (4.22%), A2-B46-DRB1*09 (3.77%), A33-B58-DRB1*17 (3.01%), A33-B58-DRB1*13.1 (1.81%) and A11-B75-DRB1*12 (1.51%). The HLA haplotype distribution of Jiangshu-Zhejiang-Shanghai Han population, its own genetics characteristic, so it suggests this population was between southern and northern Han population. The HLA polymorphism of Chinese Han population is more abundant in East Asian populations.

A21.4 Association of HLA class I with severe acute respiratory syndrome coronavirus infection
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Background: During the Taiwan epidemic of severe acute respiratory syndrome (SARS), many health care workers were infected. In an effort to establish a screening program for high-risk personnel, the distribution of HLA class I and II alleles in case and control groups was examined for the presence of an association to a genetic susceptibility or resistance to SARS coronavirus infection.

Methods: HLA allele typing was performed on 37 cases of probable SARS, 28 fever patients excluded later as probable SARS, and 101 non-infected health care workers.

Results: When analyzing infected SARS patients and high-risk health care workers, independent studies are needed to test these results.
only supplied around 1% of all apheresis platelets (Aph-PLTs) transfused in Korea and the rest were collected in hospital-based blood banks. The reasons for this are: (1) difficulty in recruiting donors for plateletpheresis, (2) hospital-based blood banks had equipped themselves with apheresis instruments and were self-sufficient, and (3) compared to platelets (PLTs), fee schedule of Aph-PLTs is exceptionally low. Realizing the duty of the KRC, which supplies about 99% of blood for transfusion, to set up a system to supply Aph-PLTs to all patients in need, in 2000 the KRC has chosen plateletpheresis as one of its major tasks for the upcoming years. To fulfill this goal we introduced new generation apheresis instruments, established a registered blood donor program, and improved the coordination system among users and KRC blood centers. With these efforts we were able to supply 11.5% (2000), 35.2% (2001) and 62.7% (2003) of all Aph-PLTs transfused in Korea. Although supply of Aph-PLTs increased tremendously within a short period of time, Aph-PLTs (1 Unit equivalent to 6 Units of platelets) still account for only about 14% of all PLTs supplied for transfusion. To further increase supply of Aph-PLTs, more donors should be recruited and feasibility of 2-Unit plateletpheresis in Korean donors should be evaluated.

A 22.5
Appropriate organisation: high efficiency
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Objective: As a supplier of vital but expensive resource of medication, BTS should be constantly modernized to fulfill all main requirements: self-sufficiency, safety and positive cost/benefit ratio. To achieve this we propose reorganization model of the current BTS in our country.
Methods: From the 2003 summer EAR and government representatives and the leading staff of the 10 BTS's were implemented to find the best solution. (1) The UK model of the NBTS was presented, (2) SOP's were made, presented and distributed, (3) All BTSs were visited, to perceive their current situation regarding equipment, staff, etc. (4) Several groups were engaged to establish the optimal model regarding safety, efficiency and economy.

Results: This study will present the process only in Vojvodina, with an estimated population of 2000 000. At the beginning of the reforms 10 BTSs existed in this region. One of them was a regional and the other nine were hospital-based services. Each of them collect, process, test and distribute blood to local hospitals. As the testing generate 70% of all costs it was wise to centralize that procedure at first. At the moment it is going to be done in four regional BTSs, each providing blood for 500 000 inhabitants. Because of lingual diversities as well as geographical and technical difficulties collecting and processing is proposed to remain in hospital based BTS.

Conclusion: Through this concept, we believe we will promote transfusion practice appropriate to the environment in which is operating.

A 22.6
Blood: from unutterably risky to sincere, or the International Development of Transfusion Medicine
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Since the UN Declaration of Human Rights much has improved. However, the fundamental right of health and education lacks in most of the world. Since the outbreak of AIDS WHO has invested much energy invested in developing safe and sustainable blood supply systems as integral part of health care. However, reality sometimes was a smarting picture – 19% of global population belongs to the more developed region. One of them were a regional and the other nine were hospital-based services.

Results: From the 2003 summer EAR and government representatives and the leading staff of the 10 BTSs were implemented to find the best solution. (1) The UK model of the NBTS was presented, (2) SOP’s were made, presented and distributed, (3) All BTSs were visited, to perceive their current situation regarding equipment, staff, etc. (4) Several groups were engaged to establish the optimal model regarding safety, efficiency and economy.

Conclusion: Through this concept, we believe we will promote transfusion practice appropriate to the environment in which is operating.

A 22.7
The impact of a 7-year program to improve transfusion practice in a district general hospital
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Blood transfusions are essential to support patients with medical conditions and to enable complicated surgical procedures to be undertaken. Transfusion is not without its hazards, with improper transfusion stated in serious hazards of transfusion (SBOT) reports as the major cause of transfusion morbidity. Reduced donor numbers and improved blood processing and testing, combined with numerous national guidelines and directives have changed attitudes towards blood transfusions. The North of Scotland Blood Transfusion Service based at Raigmore Hospital Inverness supplies all hospitals in the Highland Acute Hospitals Trust. It serves a population of 300 000 scattered over 10 000 square miles. Superb donor support guaranteed sufficient bloodstocks to cover local needs, but blood ordering was inefficient. This, along with government directive and changes in working practice led to introduction of measures to enhance transfusion practice in HAHT. Measures included Maximum Surgical Blood Ordering Schedule (MSBOS) and Minimum Transfusion Requirements for Elective Surgery (MTRES), efficient issue and turnover of blood supplies, fixed protocols and guidelines, and education programmes for all staff. Each measure was audited and compliance communicated to service users. The results indicate aspects of the programme, which most impacted on changing transfusion practices and increasing awareness of the risks and benefits of transfusion in a District General Hospital.

A 22.8
Development of a blood transfusion website in a hospital trust
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The combined Leeds and Bradford Pathology Departments provide an extensive range of tests and services to local hospitals and GP surgeries in West Yorkshire. Historically, the user-guide to these services has been contained in a ‘doctor’s handbook’, issued at induction. We are working to replace the contents of this handbook with an intranet-based website, covering all pathology resources, and aimed primarily at medical and nursing staff. A web-based pathology resource has the advantages of instant updates, greater scope with more depth and feedback via e-mail. Instead of a handbook, users will be issued with a pocket-sized card giving the address of the website and details of how to access the pathology results server. The blood transfusion section includes both local and national information. Local content includes laboratory hours and contact details, products available for transfusion and the location of emergency stock throughout the Trust. Trust policies, blood transfusion guidelines and minutes of the Clinical User Groups can be downloaded. There is useful information on laboratory tests, management and investigation of transfusion reactions, the significance of red cell antibodies, bone marrow/stem-cell harvesting protocols and links to the Hospital Transfusion Team. It also includes detailed instructions for using the BloodTrack® system. National content includes downloadable SBOT reports. Links to other relevant internet-based resources are included.

A 22.9
Reasons for elimination of blood units as a marker of transfusion service efficiency
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The aim of this study was to assess the level of efficiency of the transfusion service through the evaluation of the reasons and number of eliminated blood units.

Materials and methods: In the period of 1999–2003, 98 976 units from voluntary blood donors were typed and screened. The total number of eliminated blood units was 4988 (5.1%) and retrospectively, we evaluated the reasons.

Results: The total number of eliminated units per reason was HBsAg positive, 1372 (27.5%); HIV positive, 745 (15%); HIV positive screen, 106 (2.1%); n-phylipos positive screen, 29 (0.6%); presence of fat in the samples, 1620 (32.4%); positive Ab screen, 19 (0.4%); errors during collection, transport and processing, 1083 (21.7%). The total number of eliminated units per year is shown in the Table:
Conclusion: The significant trend of decrement of eliminated blood is mainly due to the lower number of uncontrolled units because of the presence of fat in the samples and also the mild decrement of the viral marker positivity. We may conclude that this is as a result of increased efficiency of the transfusion service, which includes better donor selection as well as improved collection, production and laboratory control system.

A 22.10
Reforming transfusion medicine in Romania: steps and results obtained
in BTC Iasi
D Ilcenco

BTC Iasi, Romania

Introduction: After the revolution from 1989, in Romania the new rulers tried to reform health care services including transfusion medicine. Since 1995 when the law concerning blood transfusion been adopted in Parliament there were introduced new methods of collecting blood, new lab tests and new management methods.

