Universal leucocyte-depletion of blood components: cell concentrates and plasma

The removal of leucocytes from red cell and platelet concentrates, and plasma units, has several advantages. Evidence is available that leucocyte-depletion to below $5 \times 10^6$ per blood component transfused results in prevention of (primary) alloimmunization against human leucocyte antigen (HLA) class I antigens, febrile transfusion reactions and transmission of infectious agents such as cytomegalovirus (CMV) and Epstein–Barr virus (EBV). There still is doubt regarding whether transfusion-induced immune modulation can be prevented by leucocyte-depletion, and there is even more doubt whether, if variant Creutzfeldt-Jacob disease (vCJD) is found to be transmissible by blood transfusion, removing leucocytes could prevent its transmission. However, there is consensus that the quality of the cellular blood components improves when leucocytes are removed prior to storage.

Based on the above benefits, there are generally accepted specific indications for leucocyte-depletion. However, there is no consensus of opinion on the question of whether leucocyte-depletion should be universal, i.e. whether all red cell and platelet concentrates, as well as plasma units, should be routinely leucocyte-reduced. It seemed appropriate to obtain information on this important issue as well as on other aspects of leucocyte-reduction in an International Forum.

The questions listed below were sent to experts in the field.

Answers were obtained from 18.

**Question 1**: Are all red cell concentrates, platelet concentrates and plasma units leucocyte-depleted in your centre/country and, if not, do you intend to institute universal leucocyte-depletion in the near future?

**Question 2**: What is the motivation to implement or not to implement universal leucocyte-depletion for blood products in your country?

**Question 3**: Do you, or will you, remove leucocytes pre- or post-storage and what is the reason for your choice?

**Question 4**: Which method of leucocyte-depletion do you use, i.e. whole blood filtration, red cell in-line filtration, red cell on-line filtration, filtration of platelets single, filtration of platelet pools, filtration of plasma single, filtration of plasma pools?

**Question 5**: If you use different procedures, can you state in what percentages the above methods are used and the reasoning for it?

**Question 6**: What is your maximal storage time prior to leucocyte-depletion of red cell and platelet concentrates, and plasma units? What is the temperature of filtration?

**Question 7**: What is the upper limit of residual leucocytes in leucocyte-depleted components and with what level of confidence should this be proven in quality control (QC) data? Which counting method(s) is/are applied?

Do you have different upper limits for cellular products and plasma? If so, why? And what is the difference? What do you do when filtration fails?

**Summary of responses**

**Question 1**

In many countries > 70% of the leucocytes are removed from the red cells by removal of the buffy-coat. Further leucocyte-depletion is achieved by filtration. Countries that did not respond but have already instituted universal leucocyte-depletion are Ireland, Portugal and France.

Five of the authors (from Canada, Germany, New Zealand, Luxembourg and the UK) mentioned that universal pre-storage leucocyte-depletion of red cell and platelet concentrates is, or will soon be, mandatory. Except in Canada and the UK, this also applies to plasma for fractionation. In some countries only leucocyte-depleted platelet concentrates are transfused but leucocyte-depleted red cell concentrates are transfused upon indication (Finland and the Netherlands). In the USA, the percentage of leucocyte-reduced concentrates is increasing and the Food and Drug Administration (FDA) encourages conversion to universal leucocyte-depletion (see AuBuchon). In the other countries, leucocyte-depletion is restricted to the well-known indications (see the answers), mainly because of cost and the absence of medical evidence.

**Question 2**

The motivation in favour of universal leucoreduction is based on the generally accepted indications mentioned above. In addition, in some countries the theoretical risk of transmitting vCJD is the reason to opt for universal leucodepletion. In others, the possible deleterious effects of transfusion-mediated immunomodulation (Canada) contributed to the decision. However, other countries (USA, the Netherlands) are of the opinion that sufficient deleterious effects of transfusion-mediated immunomodulation have not yet been established. Studies are in progress to assess this.
The arguments for not (yet) introducing universal leucodepletion include the high costs of the procedure, taking into account that, in the opinion of many, the medical data have not yet provided sufficient confidence to make the extra costs acceptable. Therefore, many countries hesitate or have rejected implementation. Surprisingly, in Austria (see Mayr) the result of calculations on the cost-effectiveness was an argument for implementing leucodepletion.

**Question 3**

Virtually everywhere, leucocyte-depletion is performed prestorage, the main justification being that it allows for much better quality of the blood components (by preventing cytokine accumulation), quality control and quality assurance than when performed poststorage at the bedside.

Another argument is that spiking experiments have shown that prestorage leucocyte-depletion decreases the growth of Trypanosoma cruzi and of Yersinia enterocolitica. Storage of whole blood for a few hours after collection and subsequent leucocyte removal may allow phagocytosis of bacteria by leucocytes that are then removed before they disintegrate (see Rebulla et al., below).

**Question 4**

Clearly, many different methods are used. Filtration is mostly performed using integrated blood bag systems that have one or more in-line filters. These systems are preferred, especially for whole blood and red cell filtration. When only a small percentage of the red cells is filtered, sterile connection with a ‘dock on’ filter is more cost effective. For pools of platelet concentrates derived from buffy-coats, a filter that is integrated in the pooling set is used. In the USA, the FDA precludes the preparation and filtration of platelet pools prestorage to reduce the danger of bacterial contamination. Platelet units must be leucocyte-depleted separately and pooled just before transfusion. The reader is referred to the answers below for further information.

**Question 5**

In the majority of the countries a maximum of 24 h between collection and filtration is preferred for prestorage filtration of whole blood or red blood cells. However, the maximum storage time may vary from 4 h to 7 days.

If leucocyte-depletion is not carried out prestorage, the storage time usually extends to the maximum storage time of the blood component. Platelets are usually leucocyte-depleted within 24 h of collection of the blood, but storage may extend to 5 days. Plasma filtration is performed in very few countries and mostly as filtration of whole blood with subsequent component preparation.

There is no consistency in the temperature at which leucocyte-depletion is performed. Filtration of red cells is equally performed at 4 °C or at room temperature. For whole blood and platelets, room temperature is preferred.

**Question 6**

In most of the European countries, quality control, as described in the ‘Guide to the Preparation, Use and Quality Assurance of Blood Components’ (6th edition) of the Council of Europe, is applied. This means that red blood cells must contain < 1 x 10⁹ leucocytes in at least 90% of the tested units, platelets < 0·2 x 10⁹ leucocytes per single (whole-blood derived) unit, and < 1 x 10⁵ leucocytes per unit of apheresis platelets in at least 90% of the tested units. The upper limit of leucocytes in plasma before freezing is 0·1 x 10⁹/l. In general, the upper limit of residual leucocytes for cellular blood components is 5 x 10⁹, although in practice the number of residual leucocytes is much lower. Some countries allow double filtration when the number of residual leucocytes is too high; others require withdrawal of the product. For further details, see the answers below.

In the majority of countries, residual leucocytes are counted in the Nageotte chamber, but in some countries flow cytometry is now used. The loss of haemoglobin and platelets as a result of filtration, as well as the problem of filtration of red cells containing haemoglobin-S, should also be considered in quality control.

**Question 7**

In several of the countries the plasma fractionation industry accepts filtered plasma. In general, there are no specific requirements, but in Germany it is stipulated that the levels of clotting factors and plasma proteins should meet certain specifications. Investigations in Austria showed that these proteins remain stable during and after filtration (see Mayr).

**Conclusion**

At present, universal leucocyte-depletion is practiced in only a few countries; the reasons for not implementing compulsory 100% leucocyte-depletion are mainly financial. Several contributors are of the opinion that there is not (yet) sufficient medical evidence to justify adopting the policy of 100% leucocyte-depletion. Leucocyte-depletion is performed prestorage almost universally, the main arguments being the advantage of obtaining ‘purer’ components prior to storage with a better quality and process control.
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Question 1
In the Blood Donation Center of the Austrian Red Cross for Wien, Niederösterreich and Burgenland, all whole-blood units and all thrombapheresis units produced by separators that give a high contamination level of leucocytes, are routinely leucodepleted by prestorage in-line filtration. With minor restrictions, this applies to all other Blood Donation Centers of the Austrian Red Cross and to all centres associated with the Austrian Red Cross. These blood donation centres produce > 90% of the labile blood products in Austria.

Question 2
The motivation for the Red Cross Blood Donation Centers in Austria to set up universal prestorage in-line filtration was mainly driven by the well-established clinical benefits (higher quality of the products, elimination of cell-associated viruses, decrease in the human leucocyte antigen [HLA] immunization rate and in the frequency of febrile, non-haemolytic transfusion reactions, less platelet refractoriness, etc.), but also by the cost-effectiveness of this procedure.

Question 3
See the answers above, to questions 1 and 2.

Question 4
The methods of leucodepletion used at our Centre are as follows:
- red cell concentrates: < 1 x 10^6 leucocytes/unit;
- platelet concentrates: < 1 x 10^6 leucocytes/unit; and
- plasma units: < 0.1 x 10^9 leucocytes/l, < 25 x 10^9 platelets/l and < 6 x 10^9 red cells/l.

The quality control (QC) is performed by using fluorescence-activated cell sorter (FACS) analysis [2] on a number of samples given by the CE. So far, the QC procedure has never detected a contamination exceeding the limits. If this were to occur, a complete re-evaluation of the procedure would be initiated.

Question 5
The maximal storage time prior to filtration is 4–5 h, and the filtration temperature is ambient (20–25 °C).

Question 6
The upper limits of residual contaminating cells in the different products correspond to the figures given by the Council of Europe (CE) [1]:
- red cell concentrates: < 1 x 10^6 leucocytes/unit;
- platelet concentrates: < 1 x 10^6 leucocytes/unit; and
- plasma units: < 0.1 x 10^9 leucocytes/l, < 25 x 10^9 platelets/l and < 6 x 10^9 red cells/l.

Question 7
Our partners in industry accept plasma of in-line filtered whole blood for fractionation as we are able to prove the stability of relevant labile factors [3].

References
2 Neumüller J, Schwartz DWM, Mayr WR: Demonstration by flow cytometry of the numbers of residual white blood cells and platelets in filtered red blood cell concentrates and plasma preparations. Vox Sang 1997; 73:220–229

Canada

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Question 1
Since July 1999, all red blood cell (RBC), platelet and plasma units produced in Canada have been leucocyte-reduced by prestorage leucoreduction. This means that we have had universal prestorage leucoreduction of cellular blood products and plasma in Canada for > 2 years. For the 12 months prior to July 1999, we had in place prestorage leucoreduction of all platelet products produced, i.e. both random donor and apheresis units.

Question 2
In Canada, the primary motivation to implement universal leucoreduction was to enhance overall transfusion medicine safety, but this was unrelated to the issue of the possible theoretical risk of Creutzfeldt-Jacob disease (CJD) and variant (v)CJD.
Question 3
As indicated above, universal leucoreduction in Canada is performed by prestorage leucoreduction. Prestorage leucoreduction is being used in order to maintain optimal control of manufacturing consistency together with the best possible quality control (QC).

Question 4
Universal leucocyte-reduction in Canada is carried out using integral in-line filters. The Pall whole-blood systems are being used to leucoreduce RBCs and whole blood-derived platelets. The whole-blood system uses the WBF-2 in-line filter for the leucoreduction of RBCs. When platelets and RBCs are manufactured from the same whole-blood unit, the RCM-1 filter is in-line to leucoreduce the RBCs and the ATS-LPL filter is in-line to leucoreduce the platelets. Leucoreduced plasma units are produced as a by-product of the manufacturing process. Apheresis leucoreduced platelets are produced using the Gambro-COBE LRS apheresis system.

Question 5
The maximal storage time allowed prior to leucoreduction of random donor RBCs is 8 h for blood stored at room temperature and 72 h for blood stored at 4 °C. The maximum storage time prior to the leucoreduction of platelets is 8 h.

Question 6
The upper limit of residual leucocytes allowed for leucoreduced RBCs and apheresis platelet units is 5 × 10^6/bag. For random donor platelet units, the upper limit of residual leucocytes allowed is 0.83 × 10^6/bag. All, or 100% of units undergoing QC, are required to be below these limits. Any units found to exceed these limits are discarded. We do not have limits for plasma and thus plasma units are not quality controlled for leucocyte count.

Residual leucocyte counts on apheresis units are performed using the Nageotte chamber. These are carried out at the Blood Centres. Residual leucocyte counts on RBCs and whole blood-derived platelets are performed centrally by using flow cytometry analysis.

Question 7
The plasma fractionator institutes accept leucoreduced filtered plasma and have not indicated any specific requirements. UK plasma is not used for fractionation.

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In Canada, prestorage leucodepletion of whole blood-derived platelets was implemented in 1998. Since mid-1999, all allogeneic cellular components have undergone prestorage leucodepletion. Apheresis plasma is not leucodepleted. Leucodepletion of whole blood that is subsequently separated into red cells and plasma is performed using an in-line filter that is an integral part of the collection pack. When platelets are produced from whole blood, leucodepletion of the red cells and the platelet-rich plasma is carried out using two in-line filters that are an integral part of the collection pack. Deleucocytation of apheresis platelets is carried out during the procedure using centrifugal separation technology (Cobe Spectra LRS System). The maximum storage time prior to leucodepletion is 8 h of room temperature storage for platelets and 72 h for whole blood placed in cold storage. Plasma from whole-blood donation sent for fractionation has been leucodepleted, but this is not a requirement of the fractionator at this time.

In November 1998, Health Canada, the regulator of the blood system in Canada, mandated the two blood suppliers in Canada – Héma-Québec and the Canadian Blood Services (CBS) – to introduce universal prestorage leucodepletion of allogeneic cellular blood components by June 1999. Both blood suppliers supported the introduction of universal leucodepletion. The main reasons for implementation were:

- prevention of alloimmunization;
- prevention of febrile non-haemolytic transfusion reactions;
- prevention of the possible immunomodulatory effects of transfusion, including enhancement of tumour growth and relapse and an increase in postoperative infections; and
- prevention of transmission of leucocyte-associated viruses, such as cytomegalovirus (CMV), Epstein–Barr virus (EBV) and human T-cell lymphotropic viruses (HTLV) I/II. (Other leucocyte-associated viruses for which testing is not currently performed may eventually be shown to be of clinical significance.)

The advantages of prestorage leucodepletion are: greater efficacy of leucoreduction prior to leucocyte disintegration, greater process control and improved quality assurance. In spiking experiments, prestorage leucodepletion decreases the growth of Trypanosoma cruzi and some bacterial species, such as Yersinia enterocolitica. Although not all of these advantages have been conclusively demonstrated, it was felt that, on balance, leucodepletion would benefit transfusion recipients.

The upper limit for residual leucocytes in leucodepleted components in Canada is 5 × 10^6 for AS-3 red cells, apheresis platelets and whole blood-derived platelets. However, Héma-Québec and the CBS aim for < 0.83 × 10^6 leucocytes in whole blood-derived platelets.
Currently, quality control testing for the whole blood-derived components is performed under contract by an outside corporation, using flow cytometry on samples that are shipped in appropriate fixative. In-house flow cytometry assays are undergoing validation and will be implemented in the very near future. For all whole blood-derived components, 1% of the monthly production, or a minimum of four units if producing less than 400 units monthly, are subject to quality control (QC) counting. Results of all QC determinations of residual white counts are tracked over time and used as part of process-mapping analysis to determine if the system overall is in control or going out of control. Apheresis components have residual leucocytes determined also at a proportion equivalent to 1% of the monthly production, or a minimum of four units if producing fewer than 400 per month. When filtration has failed (i.e. the leucocyte count exceeds the upper limit) if the unit is still not transfused it is withdrawn; if transfused the data is simply tracked. We do not have different upper limits for cellular components and plasma (we do not perform QC analysis of residual leucocytes on frozen plasma).

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Question 1

The introduction of universal leucocyte depletion is not planned for the near future, neither in the County of Funen Transplantation Service (CFTS), nor in Denmark. For the proportion of blood components that are leucocyte-depleted, see Table 1.

Fresh-frozen plasma (FFP) is not leucocyte-depleted because it does not transmit cytomegalovirus (CMV) [3] and co-transfusion of intact human leucocyte antigen (HLA) class II-coated cells is a prerequisite for primary immunization against HLA-A, -B and -C. Observations of immunization towards HLA-A, -B and -C after transfusion of FFP have only recently been published [4].

Question 2

The motivation for not implementing universal leucocyte-depletion is cost-effectiveness. To establish indications for the use of leucocyte-depleted blood components, The Danish Society for Clinical Immunology arranged a consensus conference in 1994 [5]. The conclusions from this consensus conference were later confirmed by the Canadian Coordinating Office for Health Technology Assessment [6].

Question 5

In the CFTS, the maximal storage times are as follows:

1. RBC. Units are stored for 12–72 h at 4 °C before on-line filtration at (ambient) room temperature. The 12-h limit and cool-down to 4 °C is applied to ensure optimum filtration efficiency (according to the manufacturer's instructions). The upper time-limit has been set in order to prevent significant production of cytokines. As only »20% of our production of Saline-Adenine-Glucose-Mannitol (SAGM) RBCs are leucocyte-depleted, we use the degree of freedom given by the variable period of prefiltration storage to improve the logistics of these components.

2. Platelet concentrates. Buffy-coats are stored for 3–24 h at room temperature, pooled, centrifuged and then fractionated. Platelet concentrates are filtered prestorage using an on-line filter. Prestorage filtration is applied in order to prevent the production of cytokines as well as for logistic reasons (all units are filtered).

3. FFP is not filtered. Whole-blood filtration has not been an option as we depend on the buffy-coats for platelet concentrates. On-line

<table>
<thead>
<tr>
<th></th>
<th>RBC</th>
<th>Platelet concentrate</th>
<th>FFP</th>
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<tbody>
<tr>
<td>1999 Total</td>
<td></td>
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<tr>
<td>CFTS [1]</td>
<td>30 051</td>
<td>5 260</td>
<td>18</td>
</tr>
<tr>
<td>Denmark [2]</td>
<td>327 013</td>
<td>44 730</td>
<td>14</td>
</tr>
</tbody>
</table>

CFTS, County of Funen transfusion service; FFP, fresh-frozen plasma; RBC, red blood cell.

The values shown represent the total number of transfused units and the number that are leucocyte-depleted (LD). The figures for LD components may be higher as a few departments buy bedside filters themselves.
filtration has been chosen for logistic reasons. We consider that the fraction filtered should exceed 50% before in-line filtration will be favourable.

In Denmark, the professional society agrees that leucocytes should be removed prestorage to prevent HLA immunization [7,8] and the formation of cytokines [9].