Materials and methods: BTC Iasi statistic between 1993 and 2002 reported to the National Institute of Transfusion Hematology Bucharest were used.

Results: Number of blood units collected in this period had a different evolution: 25 889 in 1993; 25 531 in 1994; 18 917 in 1995; 17 712 in 1996; 16 615 in 1997; 16 472 in 1998; 17 070 in 1999; 13 343 in 2000; 15 028 in 2001 and 17 129 in 2002.

Number of ml per unit of blood was different, starting with 250 ml in 1993 ending with 400 ml per unit in 2002. Results are reflecting the political decisions taken by the Health Ministry such as: introducing compulsory tests for all units of blood for testing HCV and HTLV, permission to family donors to give their blood, price for blood, reducing number of medical staff.

Conclusions: Decedents should take into consideration some proposals regarding the budget, staff training, hemovigilance, quality control, new level of organisation of the transfusion network and implementation of a new information system all over the country.

P 22.11
Informed consent for transfusion – sample of current practices in Canadian hospitals
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The Krever Commission (97) recommended an informed consent policy on transfusion.

In November 2002 the Canadian Standards Association included this policy as a licensing requirement for hospital transfusion services. The Transfusion Ontario Programme in Ottawa conducted a survey on Paediatric Transfusion practices (November 2002–April 2003) in paediatric and general hospitals with paediatric services. Eight children and 21 general hospitals with paediatric services completed the survey. This sample data is summarized below.

| Year | Eliminated units | Controlled units |
|------|------------------|------------------|
| 1999 | 1281 (6.5%)      | 19764            |
| 2000 | 1183 (5.9%)      | 19705            |
| 2001 | 1021 (5.3%)      | 19015            |
| 2002 | 796 (3.9%)       | 20567            |
| 2003 | 707 (3.5%)       | 19924            |

Conclusion: The significant trend of decrement of eliminated blood is mainly due to the lower number of uncontrolled units because of the presence of fat in the samples and also the mild decrement of the viral marker positivity. We may conclude that this is as a result of increased efficiency of the transfusion service, which includes better donor selection as well as improved collection, production and laboratory control system.

A23 Changing clinical practice, including minimising errors

A 23.1
Utilizing the automated ABS2000 analyzer for routine transfusion service testing
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Background: In response to a transfusion service environment of anticipated increase in workload, an automated analyzer [ABS2000, Immucor] was purchased in 1999 to meet department needs. As workload increased over time, more testing categories were considered for automated processing. Due to a relatively minor percentage of routine testing (11.9%), the majority of tests in the transfusion service are categorized as stat, ASAP or trauma. Initially, only routine testing was considered for automation but with increasing workload, all testing categories of type and screen/cross match have been assimilated for ABS2000 processing.

Study: A statistical analysis was performed to determine what percentages of testing categories were being automated using the ABS2000. Data was collected over a 2-week period (7 April–20 April, 2003 inclusive). Percentages were calculated for each test category. During this study period, the automated analyzer was routinely in use and the manual test was solid phase red cell adherence for antibody screens. Blood types were tested by hemagglutination.

| Number of tests | Test category | % Manual testing | % Automated |
|-----------------|---------------|-----------------|-------------|
| 323             | Routine       | 13% (43)        | 87% (280)   |
| 353             | Stat          | 43% (153)       | 57% (200)   |
| 159             | ASAP          | 39% (56)        | 65% (103)   |
| 119             | Trauma        | 69% (82)        | 31% (37)    |

Conclusions: The use of automation has allowed the transfusion service to not only improve the routine testing processes, but also has integrated significant percentages of stat, ASAP and trauma testing into the automated process.

A 23.2
An audit of nurse’s knowledge of blood transfusion practice
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Regular training on the appropriate usage and administration of blood is an effective method of minimising transfusion errors. The authors believe there is a gap in the current knowledge of learning needs of nursing staff in the safe administration of blood. Understanding learning deficits is essential in compiling training programmes for nurses in blood transfusion. This paper highlights the findings of a prospective longitudinal survey of one hundred nurses’ knowledge of clinical practice and process in blood transfusion in a UK district general hospital. The results have informed the education programme delivered by the Blood Transfusion Practitioner and have been re-audited after a 1-year period to monitor the impact of revised training. Key areas of concern in knowledge of clinical practice and process were demonstrated but there were also areas of robust knowledge, i.e. 79% of nurses were unaware of the timing of standard blood observations but 91% acted appropriately in a suspected transfusion reaction. Audit recommendations: ensure blood transfusion training is an integral part of the Trust mandatory and induction courses; regular teaching sessions in clinical areas that are flexible and convenient for staff; develop a competency framework for nursing staff in blood transfusion practice The results of the re-audit, which will evaluate the effectiveness of the education programme, are nearing completion.

A 23.3
Analysis of therapeutic effects of blood platelet transfusion in 211 patients
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Subjective: To learn the curative therapeutic effect of platelet transfusion in hospitalized adult patients.
Method: A questionnaire was designed for registering profiles of participants, which including diagnoses increment of platelet cells after transfusion, etc. Blood routine examination was done before and after transfusion within 24 h.

Result: Two hundred and eleven patients were registered in the teaching hospital, there were 108 male patients and 103 female patients in the study, average age is 43.8 ± 23.1 years[median is 45]. Among them, 159 cases were leukemia, 31 cases were cancer, and others were ITP, thyroiditis, hepatocirrhosis, DICE, etc. 34.45% events were transfused for the treatment of established bleeding, in patients with deficiency of platelet number and/or function. In other 55.5% events, blood platelet was used for the prevention of bleeding in patients. And the other events were for the both. Myelodysplasia (89.7%) is the main cause of the etiology of thrombocytopenia. Only 4.41% were cases diagnosed immune thrombocytopenia. 1.93% cases were diagnosed platelet depleted. And none were transfusion for the congenital or acquired platelet dysfunction.

Conclusion: Platelet transfusions are mainly used for the treatment of established bleeding and for the prevention of bleeding in patients with thrombocytopenia, especially for leukemia. Myelodysplasia is the main cause for thrombocytopenia.

A 23.4

Blood components transfusion practice in a university hospital

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Continuous improvement in the use of blood components is essential to ensure the patients’ safety. We assessed the effect of education on ordering and utilization of blood components, in a university hospital.

Material and methods: Retrospective reviews were performed periodically. (1) Three 6 months periods were studied: baseline (BL), post education (PE) and follow-up (FU). Percent of blood units transfused (T)/units cross-matched (C) in different disciplines were evaluated. (2) RBCs and Plts transfusions according to audit criteria were studied in different periods.

Result:

Table 1. % T/C at three different periods

| Periods | Hemat/ onc. | Cardiac surgery | Surgery | Neurosurgery | Obst/Gyn | Medicine | Intensive care | Total hospital |
|---------|-------------|-----------------|---------|--------------|----------|----------|--------------|---------------|
| BL      | 82          | 45              | 28      | 10           | 7        | 53       | 60           | 45            |
| PE      | 82          | 49              | 45      | 26           | 13       | 55       | 61           | 54            |
| FU      | 85          | 57              | 43      | 40           | 25       | 65.5     | 63.5         | 61.5          |

Table 2. % RBCs T according to Hb levels

| Hb (g/dl) | Hemat/onc. | Medicine | Surgery | Total hospital |
|-----------|------------|----------|---------|---------------|
| <8        | 44         | 27       | 1        | 34            |
| 8-10      | 52         | 52       | 51       | 49            |
| >10       | 2          | 14       | 36       | 13            |

Table 3. % Plts T according to Plt counts

| Plt (10^9) | Hemat/onc. | Medicine | Surgery | Total hospital |
|-----------|------------|----------|---------|---------------|
| <10       | 51         | 6        | 6       | 30            |
| 10-20     | 24         | 27       | 6       | 79            |
| 20-50     | 22         | 38       | 16      | 22            |
| >50       | 3          | 27       | 73      | 28            |

Conclusions: There was a significant improvement in blood ordering patterns in surgical departments, medicine and intensive care during the three periods studied. Obs/Gyn still need improvement. There were no differences in RBCs and Plts utilization in the three periods. There are expected differences in components transfusion practice due to different patients population. Continuous education and utilization reviews are important to improve clinicians’ hemotherapy practice.