The different counties use either bedside filtration or red cell on-line filtration and filtration of platelet pools. The exact distribution of these methods is not known. The reason for using bedside filtration is financial (blood banks have fixed budgets, they are not reimbursed for delivery of filtered units). The rationale for red cell on-line filtration is partly to save theuffy-coats for platelet concentrates and partly because of logistics, as all blood banks filter less than 25% of the red cells.

The parameters asked for in question 5 are very similar to those at the CFTS for counties using blood bank filtration.

**Question 6**

In the CFTS, the European standard [10] for residual leucocytes is used in our national standards [11], i.e. < 1 x 10^6 leucocytes/unit for SAGM RBCs and < 0.8 x 10^6 leucocytes/unit for pooled platelet concentrates (pool of four).

Using flow cytometry (LeucoCOUNT; Becton-Dickinson, BD Immunocytometry Systems, San Jose, CA) we count what is equivalent to 20 μl of undiluted component. In the case of SAGM RBCs, this corresponds to a theoretical 95% confidence on counted leucocytes of 50–22 x 10^6 at the level of 1 x 10^6 leucocytes/unit (the same absolute confidence levels apply to platelet concentrates at the level of 0.8 x 10^6 leucocytes/unit).

Regarding sampling frequency, we comply with the European Standard [10], i.e. at least 10 units/month (or 1% of the production if this exceeds 1000 units) of leucocyte-depleted platelet concentrates and leucocyte-depleted SAGM RBCs are sampled. We accept that one unit in each group can exceed the 1 x 0.8 x 10^6 limits, respectively. Components in which filtration has failed are either filtered once more (and sampled for quality control [QC] analysis), or reclassified as a non-leucocyte-depleted component.

The first step in the event that more than one unit in a group shows evidence of filtration failure, is to sample and carry out leucocyte-counting in a further 10 or 20 units in the group. If filtration failure is confirmed, corrective actions will be implemented.

In Denmark the same procedure is followed as described above for the CFTS. However, many use the Nagotte method for QC analysis.

**Question 7**

The national fractionation institute will accept filtered plasma. Factor VIII and Factor IX are not produced as the majority of Danish haemophiliacs are treated with recombinant products.

References

1. Annual Report 1999. Odense, Department of Clinical Immunology, Odense University Hospital, 2000 [in Danish]
5. The Danish Society for Clinical Immunology: Danish recommendations for the transfusion of leucocyte-depleted blood components. Vox Sang 1996; 70:185–186

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Leucocyte-reduction of cellular components was introduced relatively early by the Finnish Red Cross Blood Transfusion Service. Since the introduction of the bottom-and-top (BAT) method for separation of red cells and plasma, the production of platelet concentrates has been shifted from the platelet-rich plasma method to the buffy-coat method, allowing the large-scale production of pooled buffy-coat-derived platelet components stored in platelet-additive solution (PAS II + 20% plasma). Leucocyte-depletion by filtration was instituted on a selective clinical basis to prevent febrile
transfusion reactions, human leucocyte antigen (HLA) alloimmunization and transmission of cytomegalovirus (CMV) in haematology/oncology [1,2] and in neonatology.

Question 1
Buffy coats are removed from all red cell units. Fourteen per cent of red cell units have been further leucocyte-depleted by filtration prior to distribution to hospitals. All cellular blood components for neonatal use are supplied leucocyte-depleted (red cells since 1998 and platelets since 2000). This includes divided red cell units for small-volume transfusions as well as reconstituted blood for exchange transfusions. Similarly, the red cell units prepared for intrathecal use are leucocyte-depleted. The national transfusion needs of neonatology are covered by a small proportion (less than 1%) of all red cell units, which makes the universal leucocyte-depletion logistically feasible.

A great majority of platelet components are used to support haemostasis during bone marrow hypoplasia. Alloimmunization and CMV transmission interfere with such a support and are to be avoided in patients dependent on transfusions for any longer period of time. Also the quality, e.g. pH, of leucocyte-depleted platelet products is better maintained than that of the components stored with contaminating leucocytes. In December 1999 when the distribution of leucocyte-depleted platelet components increased to ≈ 85%, a transfer to 100% of leucocyte-depleted platelet component supply was made. Platelets are filtered in pools of four units.

Contamination of plasma with leucocytes remains low as a result of the preparation method used. No further leucocyte-depletion by filtration is carried out.

There is no decision at the moment (December 2000) to implement universal leucocyte-depletion of red blood cells in our country.

Question 2
Although universal leucocyte-depletion of all red cell units would produce some potential health benefits to patients, the currently known benefits would not justify the costs thus incurred [3].

Question 3
The current procedure of component preparation starts with an overnight storage of most whole blood at room temperature. The processing, including leucocyte-depletion of platelet concentrates, is carried out within 24 h after blood donation. The BAT method implies that ≈ 90% of leucocytes are removed from red cell units during the first step of separation. Further leucocyte-depletion of red cell units by filtration may be performed during the day of processing but at the latest within 72 h after blood donation. The reason for accepting this delay in the filtration process was the improved performance of red cell filters after cold storage of the product. With current filter technology this delay can be circumvented.

Question 4
With the rapid development of filtration technology, the process of leucocyte-depletion is also changing. No whole-blood filtration is applied at our institute. Currently, component preparation is under modification to apply red cell filtration already in the process (for plasma and platelet preparation see Question 1).

Question 5
Routinely, the maximal storage time prior to leucocyte-depletion is 72 h. Filtration is performed at room temperature. Plasma is not filtered.

Question 6
The recommendations given by the Council of Europe are followed when setting the rules for quality control (QC) of leucocyte-depletion [4]. Performance is monitored accordingly. The counting methods include the Masse method [5]. Prior to the application of any new filtration process, the filter performance is studied by the Department of Product Development. Selection of filters and processes is carried out to ensure a maximal safety margin for the residual leucocytes. This aims to raise the confidence level of the leucocyte-depletion process in practice. In routine use, the performance of each filter lot is monitored by intensified testing to detect any sporadic change in filter quality. The results of QC testing are evaluated monthly, by a team of experts, for process conformity with the standards. Amongst other measures, any non-conforming unit (> 1 × 10⁹ leucocytes) is identified and a look-back is carried out to identify any process error. Currently, we are seeking a new IT solution for statistical process control.

Question 7
This question does not apply as plasma is not filtered. The issue of universal leucocyte-depletion of cell concentrates and plasma units remains one of the primary concerns of the blood transfusion community. It needs to be reconsidered, in the light of new evidence, where universal leucocyte-depletion is not yet applied.

References

The Paul Ehrlich Institute (PEI) ordered general prestorage leucocyte-depletion, which will be implemented in Germany after October 1, 2001. Introduced general prestorage leucocyte-depletion, which thereof, the respective percentage of blood components at the PEI, the ‘leucocyte-depletion question has proven the expected levels of clotting factors and plasma proteins.

The upper limit of residual WBCs is \( \leq 1 \times 10^6 \) unit of RBC or \( \leq 1 \times 10^6 \) unit of FFP. For apheresis platelets, 2–4 h after separation are accepted. Approximately 50% of all leucocyte-depletion procedures are performed at room temperature and 50% at +4 °C.

The plasma fractionation institutes accept filtered plasma, if the procedure is adequately documented and if quality assurance has proven the expected levels of clotting factors and plasma proteins.

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**Question 3**

The Paul Ehrlich Institute (PEI) ordered general prestorage leucocyte-depletion in 2001 with regard to better quality of blood products and improvement of the validation procedure. Judging from the number of applications for official registra-

**Question 2**

The motivation to implement leucocyte-depletion was initially driven by the three clinical studies that showed advantages of filtered blood over standard RBCs. The possibility of a variant Creutzfeldt-Jacob disease (vCJD) risk, carried by white blood cells (WBCs), was also discussed. The increasing number of European countries already performing general leucocyte-depletion, tipped the balance towards in-line filtration as an apparent state-of-the-art quality standard. Finally, the medicinal products industry (filters, blood bags) fuelled the discussion in terms of European harmonization and optimization of purity of blood components.

**Question 1**

At this time (November 2000), not all red blood cells (RBC), platelet concentrates and plasma units are leucocyte-depleted in Germany. Hitherto, RBC and platelet concentrates are filtered poststorage, giving a high degree of flexibility in logistics, when providing haemotherapy for patients with the ‘classical indications’. Since 1998, some blood banks have introduced general prestorage leucocyte-depletion, which will be implemented in Germany after October 1, 2001.

**Question 4**

All methods listed are used in Germany. As in question 3, no authentic production figures are known for the year 2000. However, the proportion of different components registered at the PEI, to date, are as follows: whole-blood filtration = 35%, RBC in-line = 50%, and RBC on-line = 15% (filters welded on). As for platelets, the fraction of single-donor platelet concentrates, prepared from buffy coat and by apheresis = 40%, and of pooled platelets = 60%. No figures are available for FFP.

**Question 6**

The upper limit of residual WBCs is \( \leq 1 \times 10^6 \) unit of RBC or \( \leq 1 \times 10^6 \) unit of FFP. For apheresis platelets, 2–4 h after separation are accepted. Approximately 50% of all leucocyte-depletion procedures are performed at room temperature and 50% at +4 °C.

**Question 5**

The limit of storage time prior to leucocyte-depletion is set at 24 h for whole blood, RBC, pooled and buffy-coat platelet concentrates, and FFP. For apheresis platelets, 2–4 h after separation are accepted. Approximately 50% of all leucocyte-depletion procedures are performed at room temperature and 50% at +4 °C.
Question 1
Blood products are not universally leucodepleted in Greece. At present, universal leucodepletion is not under serious consideration, although discussions have taken place in various meetings. The percentage of red blood cells (RBC) and platelet units filtered has not been documented for the country as a whole. Plasma units are not filtered.

Question 2
The advantages of leucodepletion are well known and reservations about universal leucodepletion are based mainly on its cost. Twenty per cent of blood units collected in Greece are transfused to thalassaemia patients and approximately another 20% to patients with neoplastic disease (haematological or solid tumour). In all these cases until recently leucodepletion was carried out by bedside filters.

In the University Hospital where our Centre is located, we take care of 200 thalassaemia patients transfused with 6000 units of RBC/year.

Despite leucodepletion using bedside filters, some individuals were experiencing febrile reactions. For patients with allergic reactions we were providing washed RBCs. During the last year we have instituted prestorage leucodepletion in the majority of units collected and have noted a reduction in febrile and allergic reactions without washing the units. There is no nationwide plan for leucodepletion of all units selected.

Question 3
Bedside poststorage leucodepletion is certainly less costly, although if one is to use a filter for RBC and one for platelets the cost increases.

Systems of prestorage leucodepletion of all three products (RBCs, platelets and plasma) may eventually prove cost-effective.

Question 4
In our Centre we use both red-cell in-line and bedside filtration of RBCs and filtration of platelet pools. Approximately 30% of RBC units are filtered prestorage and 30% at the bedside. We do not filter plasma. All platelets are transfused with a filter. There are two reasons for selecting in-line versus online filtration. The first is strictly technical, as we do not collect all the units we transfuse; also we tend to use the systems

of prestorage filtration for in-house collection as opposed to those collected by mobile units. The other reason is cost.

Question 5
For prestorage leucodepletion, the maximal time to leucodepletion is 24 h. Because of chronic blood shortages we have no maximal storage time for bedside leucodepletion, although units transfused to patients with thalassaemia are always < 15 days old. Filtration is carried out at room temperature.

Question 6
Residual leucocytes in leucodepleted units are counted by flow cytometry. We have accepted the quality control (QC) requirement of \( < 1 \times 10^6 \) leucocytes per unit (set by the Council of Europe), although most units are of the order of \( 1 \times 10^5 \).

Question 7
We do not have a local plasma fractionation plant. Plasma is sent to the Swiss Red Cross for fractionation. As already stated, we do not filter plasma.

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Question 1
All blood units are separated into components, including removal of buffy-coat. Leucocyte-depleted (filtered) components are prepared upon specific indication. No decision has been made on implementing universal leucocyte-depletion.

Question 2
Epidemiological data available do not appear to necessitate or justify mandatory leucocyte-depletion of all labile blood derivatives.

Question 3
The advantage of prestorage leucocyte-depletion is well known and therefore this type of the procedure is preferred. Nevertheless, some hospitals are still employing poststorage (bedside) filtration.

Question 4
Red cell on-line filtration and filtration of platelet pools are used for leucocyte-depletion. In addition, a small number of apheresis plasma units are also subjected to filtration. All the
filtered components are prepared by the methods mentioned above.

**Question 5**
The maximum storage time of red cells prior to filtration is 10 days, but the filtration is usually performed between the second and fifth day of storage. Platelets are usually filtered after 1 or 2 days, at most after 5 days, of storage. The filtration procedure is carried out at room temperature.

**Question 6**
The upper limit of residual leucocytes is $1 \times 10^6$ per unit of single platelets, as defined in the Council of Europe Recommendation. No specific upper limit has been adapted for plasma. Quality control, as a routine procedure, is carried out by counting leucocytes in a Nageotte chamber.

**Question 7**
Filtered plasma is not submitted for fractionation.

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**Question 1**
Not all blood products are leucodepleted in Israel. It is unlikely that a universal leucodepletion policy will be instituted in the near future.

**Question 2**
The consideration not to implement universal leucodepletion is largely monetary/fiscal. However, some scientific questions still remain, as there is not sufficient medical data to support such practice. Leucodepletion is therefore reserved for well-defined indications only.

**Question 3**
Both pre- and poststorage leucocyte-depletion methods are used in Israel. The Central Blood Services, which supplies over 90% of the whole country’s blood and blood component requirements, performs prestorage leucodepletion, to prevent cytokine accumulation and to standardize the procedure.

In hospitals, the majority of blood products are leucodepleted using bedside filtration, although some leucodeplete

<table>
<thead>
<tr>
<th>Central blood services</th>
<th>Hospital blood banks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage time before RBC filtration</td>
<td>24–48 h</td>
</tr>
<tr>
<td>Storage time before platelet filtration</td>
<td>NA</td>
</tr>
<tr>
<td>Filtration temperature</td>
<td>22 °C</td>
</tr>
</tbody>
</table>

Table 2: Storage time, prior to leucocyte-depletion, of red blood cells (RBC) and platelets in Israel

prestorage in the hospital blood-bank laboratory. The reason for the bedside practice is mainly financial.

**Question 4**
Out of all leucodepletion procedures:
- whole blood filtration – very few units are used in Israel;
- in-line filtration of whole blood, prior to component preparation, is currently validated at the Magen David Adom (MDA) Central Blood Services;
- almost 100% of leucodepleted RBC are obtained by ‘on-line’ filtration, when the filter is connected using sterile connecting device (SCD);
- filtration of single platelet units – very few;
- almost 100% of leucodepleted platelets are given as a concentrate of pooled six random units, which is bedside filtered;
- plasma – single or pooled – leucodepletion is not performed at all;
- single donor apheresis platelets – almost 100% of products are leucodepleted; and
- paediatric blood bags – almost 100% of products.

The reason for on-line filtration of RBC, pooled platelets, plateletheresis products and blood for babies is to provide leucodepleted products to all immunocompromised and multiply transfused recipients.

In addition, as there is no universal leucodepletion policy, inventory management is easier when on-line, rather than in-line, filters are used.

**Question 5**
See Table 2 for the maximal storage time of red cell and platelet concentrates and the temperature of filtration.

**Question 6**
The upper limit of residual leucocytes is $< 5 \times 10^3$, although most RBC units reach $< 1 \times 10^3$. At the Central Blood Services, 10% of RBC and plateletheresis products are counted, using Imagn (Biometric Imaging Inc., Sherman Oaks, CA). Currently, the Nageotte chamber method is used.

At some hospital blood banks, the residual leucocytes are counted in 5–10% of products, using the Nageotte chamber method.
method. In most hospitals, no quality control (QC) analysis is performed. Plasma is not being filtered in Israel. When filtration fails in the Central Blood Bank the products are not supplied as 'leucodepleted'. Most hospital blood banks do not count the number of residual leucocytes in RBC and plateletpheresis products.

Question 7
No, The Israeli Plasma Fractionation Institute (PFI) does not require filtered plasma.

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Question 1
Universal leucoreduction is not mandatory in our country. Accordingly, in our centre there are specific indications for leucoreduction. In the absence of regulatory requirements, we do not intend to institute universal leucocyte-depletion in our institution in the near future, for the reasons reported in a recent editorial and in a letter published in the journal 'Transfusion' [1,2]. At the biological level, we agree with a statement of a recent review by Vamvakas & Blajchman that ‘... the efficacy of WBC reduction in preventing any deleterious immunomodulatory effect(s) of allogeneic transfusion has not been established ...’ [3]. Moreover, and besides the biological issues, we do not believe that there is conclusive evidence showing that universal leucoreduction is cost effective if we consider cost effective an intervention that costs no more than US$ 50 000 per quality-adjusted life year added to the patient’s life length [4]. Our views should be considered taking into account that our red cell units are routinely deprived of the buffy-coat. This procedure causes the removal of approximately 10% of the red cell mass. In addition, we prepare platelets using the buffy-coat method, a procedure known to be associated with much lower white cell counts than the platelet-rich method [5].

Question 2
Motivation to implement universal leucocyte-depletion could be to avoid the untoward effects occurring as a result of the presence of white cells in blood components. Motivation not to implement could be the high ratio between its cost and proven benefits.

Question 3
In our selected indications, we prefer to remove white cells early during the storage period. Our interpretation of the limited existing literature is that waiting for a few hours should allow the white cells to destroy bacteria that might be present in the unit. Nonetheless, waiting for too long might cause white cell fragmentation, which in turn could cause the release of bacteria or cellular fragments not trapped by the filters used for white cell removal.

Question 4
Between January and September 2000, we filtered 11-8% of our red cell units (2451/20 708) at the bedside and another 3-4% (710/20 708) in the laboratory. The prevalent use of bedside filtration as compared to laboratory filtration stems from the large number of thalassaemic patients treated in our institution. This policy, which has been in place in our hospital for more than 10 years, has proven its safety and effectiveness [6]. Moreover, it greatly reduces the workload generated by the leucoreduction procedure. In regard to platelets, between January and September 2000, we filtered 15% of our platelet pools (874/2396) in the laboratory, at the time of preparation from pooled buffy-coats suspended in platelet-additive solution. We do not use bedside platelet filtration because cytokines and other biologically active substances released during platelet storage are not trapped by the filters. In the very limited number of cases when we need to transfuse, in emergency, a platelet product to a patient requiring leucoreduced platelets and this product is not available (less than 1% of platelet transfusions), we issue a fresh platelet pool (≤ 2 days old) because there is evidence that cytokine accumulation does not reach clinically significant levels before the third day of storage [7].