A 23.5

Changes in whole blood and red blood cell use: effect of education

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Objective: To determine the changing tendency of whole blood (WB) and red blood cell (RBC) use in our hospital.

Materials: Data of WB and RBC use from 1985 to 2003 in our hospital.

Results: Till 1992 only WB was prepared in our hospital (WB use 100%). After 1992 WB using rates decreased from 92 to 2.8%. In 1999–2001, the systematic decrease of WB use lost it force (17, 19 and 14% respectively), but in 2002 and 2003 the decrease accelerated again and we arrived a very low rate in WB use, 6.6 and 2.8% respectively.

Discussion: Education about transfusion medicine is generally insufficient in the medical curriculum in our country. Insofar as it’s possible, our blood bank staffs tries to persuade the doctors by phone to use RBC, when WB is requested. In the year 2000, WB was requested still 41% on the blood request forms, despite the rate of WB use was 19%. We began a education program; courses that takes 5 days, about transfusion medicine which is mandatory for all postgraduate doctors in training, since October 2000. Since February 2002 we arrange elective internships (2 weeks) for the 10th semester medical students also. We suppose that this education has a great influence on WB and RBC use in our hospital. Also, on blood request forms, a request for WB is a rarity today.

A 23.6

Concept to coordinate laboratory ABD: confirmation testing with ABO-identity bedside test

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Background: ABO-identity testing at the patient bedside, intended to improve transfusion safety, is increasing the logistic demands of transfusion and the workload for nurses.

Aim: A concept is presented combining ABD control of the blood bag in the laboratory with ABO-identity testing at the patient bedside.

Concept: A plastic chip containing prefixed reagents is composed of an ABD-confirmation area with four reaction chambers (A–B–D–autocontrol) and an ABO-identity area with two reaction chambers (A–B). The chip can be attached irreversibly to the blood bag. The segments of incoming blood bags are tested in the laboratory utilizing the ABD part. The chip is then attached to the blood bag, which is stored refrigerated, until it is required for transfusion. The nurse performs the bedside test with a patient sample utilizing the second (ABO)-part of the chip.

Discussion: With this concept: (1) the events of transfusion become a coordinated interaction between laboratory technician and nurse; (2) the ABD control done by the laboratory and the labelling of tested blood bags become part of an integral process; (3) the nurse is liberated from the preparation of the materials for bedside testing; (4) the result of patient bedside tests can be compared with the physically present result provided by the laboratory; (5) errors at the patient bedside are minimized due to the physical connection of blood bag and bedside test. The procedure is in conformity with the national guidelines.

A 23.7

Superficiality in the request of anti-PLT antibody research for neonatal alloimmune thrombocytopenia

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Fetomaternal haemorrhage may cause a maternal immunization against fetal human platelet antigens (HPA), determining the immunoreg of neonatal alloimmune thrombocytopenia (NAITP). NAITP can also occur in the first pregnancy, in some cases with fatal consequences. For these reasons, if the newborn shows an unexplained thrombocytopenia, the research of anti-PLT antibody is necessary. In this retrospective study, we have evaluated all screening carried out in the period between 2000 and 2003 in order to establish the incidence of NAITP in respect to clinical requests arrived at our laboratories. Anti-PLT auto- and allo-antibody research was performed with solid-phase and ELISA test in 1 200 pregnant women. In eight women alloantibodies were identified, with a specificity anti-HPA and/or anti-HLA; moreover in other seven
subjects autostainths were founded (six panreactive and one anti-HPA-5b). In our study, a real anti-PLT alloimmunization is occurred only in 0.66% of investigated pregnancies. In the remaining cases there were probably an error in the clinical indication to the test or an error in performing the test by private or public non-qualified laboratory. In the end, this situation is resulted very expensive for our Hospitals with.. On the other hand, a benefit for only few patients. In order to avoid useless or wrong tests, we suggest creating a highly specialized Regional Reference Center for NAITP to provide a better prenatal and perinatal consultation, diagnosis and therapy.

A 23.8
The development of a national clinical policy and guidelines for Serbia
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Changing the current clinical practice in order to improve quality and safety of transfusion therapy is recommended by WHO and Council of Europe. The main tool to achieve this goal is establishing the National Clinical Blood Policy (NCBP) and Clinical Guidelines (CG) for the appropriate use of blood. These will provide agreed and binding principles and directives for optimal use of blood and establish channels for coordination and collaboration between all parties involved in clinical transfusion practice. We present the Serbian experience with activities in establishing a NCBP and CG. The presentation will highlight the activities over a year to prepare a series of CG and describes the organisation of several meetings for wider consultation to disseminate the CG and prepare them for use by clinicians both at national and local levels. The presentation also describes the strategy for selection, initiation and involvement of clinicians and health authorities to ensure that the NCBP and CG are adopted as national documents to be followed by performing audits for compliance in clinical practice. Our work shows that the role of Transfusion Medicine Specialist is crucial in establishing and supporting the strategies for improvement of the quality of clinical transfusion practice. The essential strategy in formulating the NCBP and CG is to identify all important players and stakeholders as well as their role and responsibility in improving the quality of clinical transfusion practice.

A 23.9
Mobile multimedia terminal for ordering, issuing and administration of blood transfusion
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Background: Due to administrative errors in the transfusion chain between the blood donor and the patient, errors still occur. Usually, they are caused by the misidentification of the patient, his blood samples and respective blood components. Such errors can only be prevented by a reliable identification.

Methods: An information system covering all standards of safety was prepared. The system assures the rational use of blood as well as the obligatory hemovigilance. It consists of the bedside palm computers, readers and code bar generators, fixed terminals, networking systems and servers with databases of the donors, patients and blood inventory; all being wirelessly connected into a network. Its main characteristics are the bar-coded wristbands, bar-coded blood samples and electronic ordering of the blood component via a handheld device at the patient’s side.

Results: The pilot application supports ordering, issuing and infusion of a blood component. The identity of the patient and the blood component can be matched immediately before the intended transfusion. Post-transfusion events are recorded and documented, enabling the obligatory hemovigilance and traceability.

Conclusions: We expect that this or a similar system of transfusion safety assurance and hemovigilance would enable the quality of clinical and transfusion practice and we expect it to be set up soon because of its evident benefits.

A 23.10
Results of the event-reporting program: experience in an Argentinean blood bank
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Background: The event-reporting program (ERP) is an effective tool to evaluate the Quality System and also to prioritize the implementation of corrective actions. The objective of this study was to evaluate an error management system applied to our Transfusion Medicine Service.

Materials and methods: The ERP has several components: promotion of the report using a form designed for this purpose, classification, root cause analysis, corrective and preventive actions and evaluation of the effectiveness of these actions. Personnel were trained to be able to detect and report events in a no punitive environment.

Results: Events between January 2002 and December 2003 (n = 81), were reported, analyzed and placed in the following categories: transfusion process 40.7%; component preparation, storage and labeling 32%; donor suitability and collection 7.4%; incomplete data of autologous donation 7.4%; materials and equipment 7.4% and biosafety deviations 4.9%. We found that 50.7% was attributable to latent conditions while the remaining 49.3% was due to active failure. During the transfusion process, 66.6% of near miss events and the 33.3% remaining were events (11 even one of 3664 transfusions), None of the events were life threatening for the patients.

Conclusions: The development of the ERP allowed us to identify the weaknesses of critical points of the system, to establish priorities for the decision making process, to implement preventive actions and to promote communication.

A 23.11
Transfusion in clinical practice: safety or disaster?
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Transfusion medicine is a clinically oriented discipline that emphasizes patient care. The safe handling of blood and blood products is paramount. A Quality System builds on Quality Assurance for delivering an acceptable Transfusion Service. An Error-logging score can reveal acceptable and unacceptable activities over time. It may reveal where measures to improve the Service may be taken. Error-logging data were collected over 3 months and analysed. Precursor events (near misses, such as phlebotomy sample-errors) are identified through an internal tracking system. Trust wide error in specimen collection: 20 specimens incorrectly labelled each week 17% crossmatch requests and 83% requests for group/save serum. Occasional near misses detected where patient identification on sample did not match actual patient having been bled; actual errors occurred. Incompatible units of blood were transfused due to incorrectly labelling of the specimen. Risk awareness relies on good transfusion chain communication, particularly when risking overtransfusing blood products. Borderline performance within the Trust was revealed when requests are inconsistently given. The 3 months study focuses on awareness of safety issues within the daily practice of delivering an acceptable Transfusion Service. Important is the detection and correction of less serious errors. Results show basic errors are surprisingly persistent.