Question 5
All filtrations are performed at room temperature, with the objective of completing the filtration in the shortest possible time, so as not to expose the blood component to inappropriate temperatures. Red cells are filtered within the first 2–3 days of storage. Platelets are filtered during preparation, i.e. on the day after whole-blood collection (we store buffy-coats overnight before platelet preparation). We prefer not to filter apheresis platelets too early, because the possible presence of activated platelets during the early storage time could cause high platelet losses. As most platelet apheresis collections are performed in the morning, we generally postpone filtration to the next morning or, if this is not possible, to the late afternoon of the day of preparation. We do not filter plasma.

Question 6
We use the standard of less than 1 × 10^6 residual white cells per transfusion. We believe that the achievement of this value
should be proven with 95% confidence. We count residual white cells using the Nageotte method and dilute the samples 1 : 10 (red cells) or 1 : 5 (platelets) with Leucopaque (Sobioda, Montbonnot, France). Our policy for units failing the $1 \times 10^6$ limit is to select another unit and perform a new filtration. With a precious, rare unit, we re-filter it.

Question 7

Our plasma fractionation unit does accept filtered plasma, but because plasma filtration is not a requirement, they do not specify any limit in this regard.

References


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Question 1

On 26 May 1998, the Minister of Health requested that as soon as possible all labile blood products should be leuco-depleted. The Luxembourgish Red Cross undertook extensive studies on the techniques and on the available material, conducted time-consuming validations and decided to proceed in two phases for the implementation of universal leucocyte-depletion.

The first phase was finished on 17 August 1998 and leucocyte-depletion was in place for whole-blood transfusion, applicable to 55% of blood donations; those donations, intended for the preparation of only two blood components (e.g. red blood cells and plasma), are processed by in-line filtration followed by centrifugation and separation into the two finished products.

The results from the 2000 Quality Report read as follows for the finished red cell product (6-month period from 1 March 2000 to 31 August 2000):

- volume [ml per unit]: mean, 240; SD, 16; $n = 2826$;
- haemoglobin (Hb) [g per unit]: mean, 58; SD, 5; $n = 382$;
- haematocrit (Hct): mean, 0.54; SD, 0.02; $n = 382$ and
- white blood cells (WBC) [per unit]: mean, 0.25 x $10^6$; SD, 0.42; $n = 305$.

The second phase was concluded on 17 December 1998 and covered the remaining 45% of whole-blood donations, intended for the preparation of three blood components: red blood cells, plasma and platelet (PLT).

Those blood donations are first centrifuged, then separated; the plasma is frozen, the red cells are filtered ‘in-line’ and buffy-coats are prepared for later pooling and filtration to yield filtered pooled platelets. At the beginning, six buffy-coats were used to prepare a therapeutic dose, during 1999 this was reduced to five buffy-coats and since the start of 2000 the pools of platelets have been prepared using four buffy-coats.

The results from the 2000 Quality Report read as follows for the finished red cell concentrates:

- volume [ml per unit]: mean, 240; SD, 16; $n = 2643$;
- Hb [g per unit]: mean, 45; SD, 5; $n = 374$;
- Hct: mean, 0.57; SD, 0.027; $n = 174$; and
- WBC [per unit]: mean, 0.24 x $10^6$; SD, 0.43; $n = 297$.

The results from the 2000 Quality Report read as follows for the finished standard platelet product [prepared from four buffy-coats]:

- volume [ml per unit]: mean, 271; SD, 28; $n = 305$;
- PLT [per unit]: mean, 3.00 x $10^11$; SD, 0.43; $n = 951$; and
- WBC [per unit]: mean, 0.03 x $10^6$; $n = 190$.

At the same time, filtration of single donor platelet products was also put in place. Since mid-1999, these products have been prepared by cell-separation devices yielding leucocyte-depleted platelet products without filtration. The results from the 2000 Quality Report read as follows for the finished single donor platelet product:

- volume [ml per unit]: mean, 247; SD, 28; $n = 305$;
- PLT [per unit]: mean, 3.54 x $10^11$; SD, 0.66; $n = 305$; and
- WBC [per unit]: mean, 0.04 x $10^6$; $n = 190$.  

Question 2
Leucocyte-depletion gives a purer, better product resulting in fewer and reduced side-effects of blood transfusion; and it has fewer risks for the transfused patient (in terms of alloimmunization, immunomodulation, transmission of viruses and, possibly, prions causing transmissible spongiform encephalopathies (TSEs)). We believe that the introduction of universal leucodepletion has increased the quality and the safety of labile blood components and that every patient has the right to have access to the best available product.

The drawback of universal leucodepletion is the increased production cost (in the order of 25–30%) and the loss of therapeutic compounds (Hb in red cell concentrates and PLT in platelet concentrates).

The advantages of universal leucocyte-depletion are numerous and well known; it may be that for the moment some of them have not yet been proven scientifically in a definitive way. However, this does not take away anything from the overall benefit of universal leucocyte-depletion of labile blood components.

Question 3
In Luxembourg, leucocyte-depletion is performed prestorage. The minimum holding time of whole blood before processing is 4 h, the maximum is 24 h. We believe that prestorage leucocyte-depletion is a superior approach compared to poststorage or bedside leucodepletion: filtration is performed before WBC lysis and cytokine release occur to a significant extent. Blood bank prestorage filtration is performed under controlled conditions in a stringent quality-controlled environment: in the case of Luxembourg, this is assured in a continuous and reliable way by an ISO9002 certified Quality Management System (QMS).

Question 4
As mentioned above, 55% of the blood donations undergo in-line whole-blood filtration, which represents some 12 000 units of whole blood per year. Forty-five per cent of the blood donations are centrifuged and separated into intermediate products. Later on, these products undergo in-line filtration to give the finished products: this applies to some 10 000 units of donated whole blood.

Question 5
The maximal holding time is 24 h for the whole-blood units, regardless of the blood products that are prepared. The holding temperature is 20°C and the filtration temperature is 20°C (from 18 to 22°C, under controlled conditions in an air-conditioned room).

Question 6
The standards that we follow in Luxembourg are those of the Council of Europe, published in Rec.15 (95), "Guide to the Preparation, Use and Quality Assurance of Blood Components", edn. 6, January 2000 (called the ‘Guide’, the golden European standard in blood transfusion):
- for red cells, leucocyte-depleted: \( < 1 \times 10^6 \) per unit in at least 90% of the units;
- for platelets: recovered (pool prepared from four buffy-coats): \( < 0.2 \times 10^6 \) per single unit equivalent in at least 90% of the units or \( < 0.8 \times 10^6 \) per pool in at least 90%;
- for platelets: apheresis: \( < 1 \times 10^6 \) per unit in at least 90% of the units.

If filtration fails significantly, the product is considered a non-conformity and is destroyed.

For fresh-frozen plasma (recovered and apheresis, intended for fractionation) the upper limit is \( 0.1 \times 10^9/l \). In the very near future, it is intended to switch to leucocyte-depleted plasma (\( < 10^5/l \)), using different filter devices.

The counting method we use is a validated technique in the Nageotte chamber; for platelet products, a concentrated Nageotte technique is used.

Question 7
In the very near future our plasma fractionation facility will only accept leucocyte-depleted, filtered plasma for fractionation of medical products derived from human blood and plasma. The requirement will very probably be \( < 10^4/l \) plasma.

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leucocyte-depletion to reduce the risk of vCJD is by no means proven. The decision to introduce precautionary measures within New Zealand recognizes the considerable uncertainty that exists both in relation to the likely size of any epidemic within the UK and the experimental data which suggests that the disease might be transmissible by transfusion. In this context it is considered appropriate to introduce measures that, based on the available data, seem likely to reduce the risk that vCJD might be transmitted by this route. The wider benefits of universal leucocyte-depletion were also taken into account during the decision-making process. In addition to the recognized benefits of leucocyte-depletion, leucocyte-depletion should also reduce the risk of *Yersinia* transmission by transfusion – this has been a particular problem in New Zealand during the early and mid-1990s.

**Question 3**
Leucocyte-depletion will be undertaken prior to storage under controlled laboratory conditions. Prestorage leucocyte-deletion will enable the process to be more effectively quality assured.

**Question 4**
A combination approach to leucocyte-depletion will be utilized. Eighty per cent of donated blood units will be leucocyte-depleted using whole-blood filters. The filtered whole blood will then be processed into resuspended red cells and plasma for fractionation. The remaining 20% of units will be utilized for recovered platelet production. These blood units will be buffy-coat depleted. The red cell units will then be filtered by red cell filters. The buffy coats will be pooled, platelets prepared and then leucocyte-depleted using in-line filters. Recovered platelets will provide 40% of platelet requirements, the remainder will be obtained by platelethapheresis. Plasma from these whole-blood donations will not be leucocyte-depleted in the first phase of implementation. This plasma will be utilized for fractionation. Fresh-frozen plasma for clinical use will be leucodepleted. Work is currently being undertaken to determine whether this should be derived by apheresis or from whole-blood units.

**Question 5**
In developing plans for leucocyte-depletion, the New Zealand Blood Service has worked closely with the National Blood Service in England. Performance data on individual filters have been provided; this identifies the optimal timing and temperature of leucocyte-depletion for each filter type. Consistency of performance is seen as a high priority. All blood components will be leucodepleted within 48 h of collection.