A 23.12
Treatment of hypoproteinaemia in patients with renal failure and recipients of allogenic kidneys
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Patients with chronic renal failure (CRF) on hemodialysis tend to develop hypoproteinaemia secondary to diffusion of amino acids from the patient’s circulation to the dialysate. The drop in blood amino acid levels is as little as 21 ± 7% a week. Infusion of aminosteryl nephro, a solution that contains a mixture of essential amino acids and histidine, was undertaken in 18 patients. 200 ml of the medication was given 60 min prior to the dialysis, with simultaneous injection of 200 ml of glucose solution (20%). The course of the auxiliary intravenous nutrition of the last two weeks during which the patient received 1.2 l of the solution aminosteryl nephro, i.e. 66 g of protein, or 2028 kcal. The efficacy of the therapy was evaluated based on the total protein and albumin blood levels, as well as on ALT and AST activities before and after nutritional. The therapy resulted in a rise in total protein and albumin blood levels from 67.8 ± 1.52 to 74.7 ± 1.43 g/l and from 26.1 ± 0.12 to 3.46 ± 0.14 g/l, respectively. On the contrary, AST and ALT activities fell from 46.5 ± 11.2 to 24.2 ± 9.2 U and from 26.1 ± 0.12 to 28.0 ± 10.4 U, respectively. In conclusion, the auxiliary intravenous nutrition via the balanced solution of amino acids leads to not only correction of albumin blood levels but also to amelioration of the liver function suggested by a decrease in ALT and AST activities.

A 23.13
Complex analysis of the errors made by participants in China NEQAS
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Objective: In order to establish and monitoring of the overall performance of blood transfusion laboratories in China, a external quality assessment scheme (EQA)
A total of 358 subjects were included in the study. Patient materials and methods: Participates were requested to perform ABO/RhD typing, antibody screening and identification, and crossmatch test. The analysis performed was depending on the identification of the errors made by participating laboratories. Results: Overall error rates in ABO typing was 3.3% ([5/153); in RhD typing was 1.9% (6/315)]; in antibody screening and antibody identification, the total error rate was 4.0% (10/250); in crossmatch test, the error rate was 6.0% (15/250). Regarding the errors made in ABO/RhD typing, 81.8% (9/11) were due to reagents or poor techniques while the others (18.2%) made by transcription and misinterpretation. As to the errors in antibody screening, the fail negative results may amount to 100% ([10/10] and in crossmatch test fall negative was 90% [27/30]).

Conclusion: This data will reflect the current state of the performance of transfusion laboratories in China. When compare with the data from UK NEQAS and WHO IEOQS, training programs, especially immunohematological skill for laboratory personnel, need to be considered. Thus the patients will receive appropriate and safe transfusion.

A 23.14 Genotyping of CYP2C9 in transfusion practice
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Introduction: The CYP2C9 is the principal enzyme responsible for the metabolism of S-warfarin. Besides its wild type allele CYP2C9*1, two common allelic variants are known in Caucasians – CYP2C9*2 and CYP2C9*3 that in homozygous state express enzymes of very low activity.

Aim: Evaluate the influence of CYP2C9 polymorphism on the variability of warfarin dose requirements.

Materials and methods: A total of 358 subjects were included in the study. Patient group included 181 patients on warfarin therapy. Control group included 177 unrelated healthy volunteers. PCR-RFLP genotyping for CYP2C9*1, *2 and *3 alleles was performed by using Ava II, Nsi I and Kpn I restriction endonucleases.

Results: Six different genotypes of CYP2C9 were identified in the patient and control group. As many as 42% of patients and 31.2% of controls were found to have at least one variant allele. The frequency of CYP2C9*2 and CYP2C9*3 alleles in control group was 12.4 and 3.7%, in patient group 17.4 and 6.6%, respectively (no statistical significant difference P > 0.05). The patients were divided into two subgroups, lower and higher than median dose of warfarin (4.1 mg). A considerably higher frequency of variant CYP2C9 genotypes was found in the subgroup on a lower than 4.1 mg daily warfarin dose (48.9% vs.36.3%).

Conclusion: An example of clinically important genetic polymorphism of drug metabolism is the association of variant alleles of CYP2C9 with lower warfarin dose requirements.

A 23.15 Positive Kleihauers: from a true TPH to the inappropriate use of anti-D
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Background: The Kleihauer test is used to detect and quantify transplacental haemorrhages (TPH). Using three case studies, this report demonstrates that not all positive Kleihauers: from a true TPH to the inappropriate use of anti-D Ig.

Case 1: An infant was born with severe anaemia (Hb 23 g/l) due to a large TPH. The Kleihauer test is used to detect and quantify transplacental haemorrhages (TPH). Using three case studies, this report demonstrates that not all positive Kleihauers: from a true TPH to the inappropriate use of anti-D Ig.

Case 2: A positive Kleihauer test was observed in a known case of reticulocytosis. No anti-D was administered inappropriately.

Case 3: An infant was born with a positive (4+) DAT. Anti-D was eluted from the red cells. The mother had been given 10 000 IU anti-D Ig for an apparent large TPH. However, it was subsequently discovered that the mother had HPFH (Hereditary Persistence of Fetal Haemoglobin) of the Swiss Type, mimicking a large TPH. The anti-D Ig in this case was thus administered inappropriately.

Discussion: The above cases highlight the fact that a positive Kleihauer test in a pregnant woman does not necessarily mean a TPH. Although these cases are relatively infrequent, they serve as an important learning tool for the interpretation of a positive Kleihauer and also to prevent inappropriate administration of anti-D Ig.

A 23.16 Anticoagulation clinics: 1-year follow-up
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Introduction: The relation between thrombosis and bleeding, added or not to the use of anticoagulant drugs determines the success or fail in therapeutics of patients that need to maintain a permanent anticoagulation.

Objectives: To report the results of 1 year follow-up of the patients from the INCL anticoagulation clinics.

Methods: A cohort of patients from the oral anticoagulation group of the ambulatory that submit to periodical coagulation tests, aiming to maintain the target INR according to their clinical necessity.

Results: Between 2 May 2002 and 22 May 2003, we have made a survey of 1144 attended and followed patients: male, 510; female, 634; total medical meetings, 8832; average break time between meetings, 34.12 days; average meetings per patient, 7.74; average age of patients, 51.70; patients in the target INR range, 67%; patients out of the target INR range, 456 [P < 0.0001]. 13 patients presented with INR non-coagulation, but only three of them had bleeding as complication.

Conclusion: The anticoagulation clinics have created rules that lead to a higher adhesion to the use of oral anticoagulant drugs. Added to this, better therapeutic results had been achieved whether it is compared to the traditional treatment and a good security standard has been demonstrated.

A 23.17 Assessment of the rational and effective use of blood in surgery
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Introduction: The existence of a system of monitoring and evaluation is essential to assess the patterns of the clinical use of blood. The aim of this study was to evaluate the usage of blood at the Clinical Centre, Skopje; especially the ratio of crossmatch (XM) to transfused components (C/T).

Materials and methods: We evaluated the transfused surgical patients during 2002 year. We used the following indicators for monitoring and evaluation: units requested/units transfused by patient category; percentage of unfilled requests by product; blood request form and transfusion reaction report form.

Results: The number of crossmatched and transfused units and the C/T ratio is shown in the Table:

| Department          | XM   | Transfusion | C/T ratio |
|---------------------|------|-------------|-----------|
| Urology             | 979  | 780         | 1.25      |
| Abdominal surgery   | 1419 | 985         | 1.44      |
| Obstetric and gynaecology | 1375 | 560         | 2.45      |
| Neurosurgery        | 490  | 331         | 1.32      |
| Orthopaedic setting | 1001 | 331         | 2.23      |
| Intensive care      | 1881 | 1230        | 1.75      |
| Thoracic surgery    | 365  | 155         | 2.35      |
| Traumatology        | 935  | 345         | 2.71      |

The main estimated value of the C/T is 1.9. The unfilled requests are about 17%. Blood products are never issued without a blood request form. Transfusion reaction report is returned only in cases of severe transfusion reactions.