**Question 6**
The New Zealand Blood Service will adopt the United Kingdom specification for residual leucocytes, i.e. < 5 × 10⁶ white cells per unit. This requirement must be met in greater than 99% of cases. In practice, much lower levels are seen. The New Zealand Blood Service believes that if the UK specification is met then the results will also comply with the Council of Europe requirement for 90% of tested units to have a residual white count of less than 1 × 10⁶ per unit. Residual white cells will be measured using flow cytometric technology. Statistical control methodology will be utilized to monitor performance against specification.

A specification for residual white cells in plasma has not yet been determined. The requirement for universal leucocyte-depletion of plasma will be reviewed once robust and validated systems for leucocyte-depletion of fresh components are in place. The specification for residual white cells in plasma will be determined as part of that process.

**Question 7**
CSL Bioplasma undertake fractionation of New Zealand plasma. Discussions have taken place relating to the suitability of leucocyte-depleted plasma and the probable impact on the outcome of fractionation. Concern relates particularly to the production of Factor VIII. Data provided by the National Blood Service in England (Dr Lorna Williamson, personal communication) indicates that levels of Factor VIII and Factor VIII vWF are not changed significantly postfiltration using the current generation of filters. The outcome of fractionation will be closely monitored; however, it is not anticipated that any reduction in the yield of Factor VIII or other products will be seen.

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Almost 16 million people live in the Netherlands. There are eight university medical schools and approximately 110 hospitals. Since 1998 there has been a national organization for blood supply (Sanquin) with a central board of directors. Sanquin has nine blood bank divisions, which provide their regional hospitals with red blood cells (RBC), platelets and fresh-frozen plasma (FFP). One national plasma fractionation division produces the pooled plasma products. Our division (the Blood Bank Leiden-Haaglanden) provides ~10% (70 000 donations) of the national blood supply and delivers blood components to 10 hospitals, including the Leiden University Medical Centre. In the Netherlands, since the early 1980s all RBC have been buffy-coat depleted and almost all random donor platelet transfusions are prepared from buffy-coats.
Question 1
All platelet concentrates in the Netherlands are leucocyte-depleted prior to storage, and in our centre have been since 1974. As discussed in the answer below to question 3, we are of the opinion that there is sufficient evidence to prove that prestorage leucocyte-depletion of platelet concentrates is effective in preventing human leucocyte antigen (HLA) immunization, cytomegalovirus (CMV) transmission and adverse febrile reactions.

In the Netherlands, 20% of RBC are leucocyte-depleted by filtration. Our centre still adheres to clinical indications for leucocyte-depleted RBCs and produces 25% as leucocyte-depleted and 75% as buffy-coat depleted. The national product specifications state that FFP should contain $<3\times10^7$ white blood cells (WBC)/unit prior to freezing.

It is uncertain whether universal leucocyte-depletion will be performed prior to freezing and thus prestorage. We expect in the near future the introduction of universal leucocyte-depletion of RBC as a national guideline to reduce the possible transmission of variant Creutzfeldt-Jacob disease (vCJD) or because of another reason, if it can be found. It is uncertain whether universal leucocyte-depletion will apply to FFP; this will depend on the yet-unspecified indication for universal leucocyte-depletion. A national working party has been instituted to investigate universal leucocyte-depletion. For the moment one (university) hospital has decided to introduce leucocyte-depleted RBC.

Question 2
There are several groups responsible for decision-making in this country, all of which have different views: The Ministry of Health decides about the budget for the Sanquin Blood Supply Foundation. The minister for health has not yet approved universal leucocyte-depletion for the prevention of immunosuppressive side-effects of blood transfusions. She wants further information about the clinical benefits and costs. To provide this information, several studies have been put in place and are expected to be complete within 1–2 years. For the introduction of universal leucocyte-depletion in order to prevent the possible transmission of vCJD, the minister awaits a report from her advisory board.

The board of directors of the National Sanquin Blood Supply Foundation have declared for the year plan of 2000 that universal leucocyte-depletion is ‘State of the Art’, but has not yet effectuated this statement and it seems that they will effectuate their intention only when the costs are covered. A third group involved in making decisions are the hospital directors and physicians: in our region only one hospital decided to use only leucocyte-depleted blood products. The director of this hospital indicated that his decision was not based on medical indications.

Question 3
Platelet transfusions. As mentioned in the answer to question 1, all platelet transfusions are already leucocyte-depleted prior to storage. The current indications for leucocyte-depletion of platelet concentrates are prevention of HLA-alloimmunization [1], CMV transmission [2,3] and febrile reactions occurring as a result of the presence of soluble pyrogens [4]. These indications require quality control that cannot be performed in the case of bedside filtration. Moreover, there is evidence that bedside poststorage filtration offers no benefit compared to non-leucocyte-depleted components, with respect to immunization [5] or febrile reactions [6] and perhaps bedside-filtered platelet transfusions are suboptimal with respect to transmission of CMV [7].

Red cell transfusions. In fact, leucocyte-depleted RBCs are used for the same indications as filtered platelet transfusions. Prestorage filtration has been chosen because formation of cytokines is prevented and quality control is required. If leucocyte-depletion becomes mandatory, buffy-coat removal for RBC would no longer be performed prior to freezing and thus prestorage.

Future universal leucocyte-depletion? If universal leucocyte-depletion will be implemented in the future for red cells, it will depend on the indication of whether leucocyte-depletion will be performed before or after storage.

If immunosuppressive side-effects, such as postoperative infections and multiorgan failure, will be the reason for implementing universal leucocyte-reduction, we have previously shown that poststorage and prestorage leucocyte-reduction give similar results. HLA antibodies, postoperative infections and other complications occurred at a similar rate regardless of whether pre- or poststorage leucocyte-depleted RBC were used [8].

Therefore, if practical aspects are not taken into account and if universal leucoreduction is introduced because of immunosuppressive side-effects, bedside filtration can be defended. Because the choice (pre/post) of filtration of RBC cannot be made on the evidence currently available, for logistic reasons and to avoid the coexistence of two different leucocyte-depleted RBC products, our centre favours prestorage leucocyte-reduction.

Question 4
The buffy-coat is removed from all whole-blood donations; 60% of these buffy-coats are used for platelet production by pooling five buffy-coats and subsequently the pool is filtered. Less than 2% of all platelet products (only those HLA matched and/or CMV seronegative) are prepared by donor apheresis and leucoreduced by leucoreduction system (LRS) or filtration. Approximately 25% of buffy-coat-depleted RBC in Saline-Adenine-Glucose-Mannitol (SAGM) are subsequently filtered prior to storage. If universal leucocyte-depletion becomes mandatory, buffy-coat removal for RBC would no longer be indicated. In fact, buffy-coat removal would be contraindicated, because a large volume of red cells, representing
the youngest population of RBC, would be unnecessarily removed. Ideally, a platelet-sparing leucocyte filter, suitable for pooled platelet preparation, should be used. If such a filter is not available and it is considered unethical to ask donors for platelet apheresis, while throwing the platelets from whole blood away, a suboptimal solution is to remove the buffy-coat only from the blood needed for platelet preparation and use an in-line whole-blood filter for the other products.

This implies, for the Netherlands, that 50% of whole-blood donations can be filtered by using in-line filters, to reduce the loss of red cells from units of which the buffy-coats are not needed for platelet production.

**Question 5**
The storage times and temperatures of RBCs and platelets are shown in Table 3.

**Question 6**
There are national requirements for the definition of leucocyte-depleted components. These are:
- RBC: WBC < 5 × 10⁹; 95% confidence;
- platelet transfusion product (pooled or single donor): WBC < 5 × 10⁹; 95% confidence;
- plasma: WBC < 30 × 10⁹.

The counting method we use for quality control (QC) analysis of leucocyte-depleted RBC and platelets is flow cytometry; however, according to national guidelines Nageotte can also be used.

**Question 7**
There is no national decision as to whether or not plasma filtration is indicated and, if so, whether the plasma fractionation division would accept filtered plasma, as the quality parameters for filtered plasma have not yet been officially communicated.

**Acknowledgement**
We thank Dr Walter Mook for his assistance in analysing the production data.

**References**

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| Table 3 | The storage times and temperatures of red blood cells (RBCs) and platelets |
|-----------------|-----------------|-----------------|------------------|
| **RBC: storage time and temperature before filtration (h)** | **Median** | **Average ± SD** | **Range** |
| Whole blood at 20–24 °C | 7.9 | 11.4 ± 6.4 | 1.6–25 |
| RBC buffy-coat depleted at 2–6 °C | 17.6 | 13.8 ± 8 | 0.2–37 |
| Total storage time from donation to filtration² | 25 | 24.6 ± 4 | 5–37 |
| **Platelets: storage time at 20–24 °C before filtration (h)** | | | |
| Whole blood³ | 7.9 | 11.4 ± 6.4 | 1.6–25 |
| Buffy-coats (prepooling)⁴ | 4.4 | 8 ± 7 | 0–24 |
| Buffy-coats (pooled) | 1 | 3.7 ± 56 | 0.5–29 |
| Total storage time from separation of WB to filtration⁵ | 12 | 12 ± 8 | 1–35 |

* bimodal distribution with peaks at 6 h and 18 h.
* bimodal distribution with peaks at 7 h and 19 h.
* This distribution is normal, whereas all other distributions are bimodal.
* bimodal distribution with peaks at 4 h and 13 h.
* bimodal distribution with peaks at 5 h and 14 h.