Conclusion: The usage of blood in surgery is not satisfactory rational, and the supplies of blood are not adequate to meet demands. This is partly due to the lack of transfusion committees, which are essential for the effective and rational use of blood.
A24.1 Transfusion complications and haemovigilance

A 24.1 Adverse event reporting system for blood and blood products
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The development of the Manitoba Adverse Event Reporting System (AERS) is part of a National Transfusion Transmitted Injuries Surveillance System (TTISS) sponsored by Health Canada.

Objectives: (1) Monitoring trends in order to identify any increases in the type and magnitude of known risks; (2) Assessing the magnitude of new transfusion risks, including emerging pathogens; and (3) Monitoring the effectiveness of efforts to reduce transfusion risk.

Method: (1) Creation of an electronic events reporting system; (2) Creation of an electronic report to the responsible authorities; (3) Initial pilot study followed by province-wide implementation; (4) Qualitative and quantitative analysis of data.

Results: General evaluation of first one-year period showed that only 40% of reports were complete. 18.7% were missing product and modifier codes. 12.1% were missing center codes. 9.9% were using incorrect form. Analysis of adverse event investigation results showed that 16.5% of reports had failed to collect culture data. 3.4% had severity/data on transfusion data missing.

Conclusions: A targeted follow-up educational initiative will be required in order to improve the quality of data received. This will facilitate the collection and analysis of meaningful adverse event data as a basis for continuing quality improvement to our blood system.

A 24.2 The association of cytokine gene polymorphism with FNHTR in politransfused patients
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Cytokines (IL-1, IL-1Ra, IL-6, IL-10, TNF and LTA) generated by endothelial and white blood cells are associated with inflammatory responses including febrile nonhemolytic transfusion reactions (FNHTR). The occurrence of FNHTR was unpredictable and individual characteristics of each patient maybe involved. Moreover, there are some polymorphisms of these cytokine genes associated with different levels of gene expression. The aim of present study was to investigate the association of inflammatory cytokines gene polymorphisms with the occurrence of FNHTR in politransfused patients.

We studied two groups of politransfused patients: one with FNHTR occurring after and the other before 20 expositions of donors. The gene polymorphisms studied were: IL1B–511C/T and +253G/A using PCR and restriction digestion methods. All genotypes and allele frequencies were in Hardy-Weinberg equilibrium. An association of IL1RN+2 and IL1RN+2 genotype with the occurrence of precocious FNHTR \( \chi^2 = 5.360 \), \( P < 0.025 \) and \( \chi^2 = 7.996 \) with \( P < 0.02 \) was detected. This allele and genotype failed to sero-convert include low viral load anduffy-coat depletion of red cells.

A 24.3 Establishing risk assessment management system in a large blood bank
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Background: There are increasing regulatory and socio-economic demands on the quality and safety of blood components and patient diagnostics. In addition, there is the need to control the therapeutic efficiency and side-effects of transfused blood components.

Methods and results: We have therefore established a risk assessment management system (RAMS) in order to control quality and safety of more than 1.6 million blood components in our blood bank. This novel system is an essential element of our quality management systems. It measures internal as well as external errors. All errors are analysed and categorised according to their error level. Using this grading, our system defines two different types of errors, pharmaceutically derived and diagnostically derived errors. Both error-types are documented by specific error-sheets, which include the verification of the error by the internal personal as well as the external client. Subsequently, error analysis, definition of quality and safety relevance and corrective and preventive actions are defined and centrally documented. All relevant aspects and results of our system are presented to the management board. Required structural modification of processes are subsequently implemented and periodically monitored.

Conclusion: Risk assessment systems are of considerable use for improving safety and quality of blood components and related diagnostics. They can also increase the customer acceptance and satisfaction.

A 24.4 Transmission and non-transmission of HIV by blood transfusion: a case report
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Background: Blood from a regular donor was collected and processed into components during October 2002. The unit was screened for HIV-antibodies and p24-antigen.

The unit tested negative for all markers of transmissible disease. The red cell and platelet concentrate units were transfused to two patients and the fresh frozen plasma (FFP) was quarantined. In January 2003 the donor had sero-converted.

Aim: The recipients were identified through look back and tested to determine their HIV-status.

Method: Routine tests were performed on both recipients and FFP. Subsequent testing was determined by test results. The platelet recipient tested HIV-positive. Nucleotide sequence and phylogenetic analysis followed. The red cell recipient was negative. The following tests were performed 8 months post-transfusion: From HIV-1, HIV-1 and -2 ELISA, HIV-1/2 Ab-capture ELISA, HIV-1 Western Blot, HIV-1 quantitative PCR, CCR5 genotyping and in vitro HIV susceptibility. PCR was done on the FFP.

Results: Platelet recipient: Testing determined the HIV-1 subtype in donor and recipient to be identical. Red cell recipient: Results of tests done were negative. CCR5 genotyping showed a wild type CCR5 gene. In vitro PBMC's were susceptible to infection with HIV. FFP: Quantitative PCR: 12 RNA copies/ml.

Conclusion: HIV transmission is highly effective through transfusion. Reasons for failure to sero-convert include low viral load anduffy-coat depletion of red cells.

A 24.5 Introduction of the DiaMed blood audit release system on a main issue fridge
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Serious Hazards of Transfusion recommends that computerised systems be introduced in the transfusion chain to prevent ‘wrong blood’ incidents. BARS controls access to and release of units from the blood bank fridge so that only trained staff with a validated ID card can collect. In July 2003 BARS was introduced on one site where 32,000 Units are issued per annum. At present BARS is restricted to the main issue fridge where it is used for the collection of red cells only. BARS also have the ability to audit all blood movements and provide a final bedside check; these components have yet to be introduced. Introducing BARS involved targeting the training of many different staff. Problems were encountered because of a high turnover of permanent staff and number of agency. Contingency plans were required for the potential risks and failures associated with the system. 658 members of staff are trained with ongoing weekly training available. To date no situations have arisen directly as a result of BARS. An in-depth audit of its activity is underway. Following the success of BARS we will be introducing it sequentially to the issue fridge on the other site, all satellite fridges and for the collection of other blood products. Benefits thus far have included the maintenance of collection records and the availability of more information in relation to the complexities of the transfusion chain. This information can be extremely useful for clinical risk and clinical governance issues.

A 24.6 An audit of blood fridge activity
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The following activities were recorded on the blood issue fridge using the Blood Audit Release System for a 7-day period: load (L), crossmatched unit loaded; temporary removal (TR), unit removed for transfusion or storage in another facility; stock return (SR), unused unit returned; and permanent removal (PR). The number of units specified...
for each patient was also noted. During this period 2011 activities were recorded, 58% occurred during 06:00–18:00 h Monday–Friday and 28% was error data (separate analysis). 64% units were loaded, of which 70.5% subsequently underwent TR. A further 17.7% were SR. Of single unit TR, a median of 5.4 (range: 0–15) were removed in any 6-h period and occurred equally during all time periods; multiple unit TR were mostly 05:00–18:00 h Monday–Friday. SR activity (114 units) occurred almost exclusively 06:00–12:00 h Monday–Friday. Average PR was 46 Units per day (range with a peak of 82 Units on Thursday 06:00–12:00 h. 81.15% of PR activity was 06:00–12:00 h Tuesday–Saturday. The data suggests there is a high crossmatch workload where 47.2% of units are not transfused following crossmatch, with 28.5% never removed from the fridge. The high rates of SR and PR on days following elective surgical lists suggest that excess numbers of units are requested for surgery. Single unit TR occurs at all times; this implies single unit transfusions are given 24 h a day. Data from this system should allow better rationalisation and planning of crossmatch requests.

A 4.27
Usefulness of a blood donation sample archive
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The SNBTS created a blood donation serum archive several decades ago. Up until 1985 this archive was kept for around 1 year before being discarded. The advent of HIV-testing meant the possibility of infected recipients claiming they had been infected seven years previously was reduced. Thus the archive was extended to an indefinite period. More recently with the introduction of HIV NAT minipool testing, the use of the PPT tube replaced the serum archive. A survey was conducted in 2003 to ascertain the usefulness of this archive in one of our centres over a 10-year period. Main uses of the archive have been (1) the investigation of adverse transfusion events, especially suspected transfusion-transmitted infection (TTI), transfusion related acute lung injury (TRALI), (2) looks back on previous samples from confirmed positive blood donors, and (3) as a resource to assess new versions of tests for microbiological markers. Results of TTI investigations demonstrated that 45% of investigations were carried out on samples that had been stored for less than 3 years. HBV investigations were generally all conducted within that period whereas HIV and HCV investigations often involved archive samples that had been stored for around 10 years or more. For the majority of investigations, transfusion would appear to have been eliminated as the cause. Emerging diseases with extremely long incubation periods will ultimately drive for long term monitoring of its archive.

A 4.28
Three-year prevalence of immediate transfusion reactions in BRNO Faculty Hospital
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Objective: Adverse effects of transfusion – transfusion reactions are serious complications of the haemotherapy. Prevention of transfusion reactions is very important part of the hospital care. In relationship to the number of blood product units transfused a year were evaluated the trends during three years period (2001–2003). Materials and methods: BFH is the only institution of its kind in the area of South Moravia with basic, specialized and highly specialized medical care in all branches of medicine. Haemotherapy in BFH is managed according Quality Management Standard System. Results: Reports of the transfusion reactions and their analysis are in BFH managed by BFH. During 3-year period were reported 108 cases of immediate transfusion reactions, one case was characterized as serious reaction due to circulatory overload with vital functions support. No case was acute haemolytic transfusion reaction and no transfusion reaction was fatal.

A 24.9
Telemedicine in the blood transfusion service
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The transfusion services supply hospitals with blood products and pretransfusion testing. For the assurance of a 24-h service, Slovenia needs 12 permanent on-duty specialists in transfusion medicine available in its 12 transfusion centres. Rationalization of personnel and substantial increase of blood safety could be achieved, using an online teleconsultation system. The existing information system was upgraded with a teleconsultation system that enables the exchange of professional information between 12 blood transfusion centres. The exchange consists of audio-visual communication, exchange of all transfusion-related data, availability of donor and patient histories and of a unified national blood donors data bank. The visual communication allows the remote interpretation of laboratory results via live-pictures of the gel cards used for immunohematology testing. Special care is dedicated to the safety issues. In the pilot study, pretransfusion testing results were interpreted correctly when a picture of the coded gel card was transmitted to the consultant on duty in the blood transfusion centre. Appropriate expertise was offered to the sending technician, enabling a correct transfusion intervention. This was the first telemedical service of its kind in Slovenia. With appropriate organisation and by virtue of telemedicine, it is possible to perform remote professional consultations. We expect that telemedicine will find its place in transfusion medicine.

A 24.10
Reports of adverse events in blood transfusion in Slovenia in 2002–2003
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Background: In Slovenia, the idea of haemovigilance was present more than 40 years ago, when a unique transfusion medicine reporting form in its 12 transfusion centres. Rationalization of personnel and substantial increase of blood safety could be achieved, using an online teleconsultation system. The existing information system was upgraded with a teleconsultation system that enables the exchange of professional information between 12 blood transfusion centres. The exchange consists of audio-visual communication, exchange of all transfusion-related data, availability of donor and patient histories and of a unified national blood donors data bank. The visual communication allows the remote interpretation of laboratory results via live-pictures of the gel cards used for immunohematology testing. Special care is dedicated to the safety issues. In the pilot study, pretransfusion testing results were interpreted correctly when a picture of the coded gel card was transmitted to the consultant on duty in the blood transfusion centre. Appropriate expertise was offered to the sending technician, enabling a correct transfusion intervention. This was the first telemedical service of its kind in Slovenia. With appropriate organisation and by virtue of telemedicine, it is possible to perform remote professional consultations. We expect that telemedicine will find its place in transfusion medicine.

A 24.11
Incidence of acute transfusion reactions using leuco-depleted components in a tertiary care hospital
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Objective: To find out the incidence of transfusion reactions using buffy coat (BC) depleted blood components in a tertiary care hospital in India. Materials and methods: A total of 145 439 buffy coat depleted blood components (red blood cells, 55 353; platelet concentrate, 38 849; and FFP, 51 237) were prepared and transfused to the patients at Indraprastha Apollo Hospital, New Delhi, using quadruple top and bottom (Baxter) blood collection bags and opti press II between January 2000 and December 2003. 10 503 Units of whole blood were also transfused to the patient during this period. Each and every unit of blood component once issued for transfusion was accompanied by transfusion reaction report form and the transfusionist (junior doctor/nurse) was instructed to send back the form duly filled in case of any transfusion reaction. Results: A total of 173 transfusion reactions were reported and analyzed corresponding the overall transfusion reaction rate of 0.11%. Conclusion: There is decrease in the incidence of transfusion reactions with the use of buffy coat depleted blood components (0.07) compared to whole blood (0.6%). Majority of the non-haemolytic transfusion reaction were observed in multiple transfused reaction.
A 24.12
Post-transfusion viral hepatitis C among children with β-thalassemia
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Purpose of work: Studying frequencies of meeting post-transfusional viral hepatitis C among children with β-thalassemia and finding ways to prevent this.

Materials and methods: 126 kids with β-thalassemia at the age of 2–17 years old were checked up. Analyses were carried out by IFA method with ELISA kits developed by Human (Germany). Anti-HCV marker of viral hepatitis C has been identified (anti-HCV positive sera were checked up for containing of RNA HCV).

Results and discussion: Results of analysis showed that in the first group of sick people frequency of meeting anti-HCV is 48%. Most of people like this in Azerbaijan cannot receive transfusions in a proper time because of the economical position. That's why second group's sick people were divided into two groups: first does not regularly receive transfusion and the second receives transfusions twice a month. Investigations in these groups showed that maintenance of anti-HCV is 95 and 98%, respectively. We also investigated blood donors (784) and frequency of meeting anti-HCV of whose were 3.5%. Besides we investigated donors (377-friends of patients) compatible by ABO and other erythrocyte antigens (C, D, E, c, e, K, M) with a blood group of sick thalassemia people. Percent of positives among this group was 2.4%.

Conclusion: Investigations showed that frequency of meeting hepatitis C among sick people with thalassemia received hemotransfusional therapy for more than 5 years is between 95 and 98%, depending on transfusion load and also on the patient's age.

A 24.13
Microchimerism in immune competent patients related to leukocyte content of the transfused red cells
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Microchimerism may play a part in transfusion complications. The aim of this study was to see if the establishment of post transfusion microchimerism was dose dependent.

Methods: Twenty non-pregnant female patients, without known malignant or immunological diseases, mean age 68 years, receiving 2–4 (±1 from a male donor) red cell concentrates (RCCs) during elective surgery, were included. Ten received buffy coat depleted (BC) RCCs, leukocyte count 107–108 per unit, and 10 received RCCs leuko-reduced (LR) by filtration, leukocyte count <106 per unit. EDTA samples were collected before 1 week and 6 months after transfusion, were frozen and stored at −80°C. Genomic DNA was isolated and PCR performed using four primer sets amplifying markers on the Y chromosome.

Results: Microchimerism were detected in eight out of the 20 patients studied. In three patients microchimerism were detected before transfusion. These patients had given birth to 1–2 boys each, but did not have a transfusion history. Two patients receiving BC RCCs and two patients receiving LR RCCs had detectable microchimerism one week after transfusion. One patient receiving LR RCCs had detectable microchimerism after 6 months. No patient had detectable microchimerism in more than one sample.

Discussion: We did not find that the establishment of microchimerism after transfusion was dose dependent. This may explain the difficulties in showing that leukoreduction reduces immunosuppression more than buffy coat removal.

A 24.14
Haemovigilance: experience with voluntary vs. compulsory reporting systems
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Method: All reports of ‘wrong blood to the wrong patient’ were studied for year 2000–2002. Such reports were found in one compulsory system, requiring immediate reporting of all incidents leading to death or serious injury to the patient, or events that could have led to serious injury to the patient. Reports were also found in the voluntary system that require reporting, at the end of the year, of all haemolytic transfusion reactions.

Results: A total of ten incidents of ‘wrong blood to the wrong patient’ were reported. Three incidents were reported in both systems. Two incidents were only reported in the compulsory system and five incidents were only reported in the voluntary system. Some patient needed intensive treatment of a transfusion reaction, but no deaths were reported.