Red blood cells (RBCs) are filtered at room temperature, the temperature of = 50% of the RBCs is 2–6 °C and in 50% between 2 and 20 °C. Plasma filtration: not applicable.

WB, whole blood.
Question 1
In Poland, leucocyte-depleted plasma is not commonly used.

According to Polish regulations, leucocyte-depleted red blood concentrates and platelet concentrates are indicated in the following cases:
- to prevent human leucocyte antigen (HLA) immunization (in multiple platelet concentrate recipients, of peripheral blood stem cells [PBSC] bone marrow and other organ transplants);
- to prevent repeated non-haemolytic febrile reactions (in recipients with at least two such reactions in their medical history);
- to prevent post-transfusion cytomegalovirus (CMV) infection, if red blood concentrate or platelet concentrates from CMV-negative donors are unavailable (for intra-uterine, newborn and post-transplant transfusions, as well as for other patients with immunological disorders).

According to Polish regulations, blood banks are obliged to filter and irradiate all red cell concentrates and platelet concentrates intended for intrauterine transfusion and for newborns. In all other cases, the decision for leucocyte-depleted transfusions depends on the physician. Leucocyte-depleted components are then ordered from blood banks or bedside filters are used.

In Poland, leucocyte-depleted components represent 15% of all transfused red blood cells (RBCs); 70% of platelet concentrates are leucocyte-depleted.

Question 2
As no cases of variant Creutzfeldt-Jacob disease have been registered in Poland, leucocyte-depleted blood components are not obligatory.

Question 3
Leucocytes are removed from RBCs by poststorage filtration only after cross-matching and directly prior to transfusion. In regions servicing transplantation centres (especially bone marrow transplantation centres) the demand for leucocyte-depleted platelet concentrates is the greatest. Apheresis platelets are prepared with cell separators (e.g. LRS system Cobe-Spectra), or kits with an integral filter are used. Platelet pools are filtered in closed prestorage systems. In other centres, leucocytes are removed from platelet concentrates by poststorage filtration and on the doctor’s recommendation.

Question 4
Red cell on-line filtration and filtration of platelet pools are used. Plasma is not filtered.

Question 5
In Poland, leucocytes are removed from RBCs within 48 h of donation and not later than 7 days after donation. For platelet concentrates, filtration is possible at any time during the storage period, i.e. 5 days, although it is recommended that leucocytes are removed in the early storage period, i.e. during the 6-h postdonation (for platelet concentrates produced from PRP) or 24 h postdonation (for platelet concentrates produced from the buffy-coat).

Filtration temperature is according to the manufacturer's recommendations. Filtration is performed at room temperature, in the case of RBCs usually directly after removing from the refrigerator.

Question 6
The upper limit of residual leucocytes in leucocyte-depleted components is $1 \times 10^6$/RBC unit or apheresis product and $0.2 \times 10^6$ of single platelet concentrate unit obtained from whole blood.

For leucocyte counting, the Nageotte chamber is used (with a sensitivity of 0.2 white blood cells [WBC]/µl for RBCs and 0.1 WBC/µl for platelet concentrates). One per cent of components produced undergo quality control; this represents at least 10 per month. Components with $> 1 \times 10^6$ WBC may be used exclusively for recipients with no indication for treatment with leucocyte-depleted blood components. The upper limit of leucocytes in plasma is $0.1 \times 10^9$/l. This is according to the Council of Europe recommendations.

Question 7
Plasma is not filtered.

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**Question 1**
At the end of 1999, leucocyte-depletion was not routinely carried out as part of the production process, except for apheresis platelets where it is part of the harvesting. Bedside filtration was performed in some instances, while in other cases the blood banks filtered the product on request. These were, however, all poststorage filtrations. At the end of 1999 there was a national decision to perform prestorage leucocyte-depletion for specific indications, which are as follows:

- patients considered at risk for transfusion-transmitted cytomegalovirus (CMV) should receive leucocyte-depleted blood components;
- patients with severe aplastic anaemia who are potential haemopoietic transplant recipients should receive leucocyte-depleted components from the inception of transfusion support;
- leucocyte-depleted blood components should be used for intrauterine transfusions and for all transfusions to infants less than 1 year of age; and
- all apheresis single donor units must incorporate leucocyte-depletion as part of the process.

We will also perform on-demand filtration if the treating Physician orders this.

In 2000 the process of validating the filters, products and processes started. The routine filtration of blood components for the indications listed above will become operational by the end of this year or early next year, depending on the various operational regions.

**Question 2**
On a national basis, after reviewing everything available on filtration, at the present moment we decided that we could not justify, in the South African scenario, universal leucocyte-depletion. First, there are no clear evidence-based indications for universal leucocyte-depletion; and second, the cost is astronomical. The money, if it were available, that would be spent on universal leucocyte-depleted products for South Africa could be utilized for more critical needs.

**Question 3**
Prestorage leucocyte-depletion will be carried out. The reason for this is that the process and products can be controlled and quality guaranteed, whereas for bedside filtration this is not possible.

**Question 4**
We will perform component leucocyte-depletion, and not whole-blood filtration, in order to have platelets available.

Large amounts of our platelet needs are still supplied by non-apheresis procedures. Where a physician wants to use filtered whole blood (e.g. exchange transfusion) this will be possible.

We have also decided not to use collection bags with in-line filters owing to the cost and potential wastage caused by seropositive donations. Currently, the local manufacturer of blood bags does not produce an in-line filter blood bag. Filters will therefore be sterile docked to the component bag after processing and then filtered.

As far as filtration of platelets is concerned, this will be performed using platelet pools prepared by the buffy-coat method.

There might be filtration of single platelet units, in certain regions, for paediatric use. This will be in areas where there are no apheresis facilities. We will only perform filtration of plasma on demand.

**Question 5**
The cut-off times we have set, by which a unit of blood must be filtered, are between 4 and 48 h after collection. At this stage we anticipate that the filtration will be performed at 4 °C.

**Question 6**
This is the first time that we have embarked on the process of prestorage leucocyte-depletion and because of that we have decided to be conservative and set the residual leucocyte count at \(< 5 \times 10^6\). This value will be reviewed once we are comfortable with our products.

As far as quality control (QC) is concerned, we have decided to follow the standards set by the Council of Europe, 6th edition, January 2000. Once we have sufficient data of our own, we will review these QC standards. The current QC is therefore as follows:
- filtered red cells: 1% of total production with a minimum of 10 units per month; and
- filtered platelets: 1% of total production with a minimum of 10 units per month.

Ninety per cent of the product must meet the QC standard. For counting we are using the Naegotte counting-chamber technique. We will evaluate the use of flow cytometry and the access to these facilities later.

The upper limits for residual white cells are the same for all products.

If filtration fails the QC analysis, we will investigate and handle the product accordingly.

**Question 7**
As plasma filtration is not being performed routinely, the fractionation centres have not been supplied with these products. They are, however, starting their investigations and once this is complete, will present their requirements.
Question 1
We perform partial depletion of all our blood units using the buffy-coat system.
In a reduced number of red cells and platelet concentrates, selected for clinical reasons (for patients with known or suspected leucocyte antibodies or whose transfusion requirements make it necessary to prevent leucocyte alloimmunization or cytomegalovirus [CMV] infection) we supply red cells or platelets depleted by filtration.

Question 2
There is no doubt that Blood Transfusion Centres have to supply the purest products, without forgetting the cost-effectiveness of these measures, and it is important that we have the same economic and social scenario as other European Union (EU) countries. Therefore, we plan to introduce universal leucodepletion gradually, until we have more relevant scientific data, in order to raise us up to the same level as other countries.

Question 3
We use presaturation filtration following the recommendation of the Spanish National Blood Transfusion Commission and the European Commission. This procedure is performed in the Blood Transfusion Centre to ensure high and consistent quality. All the hospitals we supply receive blood filtered under these conditions.

Question 4
In those cases where there is a clinical reason for white cell depletion, we use red cell on-line filtration, filtration of platelet pools and leucocyte-free platelet apheresis.

Question 5
The maximum storage time is 18 h at 22 °C. We always filter at 22 °C.

Question 6
The upper limit of residual leucocytes in leucocyte-depleted components is < 1 × 10^6 per unit. We perform quality control testing in 1% of the leucocyte-depleted units. We use flow cytometry for counting the residual leucocytes in leucocyte-depleted components. We do not filter plasma.

Question 7
The technical requirements in our contract with the pharmaceutical industry, which handles plasma fractionation in our country, at present do not include filtered plasma.

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Question 1
Currently, both leucoreduced and non-leucoreduced components are transfused in the United States. Because of pressures from some blood suppliers and statements from federal agencies, the proportion of components that are transfused as leucoreduced has risen to > 30% and continues to rise. Some blood centres have converted to providing all cellular components as leucoreduced, but the majority offer both kinds of components. (Leucoreduced plasma is not routinely produced except for leucoreduction as part of the production of solvent-detergent plasma, a component which holds only a small share of the US market.)
The decision of whether to transfuse blood components as leucocyte-reduced is currently regarded by most transfusion medicine physicians in the United States as the practice of medicine. As such, the decision is thought best to be made by the physicians caring for the patient – individually or corporately, such as the medical staff of a hospital. The Food and Drug Administration (FDA) is currently encouraging conversion towards universal leucoreduction, and several major blood supplying organizations have indicated their intent to convert all transfusing facilities to leucoreduced components as soon as possible. The rate of conversion and the end-point of the process remain to be determined, however.