Discussion: The scope of the reporting systems is different. The voluntary system only registers incidents where a haemolytic transfusion reaction has been identified by the blood bank. The compulsory system requires reports of all incidents; even those that did not, but could have led to serious damage. Transfusion of blood to the wrong patient must fall into this category. Despite this, the voluntary system has received more reports than the compulsory system. From 2004, a voluntary system requiring immediate reporting to a special interest group is established in an attempt to improve reporting.

A 24.15
Fatal ABO mismatch masked by massive transfusion
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Blood group reference laboratories deal with a range of requests from simple investigations to the more complex and difficult, some of which test the resources and expertise of the staff involved. On occasions these difficult cases can have unexpected answers and in some adverse outcomes. We report a case of an ABO mismatch complicated and masked by massive transfusion.

Case details: An urgent request was referred to the SBGRL to assist in providing compatible red cells for a 75-year-old female post surgery for an aortic arch repair. On testing all panel cells were reactive and initial attempts to even provide ‘crossmatch compatible’ units were unsuccessful. Patient died shortly after referral request received. Due to some observed discrepancies in the testing of this referral sample, the pre-transfusion sample was requested from the referring laboratory. Extensive testing over several days, including the eventual retrieval of historical data, provided a solution to this complicated case. Death attributed to massive incompatible ABO (and other antibodies) transfusion. There were clinical retrospective observations that were not, at the time, suspected as being suggestive of the manifestations of an incompatible transfusion. Failure of correct sample collection procedure leading to a double sample ID error was determined as the cause of this unfortunate event.

P 24.16
Male only fresh frozen plasma to reduce risk of transfusion-related acute lung injury
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Although the aetiology of TRALI is thought to be multifactorial, in >80% of UK cases where FFP is implicated there is a strong association with the presence of donor leucocyte antibodies (LAbs). LAbs are more common in female donors and develop as a result of pregnancy. Measures that reduce transfusion of LAbs are likely to reduce TRALI risk. Options were considered for reducing the risk of TRALI from FFP. The best option was to select male donors. We therefore investigated the feasibility of production of male FFP. To meet Council of Europe quality requirements, FFP is frozen on the day of collection (day 0). Blood centres aimed to maximize the number of male donations processed on day 0. Donations were identified manually at donor sessions as coming from male or female donors enabling selection of male donations for specific process streams resulting in FFP production. Male donor FFP increased from 56% in October 2003 to 58% in Jan 2004 and was maintained above 90% since November 2003. For the same period, the number of all male plasma components (FFP, cryo-depleted plasma, cryoprecipitate, plasma for platelet pooling) produced, increased from 6597 (54.8%) to 8886 (72.8%) per week. It is feasible to increase the proportion of male FFP by a multidisciplinary operational approach. Further changes to staffing and pack usage will facilitate an increase in the proportion of other male plasma components. Impact on TRALI incidence will be monitored through the UK haemovigilance scheme.

A26 Transfusion and alternatives in medical patients

A 26.1
Treatment of patients with tumors by transfusions
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Treatment of patients with tumors by transfusion in most cases is the only way of their treatment, which includes use of different components of the blood and derivatives of plasma. The aim of this study was retrospective analysis of use of blood components in patients with tumors, malignant and benign as well. Material for this study was collected in University Clinical Centre of Kosova, during the period of time of 1 year.
A26.2 Management of anemia in patients with chronic renal failure on permanent haemodialysis
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Background: Each patient on haemodialysis (HD) suffers from anemia more or less. A work-up for a diagnosis of anemia should be considered when the Hb is <11 g/dl (Htc < 33%). The aim of this study is to point out how these patients were treated for anemia.

Materials and methods: The data were used from the database of the Nephrology Clinic for the year 2003. The following parameters were investigated: levels of Hb, Htc, Er, serum iron, TIBC, ferritin, TSAT and iPTH.

Results: There were 197 HD patients, of which 156 (79.2%) on regular Epoetin beta therapy (Epo). The target Hb/Htc concentration for Epo treatment was 11 g/dl (33%). Epo was given subcutaneously at the end of each HD usually divided in three doses weekly (50–80 IU/kg/week). 112 patients received supplemental iron, which was given to maintain a TSAT > 20% and a serum ferritin level > 100 mg/ml, so that HD patients can maintain an Hb concentration > 11 g/dl with or without Epo treatment. There were 43 (21.8%) HD patients who received blood in 2003. Starting dose for Red Blood Cells transfusion was Hb <85 g/dl (Htc < 28%). 22 of them received blood only once (1–2 Units), but 21 of them had multiple transfusions during the year (from three to 10). The reasons for transfusion were acute blood loss and inadequate response to Epo.

Conclusion: Epo has the main role in anemia treatment of the HD patients, and it should stay that way. Red blood cells should be always limited to circumstances in which anemia is not otherwise reversible.

A26.3 Blood transfusion therapy: blood component and blood product administration
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This poster developed by the author and distributed widely to hospitals in New Zealand used as a guideline and reference for junior doctors (and often some seniors too) and mostly referred to by paramedical staff such as nurses and midwives. They have found it extremely useful to have information at a glance to help in their busy routines and follow-up more complex issues with respective blood banks or a specialist in this field in addition to guidance regarding transfusion of blood and blood products, product specification (as currently used in New Zealand) storage, addition of medicines and appropriate administration of these materials, there is guidance with regards to good transfusion practice.

A27 Transfusion and alternatives in surgical patients

A27.1 Use of blood, blood products and substitutes in women undergoing radical hysterectomy by Wertheim–meigs
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Background: Each patient should be given the right blood component. These way unnecessary and potentially dangerous transfusions of components that are not needed will be avoided. The aim of the study is to show how the women who undergo radical hysterectomy (HTA) were managed.

Materials and methods: This is a retrospective study, where data were taken from the Clinic for Obstetrics and Gynaecology in Skopje for the year 2003.

Results: There were 118 women who had had radical operation (HTA) because of invasive carcinoma of cervix uteri in 2003. The average blood loss during one operation was 400–600 ml and the trigger for erythrocyte (Er) transfusion was Hb < 90 g/dl. Because of hypoproteinemia they received fresh frozen plasma (FFP) and albumin (Alb). 62 women received 1 Unit of Er, 18 women received 2 Units during the surgery. 35 women received 1 Unit of whole blood, either because of need for all blood components or unavailability to provide separated blood components. There are only three women that did not receive therapy with blood during the surgery. All of them, also, received 1500–2000 ml crystalloid or colloids each. Postoperatively, they received 271 Units of Er (2.1 Unit/patient), 2000–2500 ml of substitutes each of them, 110 Units of FFP in 85 (72%) patients (1.3 Unit/patient) and 195 Units of 9% albumin in all 118 patients (1.65 Unit/patient).

Conclusion: We should be more restrictive in the use of blood, blood components and the substitutes and we should use them only when it is highly necessary.

A27.2 Data on clinical use of blood in surgery in university hospital in Tirana
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Objective: Assessment of the effectiveness of the blood transfusion based on the clinical and the laboratory parameters.

Materials and methods: A total of 67 patients were studied. Age, 16–60 years; normal volunteers; no active anemia. No pathology of the CV system, pulmonary, blood clotting. Patients were divided in three groups: 53 patients transfused with one unit of blood, 10 patients 2 Units, four patients 3 Units.

Results: Lab findings after the transfusion: increase of the number of the RBC, Hct, Hb in the group I. In the group II there was a statistically significant increase comparing to the group I. In all the groups there was a marked improvement of the respiratory and I/R. No significant changes in MBF were found after the first transfusion. In the groups II and III there was not found statistically significant improvement of the parameters after the first blood unit after transfusion. After transfusion there were found no statistical changes in the clinical parameters between the three groups.

Discussion: The transfusion of one unit of blood in the patients with acute anemia improves clinical parameters. Lab parameters improve according to the quantity of the blood units transfused. Clinical parameters remain substantially unchanged after the transfusion of the first unit of blood.

Conclusion: The transfusion should be considered only as a part of the treatment of the patients with anemia. The blood quantity should be as small as possible. The value of Hb should not be considered as decisive.