Question 2
In the United States, the primary motivation stated in promoting conversion to ‘universal’ leucoreduction relates to transfusion-related immunomodulation (TRIM). Filter manufacturers, blood centres promoting conversion and physicians who support the concept of universal leucoreduction, project fewer complications following transfusion, particularly reduced bacterial infection rates, and thus lower healthcare costs, even after the charges for leucoreduction are considered. There is little debate that selected groups of patients, particularly those who will be multiply transfused, will benefit from leucoreduction of components through
practices makes prestorage leucoreduction a technique that the emphasis on process control and good manufacturing US, but the trend appears to favour the former. Increasing Both pre- and poststorage leucoreduction are used in the analyses from several perspectives have estimated the marginal costs to convert from selective to universal leucoreduction in the US at $500 million annually, or 25–30% of the country’s entire ‘blood bill’ [1]. With federal healthcare reimbursements and most private insurance companies limiting (and reducing) their payments to hospitals over the last several years, this dramatic increase without a documented health benefit attributable to the conversion has led to opposition of conversion to universal leucoreduction without additional data [2]. While several professional organizations have been lobbying to increase federal expenditures to reflect the costs of safety interventions implemented over the last decade, others have noted that, if additional funds were made available, there are interventions other than universal leucoreduction to which these resources could be applied with greater impact. For example, documentation that 10–20 US patients die annually from receiving the wrong unit of blood or that an equal number succumb to transfusion with a bacterially contaminated unit appear to be more pressing problems than TRIM, for which conclusive evidence of clinical effect remains elusive.

Applying leucoreduction to interdict prion carriage via leucocytes has not received much support in the United States. Demonstration that prions exist free in plasma and a conclusion by the FDA’s Transmissible Spongiform Encephalopathy Advisory Committee [3] that leucoreduction would be ineffective in preventing transmission of variant Creutzfeld-Jakob Disease (should blood be a transmission vector of this disease) have bolstered the contention that this potential risk should not be a rationale for universal leucoreduction.

Many in the US are awaiting the results of a trial of the effect of universal leucoreduction, which was recently concluded at Massachusetts General Hospital. All patients lacking a specific indication for leucoreduction were prospectively randomized between standard and leucoreduced components; length of stay post-transfusion and mortality are among the primary outcome measures being analysed at present. As this is the first randomized, controlled trial of the universal application of leucoreduction in the US, its outcome is expected to have a large impact on the thinking of physicians and regulators.

**Question 3**

Both pre- and poststorage leucoreduction are used in the US, but the trend appears to favour the former. Increasing the emphasis on process control and good manufacturing practices makes prestorage leucoreduction a technique that fits better into the documentation requirements that have been adopted in blood banking. In particular, the lack of a simple and accurate means of performing quality control on the bedside leucoreduction process has further pushed blood bankers toward prestorage filtration. However, a recent review of the benefits of pre- versus poststorage leucoreduction failed to identify one as clearly advantageous over the other [4]. Concerns about hypotensive reactions in patients receiving units filtered at the bedside and thus possibly containing vasoactive agents (e.g. bradykinin), and the generation of interleukins (particularly in platelet units) during storage, are cited as additional reasons to move the filtration process to shortly after collection.

**Question 4**

A variety of leucoreduction processes are utilized in the US. Most notably, however, FDA guidelines intended to limit the growth of any contaminating bacteria preclude prestorage pooling (by use of a sterile connecting device) of platelets derived from whole-blood units and subsequent filtering of the pool [5]. Although such an approach might save time and money, platelet units must be filtered and stored individually and can be pooled only shortly before transfusion.

**Question 5**

There is, as yet, no uniform definition of the time after collection during which leucoreduction filtration must be performed in order for the process to be considered as having been conducted ‘prestorage’. Manufacturers’ directions serve as de facto regulations through their FDA approval and, of course, must be followed. Some allow filtration of red cells to be conducted for up to 5 days at the beginning of the storage period or up to 3 days for platelets, and the FDA has not yet issued guidance defining a different (shorter) period as necessary for the ‘prestorage’ appellation. (The FDA has verbally stated that the filtration should occur as early as possible, for example, within 24 h of collection for platelets.) Similarly, both room temperature and cold filtrations are performed; however, the latter appear to be used more commonly.

**Question 6**

Quality control schemes for leucoreduction are still under development in the US. Current Standards of the American Association of Blood Banks define a leucoreduced component as one containing fewer than $5 \times 10^6$ leucocytes, but the confidence that this requirement is met with a particular level of conformity is not stated. The FDA has suggested in public presentations that it may issue guidance indicating that one should have at least 95% assurance that at least 95% of leucoreduced units contain no more than $1 \times 10^5$ leucocytes; however, no official pronouncement of this target has yet occurred. The Association for the Advancement of Medical Instrumentation is developing a standard for leucoreduction.
filters that may address the issue of filter performance, but the document is only in draft form at this writing. The ISBT’s Biomedical Excellence for Safer Transfusion (BEST) Working Party is also considering how best to announce product requirements and process requirements to ensure that clinically necessary targets are achieved in a predictable manner.

While high degrees of assurance and conformance may be achievable with current technology, does performing all the quality control leucocyte enumeration that they imply confer benefit to patients? Bedside filtration (with its recognized potential for incorrect application) using an earlier (less-efficient) generation of filters provided effective avoidance of cytomegalovirus (CMV) transmission [6]. Therefore, extensive quality control efforts for the leucoreduction process may not be necessary. Furthermore, additional development of quality control schemes will be required if the country does convert to universal leucoreduction, as a ‘failed’ unit would not be able to be transfused as a ‘standard’ unit and as FDA guidelines preclude repeat filtration because of reduction of the cellular content of the unit [5]. These considerations become more problematic when recognizing that some combinations of filters and haemoglobin S-trait donors may result in unexpectedly high clogging or filter failure rates, and thus provision of units lacking certain antigens that are much less prevalent in African-American donors may become more difficult [7].

The Nageotte counting chamber is used most frequently in quality control counting of residual leucocytes, particularly now that an automated system of leucocyte enumeration is no longer available. Few blood-collecting organizations have a flow cytometer available to them, although some filter manufacturers provide automated counting services to their customers.

Question 7
Not applicable.

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Question 1
All units are leucocyte-depleted. The four UK Transfusion Services implemented universal leucocyte-depletion during 1998/99, completing the implementation project at the end of October 1999.

Question 2
Universal leucocyte-depletion was implemented as a blood safety precaution against the hypothetical risk that variant Creutzfeldt-Jacob disease (vCJD) might be transmitted by blood transfusion. This decision was taken by Secretary of State for Health. Until that decision was made, a policy of selective leucocyte-depletion was being followed.

Question 3
Leucocytes are removed prestorage in blood centres for several reasons. First, it is possible that cell-bound PrP scrape (PrPsc) or cell-associated viruses could escape the leucocyte-depletion process if this was performed after the cells had fragmented or had released microvesicles. Second, prestorage leucocyte-depletion is associated with less cytokine release and thus produces components less likely to be the cause of febrile transfusion reactions. Finally, prestorage leucocyte-depletion allows much greater standardization of the process and the ability to perform full quality control (QC) monitoring.

Question 4
The National Blood Service, which covers England, uses a mixture of techniques. All filters are now used in-line, i.e. are integral to the packs. Whole-blood filtration is used where platelet production is not required, generating leucocyte-depleted red cells and leucocyte-depleted fresh-frozen plasma (FFP). Some leucocyte-depleted red cells and FFP are also produced via separate red cell and plasma filtrations, following removal of theuffy-coat. Leucocyte-depleted platelets are produced by filtration ofuffy-coat pools, and also by apheresis using a mixture of Haemonetics MCS+ and GAMBRO Spectra.

This approach was chosen for consistency with the blood processing methods in use prior to universal leucocyte-depletion. To accommodate the change, the proportion of platelets obtained by apheresis has been increased to 50% and consequently the proportion of single donor units going through the process of buffy-coat removal has been reduced to 35%.

The cost of apheresis platelets is minimized by obtaining double-dose collections from many donors. Apheresis platelets do not differ in quality from those derived from pooled buffy-coats, but they do reduce donor exposure, ease the availability of human leucocyte antigen (HLA)/human platelet antigen (HPA)-matched platelets where they are required, and minimize the number of red cell units showing ‘double’ loss of red cells, which is consequent on combining the process of buffy-coat extraction with separate red cell filtration.

**Question 5**
The maximum storage time for red cell units prior to filtration is 48 h. In practice, filtration is either carried out on the day of collection at ambient temperature, or on day 1 on whole blood or red cell units that have been held overnight at 4 °C. Platelet filtration is carried out on pooled buffy-coats on day 1. Plasma is filtered and frozen within 8 h of collection, for use as single-unit FFP.

**Question 6**
The upper limit is $5 \times 10^6$ in each leucocyte-depleted component. This must be achieved in 99% of units with 95% confidence. A sampling regimen based upon statistical process monitoring is used, as per BEST guidelines. Counting is performed by flow cytometry using nationally standardized procedures and control samples. An internal quality assurance scheme has been established for all QC parameters, and an external quality assurance scheme for leucocyte counting has been developed with UK NEQAS. The upper limit of $5 \times 10^6$ is the same for all products.

When filtration has failed, components are withdrawn.

**Question 7**
No UK plasma is used for fractionation, so this question does not apply.

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