A27.3 Perioperative transfusion requirements in blood group O compared with non-O orthopaedic patients
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Aim: The lower levels of vWF described in blood group O versus non-O (group A, B and AB) appears to place group O patients at an increased risk for perioperative blood loss, although its clinical significance in transfusion strategy is not known.

Methods: A retrospective audit of 169 and 154 patients who underwent total hip (THR) and knee replacement (TKR), respectively, by one team of surgeons during a 3 years period was performed. We analysed data regarding patient’s characteristics, ABO-type and RBCs-transfused, which were collected from patient’s charts and Transfusion Service’s records.

Results: Patient characteristics regarding age, sex, preoperative and postoperative Hct were similar between the groups. There was no significant difference in perioperative transfusion requirement between groups O and non-O in THR and TKR, (1.93 ± 1.28 vs. 1.65 ± 1.15 Units/case; P = 0.94) and (1.47 ± 1.1 vs. 1.32 ± 1.02 Units/case; P = 0.43), respectively. The number [%] of patients not transfused for the groups for THR was [20 (19.6) vs. 41 (24.6); P = 0.184] and for TKR [23 (30.9) vs. 28 (35); P = 0.38]. The maximum number transfused per case was seven for THR and four for TKR.

Conclusion: Our study revealed no significant difference in perioperative transfusion requirement between groups in both operations. Stress induced increased in vWF in the surgical patient may account for the lack of difference between groups. No difference in transfusion services practice appears necessary based on patients ABO blood type.
A27.4 Evaluation of transfusion requirement for patients undergoing orthopaedic surgery

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Background: Specific information on blood product utilization could be used to ensure optimal transfusion procedure. The knowledge of the number of patients transfused for different procedures could more closely match the preoperative order to the expected operative use and reduce excess cross-matching blood. Information collected included data of our Blood Bank from 3 April to 3 October: categories of operations, gender, age, preoperative and pretransfusion hemoglobin level.

Subjects and methods: A consecutive cohort of 161 patients undergoing surgery for hip fracture, 86 for hip replacement, 31 for knee fracture and 27 for knee replacement were evaluated.

Results: Mean age for patients given transfusion was 71. Woman had a higher overall transfusion rate than men (64% vs. 36%). A total of 39.5% hip replacement, 36.1% hip fracture, 22.2% knee replacement and 12.9% knee fracture received allogenic transfusion.

Conclusions: This evaluation allows identifying significant waste related to blood utilization practice in hip operations. Estimating expected perioperative blood waste is critical for establishing an effective transfusion strategy. Hospitals must collaborate with the regional blood supplier in a statewide transfusion practice and improvement initiatives for not increase blood waste.

A27.5 Iron deficiency erythropoiesis in autologous blood donors treated with EPO

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In patients undergoing and aggressive PARD schedule before major elective surgery often is to increase, to increase the number of collected blood units (BU), the administration of EPO and iron. The presence of iron deficiency erythropoiesis (IDE) may reduce the yield of these procedures. 37 patients undergoing open-heart surgery were considered. The protocol foreseen for collection of 5 BU in 4-5 weeks, with administration of iron and EPO. Complete blood cells count (RBC, HB, HCT, MCV, MCH, CHCM, WBC, PLT), reticulocytes indexes (% RA content, MCH, MCHC, % of hypochromic cells) and iron metabolism markers (iron, transferrin, transferrin saturation index, ferritin, soluble transferrin receptor) were studied before and after the protocol. 163 BU were collected (mean: 4.4), only 14 BU (9%) were discharged, five patients (14%) needed of allogenic blood (mean: 3.3 BU). In routine blood cells examination a significant diminution in RBC, HB, Hb was observed. Among reticulocytes indexes an increase of number and of the percentage of younger cells was observed together with an increase of the percentage of hypochromic reticulocytes. Among iron metabolism parameters only an increase of serum soluble transferrin receptor was observed. In authors' experience, despite iron i.v. supplementation, the insufficiency of IDE was observed. The best markers to recognize this situation is the serum transferrin soluble receptor, the study of new reticulocytes indexes may be useful too.

A28 Transfusion in neonates and children

A28.1 Transfusion therapy in children: as good as it gets?

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The aim is to present the rate of transfusion therapy at the Children’s Hospital Zagreb, distribution of transfusion treated children according to clinical departments, and blood products most commonly used from 1999 till the end of 2003. During 2002 we started to analyze data on transfusion therapy at meetings or individually with clinicians in order to change evidence-based unjustified blood treatment. Data were obtained from medical documentation. During the study period 2679 patients underwent ThiS pretransfusion testing. A total of 3739 crossmatch tests were performed, of which 66% of units were transfused. A total of 75 neonates and 1086 children were treated with blood and blood products: 603 of them at Intensive Care Unit, 182 at Department of Solid tumours, 250 at Surgical departments and 106 at Department of Pediatrics. Analyzing the use of particular blood products, platelets were given to 197 patients, red cells products to 802 and FFP to 390 patients. In last 2 years there are significant less unjustified transfusion treatments, especially with FFP. The required improvement of transfusion therapy was achieved by long-term monitoring of transfusion therapy, analysis of the present state, and evidence-based recommendations for rational use of blood products. Tight cooperation between clinicians and transfusionists is a precondition for choosing the best solution for transfusion treatment. There is still a space for the improvements.

A28.2 HDN surveillance: experience in an obstetric hospital, Turin, Italy

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In our department 11 147 deliveries have been screened. Those with alloimmunization were (0.44%): 20 anti-d, three D + C, one D + C + Jka, 11c, seven E, one e, three Kell, one Kell + Jkb, one E + Fya, one E + Jka, one M. Antibodies’ (Ab) titration was performed with microcolumn method. In some cases was possible to confirm titration with IIF method. Abs’ titration at delivery was ranging between 1/4 and 1/4000 for anti-D Ab. The four ‘Kell’ patients showed an Abs’ titration not very high (1/32–1/64) while all the ‘c’ patients had a low quantity of Ab (1/8–1/16). Patients who needed the hardest care were those with anti-D Ab. Three out of 20 anti-D patients showed fetal anaemia and hydrops fetalis in the second trimester and required intrauterine transfusion. All three babies received exanguino transfusion at delivery. Haemoglobin (Hb) at delivery was ranging from 4 to 6 g/dl and mean HCT was 20.6%. Bilirubin had a peak of 20 g/dl. Later they received RBC and EPO. At 3 months, two out of three babies showed a persistent high anti-D titration and an apparent zero Rh-negative blood group. Indirect IgG subclass determination showed a persistent high titration of IgG1 and IgG3, confirming they are the best value correlated with haemolytic risk. All newborns received therapy with intravenous IG and IV. At five month they did not show abnormalities, Hb and bilirubin range was in the norm. What we can observe is that a close collaboration between gynaecologists, newborn assistants and transfusion specialists was successful for all HDN patients.

A28.3 Audit of neonatal transfusion practice in India

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Aims: Blood transfusion is essential component of management in sick and very low birth weight (VLBW) and low birth weight (LBW) babies in Neonatal Intensive Care Unit (NICU). Therefore, we assessed the transfusion practice and trend in NICU.

Materials and methods: Records of all admission in NICU at Sunder Lal Jain Hospital were prospectively studied and analysed for frequency and volume of transfusion according to birth weight and gestation between 2000 and 2003.

Results: A total of 431 (22.2%) neonates out of 1936 received transfusion. 56.4% neonates who received transfusion, were transfused repeatedly. The need of multiple transfusion was more in VLBW and LBW babies. 72.9% VLBW and LBW babies required multiple transfusions. 70.4% transfusions were given during first 2 weeks of life. Mean of transfusion volume was 72 ml per child. The amount of blood transfused was 83.0 ± 9.4, 68.1 ± 3.1, 131.4 ± 12.4 and 112.1 ± 107.8 ml in babies with birth weight <1500,1500–1999, 2000–2499 and >2500 g, respectively. During study period, transfusion in neonates decreased from 26.3% in 2000 to 15.6% in 2003.

Conclusion: A significant number of neonates in NICU require blood transfusion. Incidence of transfusion decreased due to strict vigilance for need of transfusion and reduction in sampling loss. Multiple donor exposure due to repeated transfusion need to be taken care for future action; to reduce the multiple donor exposure and to reduce the load of transfusion transmitted diseases.